

Pattern and microbiological characteristics of diabetic foot ulcers in a Nigerian tertiary hospital

Obumneme Anyim¹, Christian Okafor², Ekenechukwu Young², Ijeoma Obumneme-Anyim³, Chidimma Nwatu²

1. Department of Medicine, Enugu State University of Science & Technology Teaching Hospital, Parklane, Enugu, Enugu State, Nigeria.
2. Endocrine Unit, Department of Medicine, University of Nigeria / University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State, Nigeria;
3. Department of Paediatrics, University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State, Nigeria.

Abstract:

Purpose: To determine the pattern and bacteriological characteristics of diabetic foot ulcers in patients attending a tertiary health care facility.

Method: 160 Patients with Diabetes Mellitus foot syndrome were recruited, out of which 52 had diabetic foot ulcers. Relevant clinical, biochemical, and microbiological evaluations were carried out on the subjects. Data analysis was done using SPSS version 20. p value was set at <0.05.

Results: 52 (32.5%) out of 160 subjects with Diabetes Mellitus Foot Syndrome (DMFS) had diabetic foot ulcers. Poor glycaemic control (mean HbA1c = 9.2 (2.7) %), and abuse of antibiotics (76.9%) characterized the subjects. Foot ulcers mainly involved the right lower limb and followed spontaneous blister formation (50%). Microbiological culture pattern was polymicrobial (71.2%); predominantly anaerobic organisms (53.3%). Gram positive and negative aerobic isolates yielded high sensitivity to common quinolones (76% - 87.8%). The gram positive and negative anaerobic isolates were highly sensitive to Clindamycin and Metronidazole respectively (80.2% - 97.8%). High sensitivity (>80%) yield for gram negative anaerobes was recorded for Imipinem and Ampicillin/Sulbactam.

Conclusion: Diabetic foot ulcers (DFU) contribute about one-third of DMFS. The bacteriological isolates from these ulcers are mainly polymicrobial with high sensitivity to common antibiotics. The need for appropriate use of antibiotics should be advocated among the patients.

Keywords: Diabetes mellitus, antibiotic sensitivity, Nigeria.

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Introduction

Many complications affect people living with diabetes mellitus (DM), of which diabetic mellitus foot syndrome (DMFS) is one of the most devastating and indeed seems

to be a common cause of prolonged hospitalization. The costs associated with diabetic foot ulcers (DFUs) can be tremendous for the patient, the family, the health care system and the society at large.¹

Available evidences on DMFS show that the outcomes have not changed much in the past 30 years, despite huge advances in the medical and surgical treatment of patients with diabetes.²

More than 80,000 amputations are performed yearly on diabetic patients in the United States, and approximately 50% of the people with amputations will develop ulcer-

Corresponding author:

Christian Okafor,
Department of Medicine, University of Nigeria /
University of Nigeria Teaching Hospital, Ituku-Ozalla,
Enugu, Nigeria.
Email: christian.okafor@unn.edu.ng

ations and infections in the contralateral limb within 18 months. An alarming 58% will have a contralateral amputation 3-5 years after the first amputation while the 3-year mortality after a first amputation has been estimated to be as high as 20-50%.²

In Nigeria, it was observed that more males in their prime presented with DMFS than females.¹ The average cost of managing a single ulcer is about \$8,000, and with infection, the cost rises to about \$17,000.² When it involves a major amputation, it further rises to \$45,000.² Ogbera et al³ in Nigeria (Lagos) about a decade ago found the mean cost for successfully treating a patient with DMFS to be Nigerian Naira (NGN) 180, 581.60 (equivalent to US\$1,389.83 at 1USD=NGN 129.93 using the exchange rate as at when the study was done). The total costs incurred ranged from NGN 20,400.00 (US\$157.00.) to NGN 278,029.00 (US\$2,139.84). With worsening inflation rates, these values would have increased, and with the present exchange rate (1USD to NGN 306), the mean cost for successfully treating a patient with DMFS would be estimated to stand at NGN425,287.98. The National Bureau of Statistics of Nigeria had in February 2012 released figures that showed that about 112 million Nigerians (67.1% of the country's total population of 167million) lived below poverty level—that is living below US\$1.00–US\$1.25 per day.⁴ This would place the cost of caring for DMFS beyond the reach of the average Nigerian. In addition to the direct costs of treatment, it is important to remember the indirect costs relating to loss of productivity, loss of quality of life and sometimes mortality.

Two hospital-based studies in Nigeria have demonstrated that diabetic foot disease accounted for the majority of non-traumatic amputations performed, ranging from 22.3% to 29.3%.^{5,6} The combination of neuropathy, ischaemia and direct adverse effects of DM on hosts defense mechanisms make patients with diabetes particularly vulnerable to foot infections and gangrene; often resulting in limb loss.⁷ Foot infections in diabetic patients are usually polymicrobial.⁸ *Staphylococcus aureus* was the commonest isolate reported in some Nigerian studies.^{9–11} Early identification of the foot at risk for ulceration would prompt early and effective treatment. Moreover, bacteriological and sensitivity patterns change and there is a need for periodic studies to keep abreast with these changes.

Since microbial culture and antibiotic sensitivity results cannot be generated in less than 48 hours (and may, on some occasions, take considerably longer), a knowledge of the antimicrobial sensitivity pattern of most bacteriological isolates in the locality would be helpful in deriving early empirical antimicrobial therapy for diabetic limb infections. This may help to reduce the socio-economic burden of the disease, amputation rates and mortality among diabetic patients.

The aim of this study was therefore to determine the burden and bacteriological characteristics of diabetic foot ulcers seen in patients attending our tertiary health care facility at Enugu, Nigeria.

Materials and methods

This was a cross-sectional descriptive hospital-based study performed over a six months period from November 2013 to April 2014 at the University of Nigeria Teaching Hospital, Enugu. Over this period, a total of 1,597 patients with diabetes mellitus were seen.

Patients were selected from the Diabetes Clinic attendance and Ward admission registers based on fulfilling the following criteria: age \geq 18 years, presence of DMFS (either diabetic foot at risk, (including evidence of neuropathy and peripheral vascular disease) and / or foot ulceration, gangrene or amputation) after detailed examination, and giving of consents. Detailed examination outside clinical evaluation, involved examination using 10-g Semmes-Weinstein monofilament (SenSitest Monofilament, ES-A58.426.677), biothesiometer (Bio-medical instrument Co., Newbury, Ohio, USA) and a Hand held Eden ultrasonic pocket Doppler (Shanghai International holding corp. GmbH, Shanghai, China). 160 patients who fulfilled the criteria above were consecutively enrolled into the study.

Ethical approval was obtained from the Health Research Ethics Committee of the Teaching Hospital.

A structured pre-tested questionnaire was administered to the consenting patients by the investigators and trained assistants. This assessed information such as age, sex, occupation, presence of hypertension, duration of diabetes, smoking, features of peripheral vascular disease (intermittent claudication, cold feet and rest pain), features of neuropathy, duration of lower limb ulceration, and history of previous amputation. Out of the 160 subjects, 52 (32.5%) subjects were identified to have DFU(s) which

were now further evaluated clinically and microbiologically. These ulcers were graded using both the University Of Texas Classification of Diabetic Foot and Wagner's classification system. Ulcers were assessed for signs of infection (swelling, exudates, surrounding, cellulitis, odour, tissue necrosis and crepitation) Plain radiograph was performed on all the subjects with limb ulcer to assess for osteomyelitis.

Microbiological procedure

Two sets of deep wound samples were obtained by rolling two sterile swab sticks one after the other over the surface of the sampling site, after debridement of superficial exudates. One swab specimen was immediately transferred into a thioglycollate medium, and sent with the second specimen to the microbiology laboratory for analysis under the supervision of a Medical Microbiologist. Samples were inoculated into Robertson's cooked meat media, and were incubated at 37°C for 48 hours. Sub-cultures were made from the Robertson's cooked media into different media using standard wire loop. For aerobic cultures, sub-cultures were made from the top of Robertson's medium into sheep blood agar and MacConkey agar and incubated at 37°C for 24 hours. For anaerobic cultures, sub-cultures were made onto Rogosa agar and anaerobic basal agar (Oxoid CM0972) supplemented with 5% horse blood. The medium was inoculated by surface plating to obtain single colonies. These were incubated in anaerobic jar at 37°C for a minimum of 72 hours. Anaerobic condition was achieved using the Oxoid AnaroGen Atmosphere Generation System (AN0025)®. Anaerobiosis was monitored with the help of a biological indicator (failure of growth of pure isolate of *Pseudomonas aeruginosa*, a strict aerobe) and a chemical indicator (methylene blue).

The jar was opened after 72 hours. The plates were examined with the help of a hand lens and each colony type recorded. Each type of colony was picked and sub-cultured onto Columbia blood agar for purity anaerobically. If no growth was obtained after 72 hours then re-incubation was done for at least 7 days after which if still no growth, a negative report was then given.

Colony characteristics were noted in case of any growth. Identification of micro-organisms was done using Gram stain and other biochemical tests from Columbia blood agar and Gram stain.

The isolated organisms were inoculated onto blood agar plates and anti-microbial susceptibility testing was carried out using the modified Kirby-Bauer disc diffusion

method.¹² Discs for available, anti-microbial agents were used. Attempts were made to incorporate discs representative of different classes of anti-microbials. Disc of the following anti-microbials were used: Ceftriaxone (30µg), Cefprozime (30µg), Cefoxitin (30µg), Gentamicin (10µg), Amoxicillin/Clavulanate (30µg), Cefuroxime (30µg), Nitrofurantoin (100µg), Ceftazidime (30µg), Ciprofloxacin (10µg), Ofloxacin (10µg), Pefloxacin (30µg), Clindamycin (2µg), Ampicillin/Sulbactam (10/10µg), Imipenem (10µg), Clarithromycin (10µg), Ampicillin (30µg), Erythromycin (10µg), Ampicillin/Cloxacillin (30µg), Cefixime (5µg), Levofloxacin (10µg), Norfloxacin (10µg), And Metronidazole (5µg). Following overnight incubation, the culture was examined for areas of no growth around the discs (zones of inhibition). The anti-microbial sensitivity pattern for different bacterial isolates were documented.

Data analysis

Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 20 (IBM Corp, 2011. Armonk, NY) Qualitative data were described as proportions or percentages, cross tabulation was used where necessary. Quantitative data were reported as mean and standard deviation, or as median and inter-quartile range in case of a skewed distribution. Test of significance for differences for quantitative and categorical variables were tested with T-test and Chi square analyses respectively. A p value of < 0.05 was considered as significant.

Results

General characteristics of study population

Subjects with DFU were 52 (26 male and 26 female participants respectively) out of 160 with DMFS. DFU therefore contributed 32.5% of DMFS among these subjects. The age range of the participants was 40 to 89 years. The age group with the highest number of participants was 41 – 60 years (57.7%; 30/52). The mean age at diagnosis of DM was 47.8±12.7years while the mean duration of DM was 11.5±7.3 years. Some of their characteristics are presented in Table 1. According to Table 1, male participants were older but diagnosis of diabetes mellitus was significantly earlier in females. Systolic blood pressure (SBP), and obesity (generalized and central) was commoner among the females. Glycaemic control was generally poor and had no significant gender difference. Majority were traders, and only able to attain primary education but these were not significantly associated with gender.

Table 1: Biochemical, Socio-demographic and anthropometric characteristics of the study population

Characteristics	Mean (SD)			P value
	Males (n=26)	Females (n=26)	Total (n=52)	
Age (years)	61.7 (9.7)	58.1 (11)	59.9 (10.5)	0.221
Age at diagnosis of DM (years)	52.6 (11.5)	43 (12.1)	47.8 (12.7)	0.005*
Duration of DM (years)	9.5 (7.7)	13.6 (6.3)	11.5 (7.3)	0.043*
BMI (kg/m ²)	24.2 (4.4)	28.5 (4.9)	26.4 (5.1)	0.005*
WC (cm)	88.8 (11.1)	96.6 (9.8)	92.8 (11.1)	0.019*
SBP (mmHg)	129.8 (12.9)	146 (25.4)	137.9 (21.6)	0.006*
DBP (mmHg)	79.2 (12)	82.7 (10.5)	81 (11.3)	0.268
FPG (mmol/L)	13.1 (5.9)	10.2 (6.2)	11.7 (6.2)	0.089
HbA1c (%)	9.7 (2.5)	8.7 (2.9)	9.2 (2.7)	0.192
	N (% within gender)		N (% of total)	
Occupation				
Traders	13 (50)	9 (34.6)	22 (42.3)	
Civil servant	2 (7.7)	7 (26.9)	9 (17.3)	
Farmers	3 (11.5)	4 (15.4)	7 (13.5)	
Retired civil servants	3 (11.5)	3 (11.5)	6 (11.5)	
Artisans	5 (19.2)	-	5 (9.6)	0.386
Unemployed	-	3 (11.5)	3 (5.8)	
Educational Status				
Nil	1 (3.8)	2 (7.7)	3 (5.8)	
Primary	14 (53.8)	12 (46.2)	26 (50)	0.26
Secondary	7 (26.9)	3 (11.5)	10 (19.2)	
Tertiary	4 (15.4)	9 (34.6)	13 (25)	

*Significant ($p < 0.05$) differences between males and females

Characterization of foot ulcers

Wagner's grade 1 was the commonest grade of ulcer among the participants. The right lower limb was the more commonly affected of the two limbs (29/52 (55.8%)).

Radiological features of osteomyelitis were present in 11 (21.2%) of the participants and they all had a Wagner ulcer grade between 3 and 5. Table 2 below shows the distribution of the ulcers according to Wagner's grading.

Table 2: Wagner's distribution of the DFU among the participants

Wagner's Grade	Description of the ulcer	Frequency	Percent
1	Superficial ulcers	17	32.7
2	Deep ulcers	16	30.8
3	Ulcer with bone involvement	12	23.1
4	Forefoot gangrene	4	7.7
5	Whole foot gangrene	3	5.8
	Total	52	100.0

Among the causes of the ulcers as shown in Figure 1, spontaneous blisters was the commonest. The median duration of ulcer before hospital presentation was 6.5 (0.4-52.0) weeks. Forty (76.9%) individuals with DFU

had received antibiotics before presentation. Ampicillin/Cloxacillin was the most common drug used by these individuals (30% (12/40)). Thirty-one (59.6%) of them had the last dose of antibiotic more than a week before recruitment into the study.

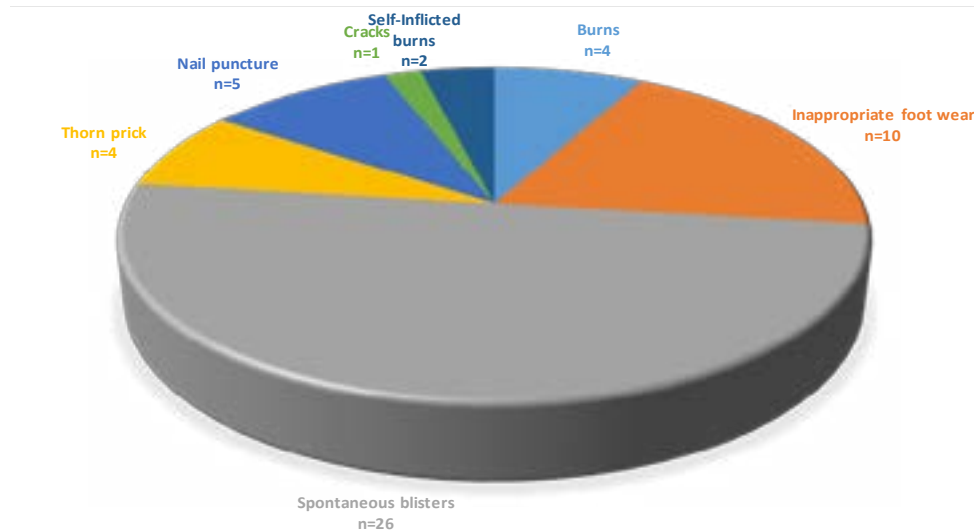


Figure 1: Distribution of causes of ulcer among study participants

Pattern of bacteriological flora in the participants with diabetic foot ulcers

A total of 137 organisms were isolated from the participants. Approximately 71.2% were polymicrobial. Sev-

en percent grew only anaerobes, 5.8% only aerobes and 15.4% were sterile cultures. The various types and frequencies of the isolated organisms are shown in Table 3 with anaerobes constituting a greater majority of the isolated organisms (53.3% (73/137)).

Table 3: Types and frequencies of isolated organisms

Types of isolated organisms	Frequency	Percent
Aerobes		
<i>Corynebacterium spp</i>	9	14.1
<i>Proteus mirabilis</i>	9	14.1
<i>Pseudomonas aeruginosa</i>	8	12.5
<i>Staphylococcus aureus</i>	11	17.2
<i>Coagulase negative Staphylococcus aureus</i>	2	3.1
<i>Streptococcus pyogenes</i>	10	15.6
<i>Enterococcus faecalis</i>	4	6.3
<i>Escherichia coli</i>	6	9.4
<i>Klebsiellae pneumoniae</i>	4	6.3
<i>Morganella spp</i>	1	1.6
Total	64	100
Anaerobes		
<i>Propionibacterium spp</i>	13	17.8
<i>Bacteroides spp</i>	21	28.8
<i>Eubacterium spp</i>	2	2.7
<i>Peptostreptococcus spp</i>	15	20.5
<i>Fusobacterium spp</i>	7	9.6
<i>Prevotella spp</i>	6	8.2
<i>Closteridium spp</i>	4	5.5
<i>Lactobacillus</i>	1	1.4
<i>Anaerococcus spp</i>	4	5.5
Total	73	100

Susceptibility pattern of the isolated organisms

The susceptibility pattern of the isolated bacteria revealed that the gram positive aerobic organisms showed high sensitivity to levofloxacin and Norfloxacin while ma-

ajority of the Gram negative aerobes were highly sensitive to Pefloxacin and Ciprofloxacin (Table 4). The highest sensitivity for anaerobes was recorded for clindamycin and metronidazole (Table 5).

Table 4: Susceptibility pattern of isolated aerobic organisms

Gram positive	Susceptibility pattern (%)									
	Eryth	Ceft	A/Cx	Cef	Levo	Norf	Gent	Oflo	Clin	Cipr
<i>Corynebacterium spp</i>	100	100	55.6	100	100	100	55.6	77.8	77.8	77.8
<i>Staphylococcus aureus</i>	54.5	54.5	54.5	54.5	100	100	54.5	27.3	54.5	72.7
<i>Coagulase negative Staphylococcus aureus</i>	100	-	-	-	100	100	-	-	-	100
<i>Streptococcus pyogenes</i>	60.0	60.0	40.0	ND	80	60.0	40.0	40.0	60.0	80.0
<i>Enterococcus faecalis</i>	-	50.0	-	-	50	50.0	50.0	-	50.0	50.0
Total %	314.5	264.5	150.1	154.5	430	410	200.1	145.1	242.3	380.5
Mean %	62.9	52.9	30.0	38.6	86.0	82.0	40.0	29.0	48.5	76.1
Gram negative	Nitro	Ceft	Cipr	Gent	Oflo	A/Cv	Pef	Clar	Chlo	Amp
<i>Proteus mirabilis</i>	-	55.6	88.9	55.6	22.2					
Mean %	23.3	37.8	87.8	61.1	39.4	205.5	438.9	83.3	183.3	33.3
						41.1	87.8	16.7	36.7	8.3

(Eryth) Erythromycin, (Ceft) Ceftriaxone (A/Cx) Ampicillin/Cloxacillin, (Cef) Cefixime, (Levo) Levofloxacin, (Norf) Norfloxacin, (Gent) Gentamycin, (Oflo) Ofloxacin, (Clin) Clindamycin, (Cipr) Ciprofloxacin, (Nitro) Nitrofurantoin, (A/Cv) Amoxicillin/Clavulanate, (Pef) Pefloxacin, (Clar) Clarithromycin, (Chlo) Chloramphenicol, (Amp) Ampicillin, (ND) not done

NB: Blank cells = 0%

Table 5: Susceptibility pattern of isolated anaerobic organisms

Gram positive	Susceptibility pattern (%)								
	A/S	Clin	Imip	Ceft	Ceftiz	Metro	Cefo	Amp	Pen
<i>Propionibacterium spp</i>	76.9	84.6	ND	76.9	53.8	100	76.9	84.6	84.6
<i>Peptostreptococcus spp</i>	73.3	100	ND	73.3	53.3	86.7	73.3	53.3	40.0
<i>Anaerococcus spp</i>	100	100	ND	50.0	50.0	100	50.0	50.0	50.0
<i>Closteridium spp</i>	50.0	100	ND	50.0	50.0	100	50.0	100	100
<i>Embacterium spp</i>	100	100	ND	-	100	100	-	-	-
<i>Lactobacillus spp</i>	-	100	ND	-	-	100	-	-	100
Total %	400.2	584.6	0	250.2	307.1	586.7	250.2	287.9	374.6
Mean %	66.7	97.4	0.0	41.7	51.2	97.8	41.7	48.0	62.4
Gram negative									
<i>Bacteroides spp</i>	90.5	90.5	90.5	62.0	71.4	90.5	52.4	ND	ND
<i>Prevotella spp</i>	100	100	50.0	-	-	50.0	-	-	-
<i>Fusobacterium spp</i>	71.4	100	100	71.4	71.4	100	71.4	100	71.4
Total %	261.9	290.5	240.5	133.4	142.8	240.5	123.8	100	71.4
Mean %	87.3	96.8	80.2	44.5	47.6	80.2	41.3	50.0	35.7

[(A/S) Ampicillin/Sulbactam, (Clin) Clindamycin, (Imip) Imipenem, (Ceft) Ceftriaxone (Ceftiz) Cefprozime, (Metro) Metronidazole, (Cefo) Cefoxitin, (Amp) Ampicillin, (Pen) Penicillin, (ND) not done] NB: Blank cells = 0%

Discussion

Diabetic Foot Ulcerations constituted about a third of the burden of foot lesions in the participants who were a subset of patients with DMFS. This clear demonstration of the huge burden and challenge among Nigerians suffering from complications of diabetes mellitus is similar to the high prevalence of 37.2% reported by Ajayi et al¹³ in Ido Ekiti Nigeria. It is also comparable to other findings in Africa and beyond.^{14,15} The picture however significantly varies from the figure of 1% in Europe and North America according to Boulton.¹⁶ The high cost and lack of well-trained multi-disciplinary medical personnel, facilities and standardized management protocols are possible contributory factors. Physicians also have an important role in the prevention, early diagnosis and management of diabetic foot complications. The physi-

cian should carry out early risk assessment of the feet in DM patients which can be time consuming and when factored into the abysmally inadequate doctor-patient ratio in Nigeria will affect the depth of care a doctor can provide. According to WHO the density of physicians per 10,000 population is 4.0.¹⁷ This clearly will translate to very poor specialist attention. The seriousness of this crisis is underscored by current estimates that sub-Saharan Africa is part of the world that will experience the greatest rise in diabetes prevalence over the next 20 years.¹⁸ Other patient characteristics such as poor glycaemic control as measured by HbA1c, fasting blood glucose and even a single random blood glucose has been found to be strongly predictive of subsequent ulceration and amputation.¹⁹ In this study, the mean FBG, eABG and HbA1c were above acceptable limits for good glycaemic control.

Similarly, comparable HbA1c results were documented by Dziemidok et al.²⁰ and Chinenye et al.²¹ Ogbera et al.²² found a higher mean FBG of 11.6 ± 4.7 mmol/l in Nigeria while Kibirige et al.²³ found a higher mean HbA1c of $9 \pm 2.9\%$ in Uganda. Jbour et al.²⁴ on the other hand found a lower mean HbA1c of $7.4\% \pm 1.4\%$ in Amman Jordan. Jordan was ranked by the World Bank to be the number one health care services provider in the region and among the top five in the world, as well as being the top medical tourism destination in the Middle East and North Africa.²⁵

Approximately 20% of the participants with DFU had radiological features of osteomyelitis. This is comparable with 17% found in a UK based study by Bano et al.²⁶, but lower than 34.4% found by Edo et al.²⁷ The lower prevalence in the index study, compared to the other Nigerian based study could be due to the differences in the distribution of the ulcers according to Wagner's grading method. Grades 1 and 2 were the most common ulcer grades at presentation in the index study, as against grades 4 and 3 in the study by Edo et al.²⁷

Spontaneous blister was the commonest cause of foot ulceration in this study, involving half of the study population. Unachukwu et al.²⁸, and Edo et al.²⁷ similarly found similar occurrences in their study (51.7% and 52.46% respectively). The picture is further similar to the findings of workers in Nigeria.^{22,29} Though the patients reported the ulcers as resulting from spontaneous blisters, there remains a possibility that some of the ulcers may have resulted from unnoticed micro-trauma. Spontaneous blisters may also result from the use of inappropriate footwear; this was found to be the second commonest predisposing event for DFU in this study. Ill-fitting foot wears in patients with peripheral neuropathy may result in foot ulcerations in patients with insensate feet. Disordered foot mechanics and abnormal weight-bearing in different areas of the foot in patients with peripheral neuropathy also make the foot susceptible to ulceration while wearing shoes.

Of interest also, is the fact that self-inflicted burns due to thermal injury resulting from application of hot compresses to numb feet precipitated two cases of DFU recorded in this study. Thus, there is indeed a need to ensure that better focused education on appropriate foot wears, foot care and other harmful practices be intensified among these patients.

Bacteriological pattern of diabetic foot ulcers

In the present study, a total of 137 different microorganisms were isolated from the participants, with mixed gram-positive and gram-negative species; an average of 1.23 aerobic bacteria, 1.40 anaerobic bacteria and an overall average of 2.63 organisms per case. This is similar to the findings by Unachukwu et al.⁹ where cultures yielded an average of 2.3, but lower than the findings of Citron et al.³⁰ that yielded an average of 3.8 species per specimen; this may be as a result of the larger sample size in the US study. Improved culture techniques and use of nucleic acid-based techniques for isolating organisms is another plausible reason. Polymicrobial nature of diabetic foot infections have been observed in various studies within and outside the country.^{8,9,30} The 71.2% polymicrobial isolates found in the present study is similar to the finding of 83.8% by Citron et al.³⁰ Some studies suggest that the interactions of organisms within these polymicrobial mixtures lead to the production of virulence factors, such as hemolysins, proteases, and collagenases, as well as short-chain fatty acids, that cause inflammation, impede wound healing, and contribute to the chronicity of the infection.^{31,32} In such mixtures, biofilms that impede the penetration of antimicrobial agents into the infected site may also form.³³ Thus, the presence of multiple species can have important clinical implications that should not be overlooked. In this study, gram-positive bacteria were the predominant pathogens with *Staphylococcus aureus* being the commonest aerobic isolate followed by *Streptococcus pyogenes*. Similarly, the predominance of *Staphylococcus aureus* has been demonstrated in many studies, within and outside the country.^{9-11,33,34-36} Predominance of gram-negative aerobes have been reported also by some few workers.^{37,38} These differences could be partly due to changes in the causative organisms occurring over time, geographical variations, or the types and severity of infection. Differences could further result from use of a relatively small number of specimens, and inadequate specimen collection techniques (which would fail to exclude superficial or colonizing organisms), poor handling techniques and poor preservation methods for anaerobic organism.³⁹

Anaerobes were the predominant organisms cultured overall in this study with *Bacteroides spp* being the predominant organism, followed by *Peptostreptococcus spp*. This is in contrast with the findings of some other studies, which failed to isolate anaerobes in general.⁴⁰⁻⁴² The failure to pick anaerobic bacteria in wounds may be due to several

reasons. Compared with aerobic and facultative microorganisms, the culture, isolation, and identification of anaerobic bacteria is more time-consuming, labor-intensive, and expensive and is often deemed to be too demanding for many diagnostic microbiology laboratories. Since anaerobes are often perceived to die rapidly in air, the method of specimen collection and transportation to the laboratory is assumed to be critical for maintaining viability and for effective culture, in fact the yield of anaerobic organisms depends on the method of sample collection. Among those that did use appropriate methods, one study suggested that *Bacteroides fragilis* was the predominant anaerobic isolate⁴³, while the predominance of *Peptostreptococcus spp*, followed by *Bacteroides spp* was demonstrated by Banoo et al.¹² Least sensitivity was seen with the Penicillin (Ampicillin); this may be linked to the indiscriminate use of this older group of drugs in our society, even as seen among the participants in this study.

The gram positive and negative aerobic isolates showed high level of sensitivity to the quinolones particularly Levofloxacin, Norfloxacin, Pefloxacin and Ciprofloxacin but this was not a class effect as sensitivity to Ofloxacin was poor for both groups of microbes. All the anaerobic isolates were significantly sensitive to Clindamycin and Metronidazole. These findings are similar to the observations by Unachukwu et al.⁹ in Port Harcourt, Nigeria. This tends to suggest that antibiotic susceptibility may not have significantly changed over time across these close, but variable geographic locations. Similarly, despite wide geographical variation, but similar socioeconomic state, a study done in India, also reported a comparable susceptibility pattern.⁸

Conclusion

The study has demonstrated that the burden of DFUs is still huge among Nigerians living with diabetes mellitus. The bacteriological findings also shows that DM foot infections still largely remain polymicrobial however with likelihood of isolating many anaerobes if painstaking measures are taken to collect and handle the samples appropriately. A good sensitivity to common and available antibiotics was demonstrated but the abuse/inappropriate use of antibiotics remains a major issue to tackle among the patients. The sensitivity pattern shown here and previously reported by others should be a guide to

empirical antibiotic usage among healthcare practitioners while waiting for sensitivity-guided treatment, which is the ideal practice. Intensified and focused educational initiatives on diabetes and the foot, early screening for diabetes complications and concerted efforts to improve glycaemic control may all go a long way in stemming the burden and consequences of DFUs and are therefore recommended.

Conflict of interest

None declared.

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