MICROBIOLOGICAL ASSESSMENT OF DIABETIC FOOT ULCERS FROM MAJOR TERTIARY HOSPITALS IN ABUJA, NIGERIA

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Abstract: Diabetes mellitus (DM), a debilitating metabolic and physiological disease, affects millions worldwide with diverse outcomes. Diabetic Foot Ulcers (DFUs) is one severe, debilitating complication of DM. These chronic wounds can become colonized by various bacterial species. The aim of the study was to evaluate bacteria isolated from DFUs at two tertiary hospitals in Abuja, Nigeria. The study reviewed data of bacteria isolated from DFUs over a two-year period. Gender, ages, duration of diabetes and the stage of DFUs using the Wagner scale, were considered. Chi square (χ^2) analysis determined statistically significant associations. P values < 0.05 were considered significant. One hundred and fifty-one (151) cases of DFUs were established. More females (57.3%) than males (42.7%) had DFUs. More 63 (42%) cases of DFUs were at Grade III than other stages. One hundred and sixty (160) bacterial, Gram-positive and Gram-negative aerobes were isolated. No anaerobes were found. The most isolated bacterium was *S. aureus* (52 (34.7%), followed by *P. aeruginosa* 16 (10.7%). Coagulase-negative *Staphylococcus* (CoNS) was the least isolated 4 (2.7%). χ^2 showed that differences in infecting bacteria were statistically significant ($\chi^2 = 43.74$; $P < 0.05$). More bacteria were isolated from females 87 (58%) than from males 63 (42%) ($\chi^2 = 14.27$; $P > 0.05$). Also, more bacteria were found in females 18 (11.3%) who had had DM for greater than 10 years than in males 8 (8.13%) of similar duration. This was however not statistically significant ($\chi^2 = 24.13$, $P > 0.05$). There was also no statistical significance to bacteria isolated by DFU grade ($\chi^2 = 0.47$; $P > 0.05$). Identifying prevalent bacteria of DFUs in Nigeria could help in shaping antibiotic, treatment, and other intervention policies towards effective management of DFU infections.

Key Words: Diabetic Foot Ulcers, Diabetic Foot Infections, Colonizing bacteria, Diabetics, Diabetes, Nigeria

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease, of three (3) types – Type 2, Type 1 and Gestational. Symptoms include high glucose levels in the blood, which eventually can cause damage to nerves, kidneys, eyes, blood vessels and the heart (IDF, 2023). The International Diabetes Federation (IDF) states that the prevalence of DM worldwide is near epidemic rates, with over 537 million people living with the disease and over 80% of cases in low and middle-income countries (IDF, 2023). This figure is projected to rise to between 642 to 783 million people between 2030 to 2050 (Lancet, 2023).

The burden of diabetes is reflected in the number of premature deaths from the devastating complications of diabetes. With prevalence between 0.8% - 10.3%, about 5.1 million deaths occurred from diabetes related causes in sub-Saharan Africa. About 75% of these deaths are in people under the age of 60 (WHO, 2023).

Diabetic foot (DF) refers to the variety of pathological conditions that affect the feet of individuals with diabetes, while Diabetic Foot Ulcer (DFU) is a severe complication of diabetes that affects about 15% of people with DM. It is a chronic wound that develops on the foot or lower leg, and has severe consequences such as lower limb amputation, extended hospitalization, and death (IDF, 2023; Raja *et al*., 2023).

Diabetic foot complications are a major public health problem, responsible for more hospitalizations than any other complication of diabetes. The lifetime risk of a person with diabetes developing diabetes mellitus foot ulcers is as high as 25%, and accounts for 40 - 60% of all non-traumatic lower extremity amputations worldwide (WHO, 2023).

Some factors that lead to DFUs in Nigeria include walking bare foot, improper footwear, poor foot hygiene, poor living conditions, lack of awareness and proper information amongst patients, relatives and even healthcare providers, preferences for home or herbal treatment of diabetes, and lack of facilities at primary, secondary or tertiary health care centers (Ugwu *et al*., 2019).

The burden of DFU in Nigeria is significant, and its management is a significant public health challenge. One study at a tertiary hospital in Nigeria found that DFU was the most common reason for lower limb amputation, accounting for 65.4% of cases and ranged between 12% to 53% (Anyim *et al*., 2019; Ejiofor *et al*., 2019).

Understanding the bacterial infection patterns, will help provide insight into the current clinical practices and offer empirically informed antibiotic treatment options to health workers involved in management of diabetics in health establishments of Nigeria, as well as suggestions on targeted interventions to ultimately enhance positive patient outcomes.

Materials and Methods

Study Location

Federal Capital Territory (FCT), Abuja, Nigeria

Abuja, the FCT, is the capital city of Nigeria and is the administrative and political centre of the country. Several tertiary health institutions are in the FCT. The study was conducted in two tertiary health institutions in Abuja:

National Hospital Abuja (NHA)

NHA was established in 1999 as a 200-bed space national hospital for women and children. It was expanded to a

400-bed space hospital that renders specialist services in all areas of medicine.

University of Abuja Teaching Hospital (UATH)

A 350-bed space hospital, it was established as a specialist hospital in 2006. It also functions as a tertiary health institution for the training of medical students.

Study Design

A cross-sectional observational study that assessed the infecting bacteria of DFU patients in the Federal Capital Territory (FCT), Abuja, Nigeria.

Ethical Considerations

The study adhered to ethical guidelines and principles for human research. Ethical approval was obtained from the relevant institutional review boards before data collection. Patient confidentiality was maintained by using anonymized data. Informed consent was sought and granted before commencement of data collection as necessary.

Sample Size and Study Population

One hundred and fifty-one (151) patients with DM and documented DFU were analyzed. Fifty-five (55) cases from UATH and 95 from NHA.

Data from patients included patients' demographic characteristics (age, gender, etc.), medical history (diabetes duration, previous DFU episodes, etc.), DFU characteristics (size, grade, location), and details of isolated bacteria following standard isolation, culturing, identification protocols and best practices.

Staging the diabetic foot

The grading and staging of DFU was according to the 5-grade scheme (stage) of Wagner (Shah *et al*., 2022).

Data Analysis

Descriptive and inferential statistics methods were used for analysis. Descriptive statistics tools such as frequency tables, means and standard deviation were used to describe the demographic characteristics of the respondents.

Chi Square (χ^2) analysis (IBM SPSS Version 27) was employed to test significant relationships between variables. Values of $P \leq 0.05$ were considered statistically significant.

Results

Socio-demographic characteristics of study population

The socio-demographic characteristics of participants in the study are shown in Table 1. Gender rates showed that 57.3% of were female, while 42.7% were male. The age distribution showed that 11.3% were 35 years and younger, 21.3% were 36 – 45 years, 18.7% within the ages of 46 – 55 years, 20.0% were 56 – 65 years, 28.7% were greater than 65 years old. The mean and standard deviation of ages of patients were 54.9 and ±15.3 years respectively. The duration of diabetes among the respondents showed that 0.7% had diabetes less than a year, 26.7% respondents had diabetes for about 2 - 4 years, 22.0% between 4 - 6 years, 24.0% had diabetes for 6 - 8 years, 9.3% for 8 - 10 years, while 17.3% had the condition for greater than 10 years.

The DFU grade among the participants showed that 19.3% had DFU Grade 1, 34.0% had DFU Grade 2, the majority (42.0%) had DFU Grade 3, while those who had DFU Grade 4 were 4.7%. χ^2 analyses, however, did not reveal any statistical association between the stage of DFU and the gender ($P > 0.05$).

Table 1: Medical and Demographic Characteristics of Study Population (N = 151)

Variables	Category	Frequency	Percentage
Gender	Male	64	42.7
	Female	86	57.3
Age *(years)*	26 – 35	17	11.3
	36 – 45	32	21.3
	46 – 55	28	18.7
	56 -65	30	20.0
	> 65	43	28.7
Duration of Diabetes *(years)*	1	1	0.7
	2 to 4	40	26.7
	4 to 6	33	22.0
	6 to 8	36	24.0
	8 to 10	14	9.3
	>10	26	17.3
DFU Grade	Grade 4	7	4.7
	Grade 3	63	42.0
	Grade 2	51	34.0
	Grade 1	29	19.3

Bacteria Isolated

The bacteria isolated from the 151 participants included coagulase negative *Staphylococcus* (CONS) in 2.7% patients, *B. fragilis* in 4.0%, methicillin resistant *Staphylococcus aureus* (MRSA) was isolated in 5.3%, *Proteus* spp. in 9.3% of patients, *Pseudomonas* spp. in 17.3%, *Klebsiella* spp. was found in 6.7% respondents, *E. coli* was isolated in 9.3% of respondents, Streptococci in 10.7% respondents, while *Staphylococcus aureus* was found in 34.7% of patients. (Figure 1). Again, χ^2 analyses did not show any association between gender and bacterial isolates. All bacteria had an equal chance of being isolated from both gender ($\chi^2 = 14.27$; $P > 0.05$).

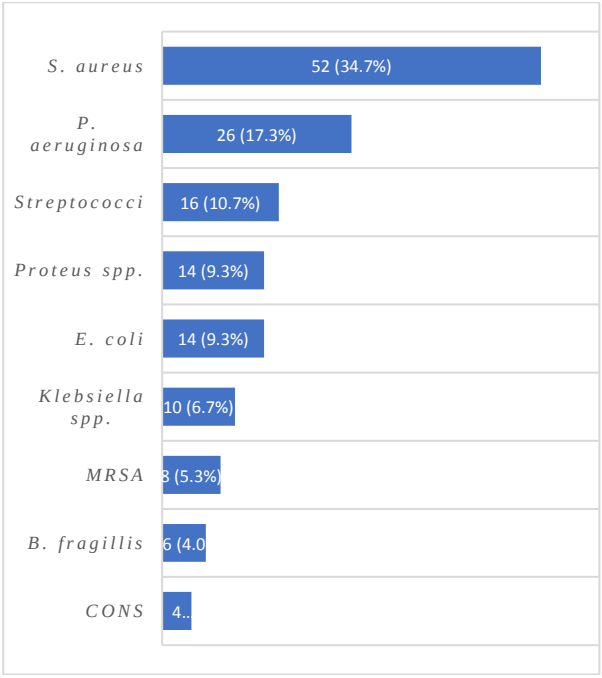

Figure 1: Bacteria Isolated from DFU Patients

Table 2 shows bacteria isolated from DFU in relation to the gender and ages of patients. In males, the age range with the most isolates was the 36 – 45-year range with 20

(12.7%). This was closely followed by 56 – 65 years at 17 (10.8%). The least number of bacteria isolated was in the 26 – 35-year group with 8 (5.1%). *S. aureus* was the most frequently isolated organism; 13 (8.7%) from the age range 36 – 45 years. In patients 65 years and above, *S. aureus* occurred at 11 (7.3%). In males, the least occurring bacteria was coagulase negative *Staphylococcus* (CoNS) 26 – 35 and 46 - 55-year groups at 1 (0.7%) each.

Conversely in females, the highest 27 (17.2%) bacterial isolation rates were in the > 65-year group. The 46 – 55-year group followed closely with 26 (16.6%). This surpassed the similar age range in males. Again, *S. aureus* was the most isolated bacterium in the 46 – 55-year group with a 10 (6.7%) rate. In females, the least occurring bacterium was also CoNS at 1 (0.7%) each in the 56 – 65 and > 65-year age ranges. Statistical analysis did not show any association between age and bacteria isolated ($\square^2$ = 23. 84; P >0.05).

Table 2: Association of Isolated Bacteria from DFU With Age and Gender

Gender	Age Range *(years)*	Prevalent Bacteria									
		S. aureus No. *(%)*	*P. aeruginosa* No. *(%)*	Streptococci No. *(%)*	*Proteus* No. *(%)*	*E. coli* No. *(%)*	*Klebsiella* Spp. No. *(%)*	MRSA No. *(%)*	*B. fragilis* No. *(%)*	CONS No. *(%)*	Total No. *(%)*
Male	26 – 35	4*(2.7)*	2*(1.3)*	0	0	1*(0.6)*	0	0	0	1*(0.7)*	8*(5.3)*
	36 – 45	8*(5.3)*	2*(1.3)*	0	2*(1.3)*	3*(2.0)*	0	1*(1.3)*	0	0	16*(10.6)*
	46 – 55	2*(1.3)*	0	1*(0.6)*	2*(1.3)*	1*(0.6)*	0	0	0	1*(0.7)*	7*(4.5)*
	56 – 65	6*(4.0)*	4*(2.7)*	1*(1.3)*	1*(0.7)*	1*(0.6)*	2*(1.3)*	0	1*(0.6)*	0	16*(10.6)*
	> 65	11*(7.3)*	1*(0.6)*	1*(0.6)*	1*(0.7)*	1*(0.6)*	0	0	1*(0.6)*	0	16*(10.6)*
Total		**31**	**9**	**3**	**6**	**7**	**2**	**1**	**2**	**2**	**63**(42.0)
Female	26 – 35	3*(2.0)*	2*(1.3)*	0	2*(1.3)*	1*(0.6)*	0	1*(0.6)*	0	0	9*(5.7)*
	36 – 45	2*(1.3)*	6*(4.0)*	2*(1.3)*	2*(1.3)*	0	3*(2.0)*	1*(0.6)*	0	0	16*(8.9)*
	46 – 55	10*(6.6)*	4*(2.7)*	2*(1.3)*	0	1*(0.6)*	2*(1.3)*	0	1*(0.6)*	0	20*(16.6)*
	56 – 65	0	1*(0.6)*	2*(1.3)*	1*(0.7)*	3*(1.3)*	2*(1.3)*	4*(2.7)*	1*(0.6)*	1*(0.6)*	15*(8.4)*
	> 65	7*(4.5)*	4*(2.7)*	6*(4.0)*	3*(2.0)*	2*(1.3)*	1*(0.7)*	1*(0.6)*	2*(1.3)*	1*(0.6)*	27*(17.2)*
Total		**22**	**17**	**12**	**8**	**7**	**8**	**7**	**4**	**2**	**87***(58.0)*

Key: MRSA = Methicillin Resistant *Staphylococcus*; CONS = Coagulase negative *Staphylococcus*

DFU stages showed that in males, *S. aureus* was the most frequent bacterium, 12 (8.0%) isolated at DFU Grade 3. *Klebsiella* spp. and Methicillin-resistant *S. aureus* (MRSA) were 1 (0.7%) each, and isolated at DFU Grades 1, 2, 4 for the former, and DFU Grades 2 and 4 for the latter respectively. However, bacteria were most isolated from 28 (18.7%) at DFU Grade 2 in males.

Similarly, in females, *S. aureus* was also the most frequently isolated bacterium among patients with DFU Grade 3 11 (7.3%), while the least isolated was also *S. aureus* at DFU Grade 4 1(0.7%). However, in females, the DFU Grade with most isolated bacteria was Grade 2 with 35 (23.3%). Table 3. No association was found between bacteria isolated and gender ($\square^2$ = 214.27; P > 0.05).

Table 3: Association of Bacteria Isolated from DFU With Gender and Stage of DFU

Gender	DFU GRADE	Prevalent Bacteria									
		S. aureus No. *(%)*	*P. aeruginosa* No. *(%)*	Streptococci No. *(%)*	*Proteus* No. *(%)*	*E. coli* No. *(%)*	*Klebsiella* Spp. No. *(%)*	MRSA No. *(%)*	*B. fragilis* No. *(%)*	CONS No. *(%)*	**Total No.** *(%)*
Male	Grade 1	2*(1.3)*	0	0	0	0	1*(0.7)*	0	0	0	3*(2.0)*
	Grade 2	9*(5.7)*	5*(3.3)*	2*(1.3)*	2*(1.3)*	4*(2.7)*	1*(0.7)*	1*(0.7)*	2*(1.3)*	2*(1.3)*	28*(18.7)*
	Grade 3	12*(8.0)*	4*(2.7)*	0	3*(2.0)*	2*(1.3)*	0	0	0	0	21*(14.0)*
	Grade 4	8*(5.3)*	0	2*(1.3)*	1*(0.7)*	1*(0.7)*	1*(0.7)*	1*(0.7)*	0	0	14*(9.3)*
Total		**31**	**9**	**4**	**6**	**7**	**3**	**2**	**2**	**2**	**66***(44.0)*
Female	Grade 1	2*(1.3)*	0	0	0	0	2*(1.3)*	0	0	0	4*(2.7)*
	Grade 2	6*(4.0)*	4*(2.7)*	5*(3.3)*	4*(2.7)*	7*(4.5)*	3*(2.0)*	4*(2.7)*	0	2*(1.3)*	35*(23.3)*
	Grade 3	11*(7.3)*	7*(4.5)*	3*(2.0)*	2*(1.3)*	0	3*(2.0)*	2*(1.3)*	2*(1.3)*	0	30*(20.0)*
	Grade 4	1*(0.7)*	6*(4.0)*	4*(2.7)*	2*(1.3)*	0	0	0	2*(1.3)*	0	15*(10.0)*
Total		**20**	**17**	**12**	**8**	**7**	**7**	**6**	**4**	**2**	**84***(56.0)*

Key: MRSA = Methicillin Resistant *Staphylococcus*; CONS = Coagulase negative *Staphylococcus*

By duration of diabetes, males who had DM for 4 – 6 years had the most bacteria isolated 23 (15.5%). This group was followed by those who had the condition for 7 – 9 years 21 (14.0%). In these two groups the most isolated bacterium was *S. aureus* at 9 (6.0%) and 10 (6.6%) respectively.

In females, bacteria were most frequently isolated from those who had diabetes for between 4 – 6 years with of 32 (21.3%). Again, they were closely followed by the 7 – 9 years duration of diabetes with 24(16.0%) frequency of isolation. However, in these groups, the most isolated bacteria were *S. aureus* 10 (6.6%) and *P. aeruginosa* 8 (5.4%) respectively (Table 4). As in other instances, no association was observed between isolates and age range ($\chi^2 = 23.84$, $P > 0.05$).

Table 4: Association of Isolated Bacteria from DFU with Duration of Diabetes

Gender	Duration of Diabetes *(years)*	Prevalent Bacteria									
		S. aureus No. *(%)*	*P. aeruginosa* No. *(%)*	Streptococci No. *(%)*	*Proteus* No. *(%)*	*E. coli* No. *(%)*	*Klebsiella* Spp. No. *(%)*	MRSA No. *(%)*	*B. fragilis* No. *(%)*	CONS No. *(%)*	**Total** No. *(%)*
Male	1 – 3	3*(2.0)*	2*(1.3)*	0	2 *(1.3)*	2*(1.3)*	0	0	0	0	9*(6.0)*
	4 – 6	9 *(6.0)*	4*(2.7)*	1*(0.6)*	4*(2.7)*	1*(0.6)*	1*(0.6)*	1*(0.6)*	1*(0.6)*	1*(0.6)*	23*(15.3)*
	7 – 9	10*(6.6)*	3*(2.0)*	1*(0.6)*	1*(0.6)*	3*(2.0)*	0	1*(0.6)*	1*(0.6)*	1*(0.6)*	21*(14.0)*
	> 10	9*(6.0)*	1*(0.6)*	2*(1.3)*	0	0	1*(0.6)*	0	0	0	13*(13.0)*
Total		**31**	**10**	**4**	**7**	**6**	**2**	**2**	**2**	**2**	**66***(44.0)*
Female	1 – 3	1*(0.6)*	4*(2.7)*	1*(0.6)*	1*(0.6)*	1*(0.6)*	1*(0.6)*	1*(0.6)*	0	0	10 *(6.6)*
	4 – 6	10*(6.6)*	2*(1.3)*	5 *(3.3)*	2*(1.3)*	4 *(2.6)*	2 *(1.3)*	2 *(1.3)*	3 *(2.0)*	2 *(1.3)*	32*(21.3)*
	7 – 9	3*(2.0)*	8*(5.4)*	4*(2.7)*	4*(2.7)*	1*(0.6)*	1*(0.6)*	2*(1.3)*	1*(0.6)*	0	24*(16.0)*
	> 10	7*(4.5)*	2*(1.3)*	2*(1.3)*	0	2*(1.3)*	4*(2.6)*	1*(0.6)*	0	0	18*(12.0)*
Total		**21**	**16**	**12**	**7**	**8**	**8**	**6**	**4**	**2**	**84***(56.0)*

Key: MRSA = Methicillin Resistant *Staphylococcus*; CONS = Coagulase negative *Staphylococcus*

Discussion

The observation that more females than males had greater numbers of bacteria associated with DFUs, and the recorded diverse age distribution of occurrence is also reported globally by The Lancet (2023). Regarding the age at which diabetes is most common, Murphy-Lavoie *et al.* (2023) affirm that the highest prevalence is in persons 65 years and older, though younger age ranges are not exempt from the disorder. These demographic details provide a context for understanding the patient population under investigation.

The data also indicated that a large proportion of patients had lived with diabetes for more than 10 years. Additionally, most had Grade 3 DFU, from which a variety of bacteria were isolated. This suggested that majority of cases were relatively severe. While cultures of normal skin typically yield a mixture of Gram-positive organisms present in benign associations, normal skin is usually not colonized with primary potential pathogens such as *Staphylococcus aureus* or γ -haemolytic streptococci. However, these organisms can attack and rapidly colonize breaks in the skin. If a chronic ulcer results, the colonizing flora may become more complex, often including aerobic Gram-negative rods and sometimes anaerobes. According to Raspovic and Kane (2014), early onset Diabetic Foot Infections (DFIs) begin with staphylococci and streptococci but rapidly become polymicrobial as the infection progresses. This agreed with the findings of this study.

In this study, *Staphylococcus aureus* was the most isolated bacterium. Anita *et al*. (2023) similarly isolated *E. coli* as the prevalent Gram-negative organism from Type-2 DM DFUs and *S. aureus* as the prevalent Gram-positive bacterium. Obuneme *et al*. (2019) at Enugu, Nigeria found *S. aureus* as the dominant bacterial isolate from DFUs, while Ako-Nai *et al*. (2006) at Ile-Ife, Nigeria documented *E. coli* as the predominant isolate and *S. aureus* as the most common Gram-positive isolate. *S. aureus* has long been associated with DFUs (Dunrach-Remy *et al*., 2016; Thurlow *et al*., 2020; Butrico *et al*., 2023). Its predominance may be because as a normal and common commensal on human skin, it rapidly becomes pathogenic under appropriate conditions, such as presented by DFUs (Chen *et al*., 2023; Murphy-Lavoie *et al*., 2023).

The isolates of MRSA in this study may not have any serious consequences for patients. Besa *et al.* (2014) did not associate any significant negative outcomes in patients from who MRSA had been isolated compared with those from who other bacteria were found. Reveles *et al.* (2016) also concluded that while *S. aureus* constituted about 86% of total DFI isolates, MRSA were only 15% of these bacteria. Antibiotics against the organism were therefore dis-proportionately overprescribed by 71% of total DFI antibiotic prescriptions. However, the rising prevalence of MRSA makes predicting antibiotic susceptibility less secure. MRSA isolated from this study may have been (and are often) acquired during previous hospitalizations or at chronic care facilities (Al-Bakri *et al*., 2021; Anafo *et al*., 2021)

Isolated bacteria that were less virulent, such as enterococci, coagulase-negative staphylococci, or corynebacteria can sometimes be ignored, especially in a mixed infection of DFUs. However, while CoNS, such as isolated in this study are low in virulence, when isolated in pure, predominant growth or from deep reliable specimens, these organisms may also represent true pathogens and have been associated with significant DFU infections (Shah *et al*., 2024). Gramberg *et al.* (2023) however states that CoNS may be more associated with higher amputation risks.

To treat and manage DFUs, whenever reliable culture and antibiotic sensitivity results are available, they should

guide selection and choice of drugs. However, when antibiotic therapy is needed before culture results are available, Gram-stained smear of carefully obtained wound specimen can provide some guidance to the etiologic agents (Amaefule *et al*., 2019). Results of studies of the microbiology of diabetic foot infections allow reasonable selections based on the bacterial pathogens commonly isolated. Patients that have a mild infection who have not previously received antibiotic therapy usually have an infection caused by only one or two species of bacteria, which are almost invariably aerobic Gram-positive cocci, with *S. aureus* most important, then γ-haemolytic streptococci (Armstrong and Lipsky, 2004). Previous studies posit that *Pseudomonas* species are often isolated from wounds that have been soaked or treated with wet dressings. Enterococci are commonly cultured from patients who have previously received cephalosporin therapy, to which they are inherently resistant. Infections in hospitalized populations are usually caused by several species, including both aerobic and anaerobic organisms (Shi *et al*., 2022; Perzon *et al*., 2023). While such considerations were not currently ascertained, this study found that most of the isolated organisms were Gram-positive. This varies with the position of Baig *et al*. (2022) that bacteria isolated from DFUs in warmer climates are Gram-negative bacilli. While it could be expected that anaerobes would be present, none were isolated in this study, though many patients were at mid-level grades of DFU staging. However, such bacteria are most frequent in wounds with necrosis, deep tissue involvement, or a feculent odor (Sadeghpour *et al*., 2019, Baig *et al*., 2022). Such conditions are common at DFUs grades 5 or 6. However, most patients in this study had not progressed to these stages. Even so, the most important pathogens in serious infections, are reported to be *S. aureus* and γ-haemolytic streptococci though a mixture of other organisms are said to be common (Anyim *et al*., 2019; Shi *et al*., 2022).

In conclusion, the bacterial infections of DFUs in two tertiary health institutions in the Federal Capital Territory of Nigeria were examined to determine a recurring pattern. Standard guidelines for DFU treatment should be developed, based on commonly isolated bacteria. These should be shared and adhered to across all healthcare institutions that provide care for diabetics and especially DFUs. This will help ensure consistency in treatment approaches.

Moreover, training and educational programs should be provided for healthcare professionals to keep them updated on the latest developments in DFU treatment and to promote evidence-based practices.

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