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Original Research Article

Diversity of microbiological pathogens in diabetic foot ulcers in an African population

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ABSTRACT

Background: Diabetic foot ulcer is a devastating complication of diabetes and the commonest cause of non-traumatic amputation worldwide. It constitutes a huge socioeconomic burden to the patient and health care system. The aim of this study was to determine the spectrum of bacterial isolates cultured from diabetic foot ulcers and assess their in vitro susceptibility to commonly used antibacterial agents.
Methods: The study was a retrospective descriptive study where records of all diabetic foot ulcer samples sent to the microbiology department for microscopy, culture and sensitivity between January 2020 to December 2021 were extracted. The bacteriological isolation and antimicrobial sensitivity tests of the isolates was carried out by standard microbiological methods.
Results: A total of sixty-one patients results were retrieved and included in this study. The commonest organism isolated was *Staphylococcus aureus* which constituted almost half 40 (46.0%) of the organisms isolated, followed by *Escherichia coli* and *Proteus mirabilis* which accounted for 23.0% and 18.4% respectively of the organisms. *Pseudomonas aeruginosa* was the least common organism and was seen in only 3 (3.4%) samples. Concerning antibiotic sensitivity, 55.0% of *S. aureus*, 50.0% of *K. pneumoniae*, 60.0% of *E coli* and *P. mirabilis* were sensitive to cefpodoxime whereas 50.0% of *S. aureus* and *K. pneumoniae*, 90% of *E coli* and 68.8% of *P. mirabilis* were sensitive to cefuroxime.
Conclusions: Diabetic foot ulcers are infected with polymicrobial organisms and *S. aureus* was the predominant organism isolated. However, there is an emerging pattern in the spectrum of antibiotic sensitivity which is an important consideration in management.

Keywords: Diabetes mellitus, Diabetic foot ulcer, Microbiology, Nigeria

INTRODUCTION

Diabetic foot ulcer is the most devastating of all the complications of diabetes. It constitutes a huge socioeconomic burden to the patient, family and health care system. It is the commonest cause of non-traumatic amputation worldwide and it is estimated that every 30 seconds a lower limb or part of a lower limb is lost somewhere in the world as a consequence of diabetes.1 Approximately 25% of patients living with diabetes will have a diabetic foot ulcer in their lifetime with a high risk of recurrence.[2] These ulcers usually develop from trauma (often trivial) on background of poor glycemic control, neuropathy, ischemia and impaired immunity and is

associated with significant morbidity and mortality causing deaths comparable to cancer.[3]

Infection is a major component of diabetic foot disease and poses a serious problem in its management. This is because they are often deep-seated and hence difficult to treat requiring several rounds of antibiotics with a high risk of development of antibiotic resistance.[4] It is estimated that about 60% of diabetic foot ulcers are infected and this increases the risk of amputation.[5] Antibiotic therapy is therefore an essential aspect of the management of diabetic foot ulcers in addition to glycemic control, offloading and wound care. The choice of antibiotic therapy is determined by several factors and initial empiric therapy is usually determined by the local sensitivity pattern.

Previous studies have shown that most of the infections in diabetic foot ulcers are polymicrobial.[5-7] Both gram positive and gram-negative aerobic and anaerobic organisms have been identified as common causative pathogens. In a study in Port Harcourt in 2005, *Staphylococcus aureus* was the commonest bacterial isolate just as was documented in several other studies.[4,5,8] The sensitivity pattern of the aerobic organisms in this study was to the fluoroquinolones and third generation cephalosporins while the anaerobic isolates were sensitive to metronidazole and clindamycin.[5] This informed the decision of using these drugs as first line antibiotic therapy in management of diabetic foot ulcers in our hospital till date.

The management of diabetic foot ulcers in our setting is challenging. This is especially because of late presentation, poor drug compliance, poverty, ignorance, religious beliefs, and cultural practices. There is also a lack of trained manpower and healthcare resources to manage diabetic foot ulcers in our setting and as such it puts a heavy burden on the healthcare system. This study aims to determine the spectrum of bacterial isolates cultured from diabetic foot ulcers and assess their in vitro susceptibility to commonly used anti-bacterial agents.

METHODS

Study area

This study was conducted in the Rivers State University Teaching Hospital (RSUTH) which is in Port Harcourt, the capital of Rivers State, South-South of the Federal Republic of Nigeria, a tertiary hospital owned and funded by the Government of Rivers State.

Study design

This was a retrospective descriptive study. All data from microbiology samples from diabetic foot ulcers sent for microscopy, culture and sensitivity from any department in the hospital between the period of January 2020 to December 2021 were retrieved. The total number of diabetic foot ulcer samples received by the microbiology laboratory during the two-year study period was sixty-one (61). Data extracted from these 61 cases included age and sex of the patients, organisms isolated and antimicrobial sensitivity pattern.

The department of microbiology receives wound swab samples collected from patients having diabetic foot ulcers, using a sterile disposable swab. The bacteria were inoculated in peptone water and streaked on a 4mm deep Mueler-Hinton agar with the aid of a swab stick and incubated for 24 hours duration. Isolates were identified and confirmed by biochemical reaction. Antibiotic sensitivity was done to determine the minimum inhibitory concentration or lowest concentration of antimicrobial agent that inhibits the growth of bacterial isolate with the Kirby-Bauer disk diffusion method where paper disks containing known concentrations of antibiotics well were applied to the plate inoculated with the test organisms and incubated for 24 hours duration. The zone diameter was measured with a ruler in millimetres and obtained values were compared with the Clinical Laboratory Standard Institute (CLSI).[9]

Data analysis

Data were entered to a personal computer and were analysed using Statistical Package for the Social sciences (SPSS, Chicago Illinois) program version 25. Results were presented as mean±standard deviation for continuous variables while categorical variables were expressed as proportions or percentages. Graphs and tables were used to illustrate results where appropriate.

Ethical consideration

Ethical approval was obtained from the health research ethics team of the hospital.

RESULTS

A total of sixty-one (61) patients results were retrieved and included in this study. The mean age was 55.74±11.18 years with a range of 34-83 years, with 14 (23.0%) persons in the young age group, 32 (54.4%) middle aged and 15 (24.6%) elderly persons. There were more females 35 (57.4%) than males 26 (42.6%), giving a female to male ratio of 1.35:1 (Table 1).

There were 87 organisms isolated from the 61 samples which gives a mean of 1.42 bacteria per diabetic foot infection. The commonest organism isolated was *Staphylococcus aureus* which constituted almost half 40 (46.0%) of the organisms isolated, followed by *Escherichia coli* and *Proteus mirabilis*. *Pseudomonas aeruginosa* was the least common organism and was isolated in only 3 (3.4%) samples and this is illustrated in Figure 1.

Table 1: Age and sex distribution of the study population.

	Frequency (%)
Age group	
Young adults (<45 years)	14 (23.0)
Middle aged (45-64 years)	32 (54.4)
Elderly (≥65 years)	15 (24.6)
Sex	
Male	26 (42.6)
Female	35 (57.4)
Total	61 (100)

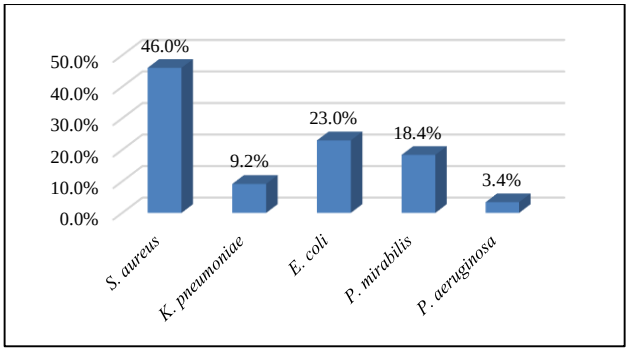

Figure 1: Bacterial isolates from diabetic foot ulcers.

Table 2: Antibacterial susceptibility pattern of isolated organisms.

Antibiotics	*S. aureus* n (%) N=40	*K. pneumoniae* n (%) N=8	*E. coli* n (%) N=20	*P. mirabilis* n (%) N=16	*P. aeruginosa* n (%) N=3
Ceftazidime	5 (12.5)	1 (12.5)	7 (35.0)	6 (37.5)	0 (0)
Cefuroxime	20 (50.0)	4 (50.0)	18 (90.0)	11 (68.8)	0 (0)
Cefixime	9 (22.5)	2 (25.0)	10 (50.0)	5 (31.3)	0 (0)
Gentamycin	25 (62.5)	3 (37.5)	10 (50.0)	9 (60.0)	0 (0)
Ofloxacin	22 (55.0)	4 (50.0)	8 (40.0)	5 (31.3)	0 (0)
Co-amoxiclav	15 (37.5)	3 (37.5)	4 (20.0)	4 (25.0)	0 (0)
Ciprofloxacin	11 (27.5)	0 (0)	5 (25.0)	8 (50.0)	0 (0)
Nitrofurantoin	9 (22.5)	3 (37.5)	9 (45.0)	10 (62.5)	0 (0)
Moxifloxacin	8 (20.0)	4 (50.0)	7 (35.0)	4 (25.5)	2 (66.7)
Cefpodoxime	22 (55.0)	4 (50.0)	16 (80.0)	9 (60.0)	0 (0)
Ceftriaxone	15 (37.5)	1 (12.5)	5 (25.0)	6 (37.5)	0 (0)
Cloxacillin	8 (20.0)	1 (12.5)	3 (15.0)	2 (12.5)	0 (0)
Erythromycin	8 (20.0)	0 (0)	1 (5.0)	0 (0)	0 (0)
Levofloxacin	0 (0)	1 (12.5)	1 (5.0)	0 (0)	0 (0)

Almost two thirds of *S. aureus* isolates were sensitive to Gentamycin (62.5%), while Cefpodoxime (55.0%), Ofloxacin (55.0%) and Cefuroxime (50.0%) showed sensitivity in about half of the isolates. *K. pneumoniae* was more sensitive to ofloxacin, moxifloxacin, cefuroxime and cefpodoxime (with 50% sensitivity each). *E. coli* was sensitive to cefuroxime (90.0%), cefpodoxime (60.0%), cefixime (50.0%) and gentamycin (50.0%). *P. mirabilis* was sensitive to cefuroxime (68.8%), nitrofurantoin (62.5%), gentamycin (60.0%) and cefpodoxime (60.0%); while *Pseudomonas* only showed sensitivity to moxifloxacin (66.7%). The antibacterial susceptibility pattern of the above organisms is shown in the Table 2.

DISCUSSION

The purpose of this study was to determine the microbiological spectrum and antimicrobial sensitivity pattern of diabetic foot ulcers. This study became very important in the face of the current challenges of managing diabetic foot infections with concerns of rapid changes in bacteriological and sensitivity patterns coupled with the burden of antibiotic resistance and the recent advocacy for antimicrobial stewardship.

In this study, culture results of 61 patients were reviewed for microbiological patterns and antimicrobial susceptibility. The study showed that the middle-aged group was most affected by the burden of diabetic foot ulcers. This is consistent with findings in most of the previous studies. A study of 56 patients in Zaria in Northern Nigeria reported a mean age of 56.2 years with a range: 48-75 years.[10] Fatima and colleagues also documented that diabetic foot ulcers were more prevalent in the age group between 40 and 60 years of age in a recent meta-analysis.[11] This finding is probably related to the fact that the middle aged group are more active, more likely to have unhealthy lifestyle and by extension poor glycaemic control and therefore at higher risk of diabetic foot ulcers.[12] This obviously has negative implications considering the fact that the middle age group constitutes the major work force of any society and country at large.

In contrast to reports from similar studies which have reported a male preponderance, this study showed that

diabetic foot ulcers were commoner in females.[5,10] Previous studies have shown that females are less likely to develop diabetic foot ulcers compared to males because they are at lower risk of developing neuropathy among other risk factors.[13] However in the presence of established risk factors, both sexes have equal risk of ulceration.[13] The reason for the female preponderance in this study is not clear, it may however reflect the fact that females are now engaging in unhealthy lifestyles which puts them at high risk of developing complications just like males. It may also be because women have better health seeking behaviour and are now relatively socially, culturally and economically independent and therefore able to present earlier for care compared to before.

This study showed that there was a very high rate of infection of diabetic foot ulcers most of which were polymicrobial. This polymicrobial nature of diabetic foot ulcers has also been reported in most of the previous studies done both locally and internationally.[5,6,14,15] Polymicrobial infections are more common in deep-seated infections especially in chronic ulcers and reflect severe disease. Hitam et al in a study of 104 patients in Malaysia reported that polymicrobial infections were associated with poorer glycaemic control, higher white blood cell count and anaemia.[16] The findings of a predominance of polymicrobial infections in this study is therefore a reflection of severe disease in the hospitalised patients. As is well known, most patients in low- and middle-income countries present late to hospital. In addition, a lot of patients attempt self-care with unorthodox medications before presenting to the hospital which can lead to wound contamination. The presence of polymicrobial infection makes antibiotic therapy more difficult and expensive because it usually requires combining 2 or more antibiotics to treat infections.

A mean of 1.42 bacteria was isolated per specimen in the current study. This is lower than 2.3 per specimen reported by Unachukwu, and 2.63 per specimen by Obumneme Ayim, both in Nigeria.[5,6] This is however similar to the findings by Miyan et al in India in which an average of 1.45 organisms per specimen were isolated from 473 specimens.[17] The reason for the lower number of isolates per specimen in this study compared to other studies could be because only cultures for aerobes were done in the current study.

Both gram-positive and gram-negative organisms were isolated from the 61 cultures in this study with majority of the organisms isolated being gram negative. The commonest organism isolated was *Staphylococcus aureus* which made up 46% of the isolates and was the only gram-positive organism isolated. This is consistent with findings from previous studies documented in available literature. In the study in Port Harcourt, 56.1% of the isolates were *S. aureus* and this preponderance was also reported in Southern and North Western Nigeria.[5,10,15] Atlaw in a multicentre study of 130 patients in Ethiopia also reported a higher prevalence of gram-positive isolates (66.7%) with *S. aureus* ranking as the highest bacterial isolate.[18] Few other studies have reported other organisms such as *Proteus mirabilis*, *Pseudomonas* species and *Escherichia coli* as more prevalent than *S. aureus* in contrast to the findings in this study.[19-21] These differences could be due to difference in patient characteristics (such as severity of ulcer and prior antibiotic use) difference in sample size, as well as specimen handling. It may also be due to geographical differences and periodic change in microbial organisms over time.

The observation that *Staphylococcus aureus* is the most prevalent isolate in this study as well as several other similar studies is not unexpected. *S. aureus* is a normal commensal of the skin and mucous membranes in humans.[22] It is also a human pathogen capable of causing severe infections and is a major culprit in nosocomial infections. *S. aureus* infection in diabetic foot ulcers have also been found to be more prevalent in the older age group, and this is probably due to age related impaired immunity in the elderly.[23] Infection with *S. aureus* is of particular concern in diabetic foot ulcers because of its potential to rapidly develop resistance to most antimicrobial agents through diverse mechanisms.[22] Unrestricted use of antibiotics has been implicated in the emergence of this antibiotic resistance which currently constitutes a major problem in antibiotic therapy in diabetic foot ulcers.

This study showed that the predominant isolate was mostly susceptible to gentamycin (62.5%) as well as cefpodoxime (55.0%), ofloxacin (55.0%) and cefuroxime (50.0%). There was however no sensitivity to levofloxacin. *E. coli* which was the second commonest isolate and predominant gram-negative organism was sensitive to cefuroxime (90.0%), cefpodoxime (60.0%), cefixime (50.0%) and gentamycin (50.0%). Sixty percent of the organisms were resistant to at least 2 antimicrobial agents with *P. aeruginosa* showing the highest degree of resistance being sensitive to moxifloxacin alone.

Various studies have shown different susceptibility patterns. Most of the previous studies in Nigeria showed that the predominant isolates were most sensitive to the quinolones which contrasts with the findings in this study.[5,6,14,24,25] In addition, Unachukwu also reported significant sensitivity to the cephalosporins in a previous study in Port Harcourt just as was observed in this study.[5] The pattern is however different in other parts of the world. In Ethiopia, the sensitivity of both gram-positive and gram-negative isolates was mainly to chloramphenicol, clindamycin and amikacin with resistance to gentamycin.[18] However, a study in cameroun reported that the gram-positive isolates were most susceptible to vancomycin (94%), pristinamycine (82%) and fusidic acid (67%) while the gram-negative isolates were sensitive to Imipenem (95%), amikacin (88%), with moderate sensitivity to third generation cephalosporins (62%).[26] Two recent independent studies

in Kuwait and Malaysia also reported highest sensitivity of gram positive and negative organisms to vancomycin and imipenem respectively.[27,28] Some older studies have reported very high sensitivity of isolates from diabetic foot ulcers to Gentamycin which supports the finding in the present study. Eregie et al in a study in Benin, Nigeria, in 2007 reported 100% sensitivity of the gram-positive isolates to both fluroquinolones and Gentamycin.[24] Ramani and colleagues had also reported earlier in a study in India in 1991, that gentamycin was the most effective antibiotic against aerobic organisms.[29]

The observations in the present study suggests a changing sensitivity and local resistance pattern in diabetic foot ulcers. It also reflects a declining sensitivity to the fluoroquinolones which have remained the first line antibiotic in empirical management of diabetic foot ulcers in our environment for over a decade, however, they are also a readily abused antibiotic. Unlike the fluroquinolones, gentamycin has not been readily available for abuse, perhaps because it is an injectable, and this may be the reason for the significant sensitivity of the predominant isolate to gentamycin in this study. Gentamicin is however an unfavourable antibiotic that is not usually prescribed in diabetic patients because of safety concerns relating to renal and ototoxicity especially in the elderly. For this reason, it may not suffice as a recommended antibiotic for empirical therapy in diabetic foot ulcers.

Limitation of the study was that culture for anaerobes was not available in the center for this study.

CONCLUSION

This study showed that most diabetic foot ulcers are infected with polymicrobial organisms. Though majority of the organisms were gram negative, *Staphylococcus aureus* was the predominant and only gram-positive organism isolated. The observation of a changing pattern of sensitivity of antibiotics compared to previous studies in our environment as well as a significant degree of multidrug resistance are of concern and should be important considerations in management of diabetic foot ulcers.

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Ethical approval: The study was approved by the Rivers State University Teaching Hospital Research Ethics Committee (RSUTH/REC/2022176)

REFERENCES

1. Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. World J Diabetes. 2015;6(1):37-53.
2. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA. 2005;293:217-28.
3. Armstrong DG, Swerdlow MA, Armstrong AA, Conte MS, Padula WV, Bus SA. Five year mortality and direct costs of care for people with diabetic foot complications are comparable to cancer. J Foot Ankle Res. 2020;13(1)16.
4. Macdonald KE, Boeckh S, Stacey HJ, Jones JD. The microbiology of diabetic foot infections: a meta-analysis. BMC Infect Dis. 2021;21(1)770.
5. Unachukwu CN, Obunge OK, Odia OJ. The bacteriology of diabetic foot ulcers in Port Harcourt. Niger J Med. 2005;14(2):173-6.
6. Anyim O, Okafor C, Young E, Obumneme-Anyim I, Nwatu C. Pattern and microbiological characteristics of diabetic foot ulcers in a Nigerian tertiary hospital. Afr Health Sci. 2019;19(1):1617-27.
7. Hena JV, Growther L. Studies on bacterial infections of diabetic foot ulcer. Afr J Cln Exp Microbiol. 2010;11(3):146-9.
8. Otu AA, Umoh VA, Essien OE, Enang OE, Okpa HO, Mbu PN, et al. Profile, bacteriology, and risk factors for foot ulcers among diabetics in a tertiary hospital in Calabar, Nigeria. Ulcers. 2013;2013:820468.
9. Gerstein AC, Rosenberg A, Hecht I, Berman J. diskImageR: quantification of resistance and tolerance to antimicrobial drugs using disk diffusion assays. Microbiology. 2016 l;162(7):1059.
10. Amaefule KE, Dahiru IL, Okpe IO, Aliyu S, Aruna A. A clinico-microbial profile of diabetic foot infections in Zaria, North-West Nigeria. Sahel Med J. 2019;22(1):28-32.
11. Fatima HK, Suhad HM. Microbiological profile, antibiogram, and risk factors of patients with diabetic foot infections: A systemic metaanalysis. Biomed Biotechnol Res J. 2021;5(3):235-44.
12. Tong T, Yang C, Tian W, Liu Z, Liu B, Cheng J, et al. Phenotypes and outcomes in middle-aged patients with diabetic foot ulcers: a retrospective cohort study. J Foot Ankle Res. 2020;13(1):1-8.
13. Dinh T, Veves A. The influence of gender as a risk factor in diabetic foot ulceration. Wounds. 2008;20(5):127-31.
14. Otu AA, Umoh VA, Essien OE, Enang OE, Okpa HO, Mbu PN. Profile, bacteriology, and risk factors for foot ulcers among diabetics in a tertiary hospital in Calabar, Nigeria. Ulcers. 2013;2013.
15. Ramakant P, Verma AK, Misra R, Prasad KN, Chand G, Mishra A, et al. Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose? Diabetologia. 2011;54:58-64.
16. Hitam SAS, Hassan SA, Maning N. The significant association between polymicrobial diabetic foot infection and its severity and outcomes. Malay J Med Sci. 2019;26(1):107-14.
17. Miyan Z, Fawwad A, Sabir R, Basit A. Microbiological pattern of diabetic foot infections at a tertiary care center in a developing country. J Pak Med Assoc. 2017;67(5):665-9.

18. Atlaw A, Kebede HB, Abdela AA, Woldeamanuel Y. Bacterial isolates from diabetic foot ulcers and their antimicrobial resistance profile from selected hospitals in Addis Ababa, Ethiopia. Front Endocrinol. 2022;13:987487.
19. Dwedar R, Ismail DK, Abdulbaky A. Diabetic foot infection: microbiological causes with special reference to their antibiotic resistance pattern. Egypt J Med Microbiol. 2015;24:95-102.
20. Amogne W, Reja A, Amare A. Diabetic foot disease in Ethiopian patients: a hospital based study. Ethiop J Health Develop. 2011;25(1):17-21.
21. Shanmugam P, Jeya M. The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. J Clin Dign Res. 2013;7(3):441.
22. Nwankwo EO, Nasiru MS. Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. Pan Afr Med J. 2011;8:4.
23. Macdonald KE, Jordan CY, Crichton E, Barnes JE, Harkin GE, Hall LM, et al. A retrospective analysis of the microbiology of diabetic foot infections at a Scottish tertiary hospital. BMC Infect Dis. 2020;20:1-7.
24. Edo AE, Eregie A. Bacteriology of diabetic foot ulcers in Benin City, Nigeria. Diabetes Int. 2007:21-3.
25. Ikeh EI, Peupet F, Nwadiaro C. Studies on diabetic foot ulcers in patients at Jos University Teaching Hospital, Nigeria. Afr J Clin Exp Microbiol. 2003;4(2):52-61.
26. Yefou MD, Jingi AM, Etoga MC, Mekobe FM, Agoons BB, Ngassam E, et al. Bacterial profile of diabetic foot infections and antibiotic susceptibility in a specialized diabetes centre in Cameroon. Pan Afr Med J. 2022;42.
27. Al Benwan K, Al Mulla A, Rotimi VO. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health. 2012;5(1):1-8.
28. Goh TC, Bajuri MY, C Nadarajah S, Abdul Rashid AH, Baharuddin S, Zamri KS. Clinical and bacteriological profile of diabetic foot infections in a tertiary care. J Foot Ankle Res. 2020;13(1):1-8.
29. Ramani A, Ramani R, Shivananda PG, Kundaje GN. Bacteriology of diabetic foot ulcers. Indian J Pathol Microbiol. 1991;34(2):81-7.

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