Bacteriological evaluation of diabetic ulcers in Nigeria

F A Orji, N C Nwachukwu, and E C Udora

Abstract

Diabetic foot ulcers are a common cause of morbidity and mortality in Nigeria. The lesions are usually infected, and this study was carried out in Enugu State, Nigeria to determine the common bacterial pathogens that infect ulcers and their antimicrobial susceptibly patterns. Deep wound swabs were collected from 71 consecutive diabetic patients admitted with foot ulcers into the medical wards of University of Nigeria Teaching Hospital, Enugu (26% of 274 total diabetes-related admissions). Polymicrobial isolates of *Clostridium* spp, *Staphylococcus aureus*, *Escherichia coli*, or *Klebsiella aerogenes* were found in 26 (53%) cases. *Clostridium* species showed susceptibility to fluoroquinolones, and high resistance to beta-lactams. *Escherichia coli* and *Klebsiella aerogenes* showed resistance to the aminoglycosides and beta-lactams. All the gram-negative organisms showed significant susceptibility to the fluoroquinolones used. *Clostridium* species, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella aerogenes* were the most common causes of diabetic foot infections in the study and the rates of antibiotic resistance were observed to be relatively high. Antibiotic susceptibility testing remains of paramount importance in the management of diabetic foot ulceration.

Introduction

The diabetic foot ulcer is one of the notable complications of diabetes. The clinical manifestations include blistering, malodorous smell, and pus production.[1] A major obstacle in the management of diabetic foot ulcers is the colonisation of wounds by virulent bacterial pathogens.

The challenge is, therefore, to use an appropriate antibiotic and it is worth noting that the attack of these virulent bacterial pathogens on diabetic wound ulcers in patients has increased morbidity and mortality rates in developing countries including Nigeria.[2] It has also increased the cost of management of diabetic ulcers, especially in resource-limited regions of the world.

In view of this challenge, we have studied the pattern of bacterial colonisation of diabetic ulcer wounds and the antibiotic susceptibility patterns of the bacterial pathogens isolated.

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Patients and methods

This was a cross-sectional study carried out at the University of Nigeria Teaching Hospital, Enugu, Nigeria, which is a tertiary health institution serving over 3.6 million people in Nigeria. The study was carried over an 8-month period.

During the study period, 274 diabetic patients were admitted, of whom 71 (26%) had foot ulcers. Clinical data on these patients were collected, and the ulcers were swabbed for bacterial culture using disposable sterile swab sticks.

The specimens were transported to the microbiology laboratory at Abia State University, Uturu, Nigeria within 2 hours of collection, inoculated on freshly prepared Mackonkey Agar, and incubated for 48 hours. Bacterial isolates were characterised and identified using methods previously described by Madigan and Martinko[3]

Antibiotic susceptibility testing was carried out on all the isolates using the paper disc diffusion technique, which has been previously reported by Chigbu and Ezeronye).[7] The antibiotic discs used and their concentrations were as follows:

- ampicillin (25 µg/disc)
- norfloxacin (10 µg/disc)
- streptomycin (30 µg/disc)
- gentamicin (10 µg/disc)
- erythromycin (25 µg/disc)
- chloramphenicol (25 µg/disc)
- ofloxacin (10 µg/disc)
- nitrofurantoin (30 µg/disc).

An aliquot (0.2 ml) of peptone water culture of the test organisms was inoculated onto a dry sterile nutrient agar plate. This was spread over the entire surface of the plate using a sterile glass spreader and allowed to dry for about 15–30 minutes. The antibiotic discs were placed 1 cm from each other to avoid the zones of inhibition from coalescing among themselves. The plates with the antibiotic discs were incubated at 37°C for 24 hours to observe the zones of inhibition produced by the antibiotics in millimeters (mm). The standards of the National Centre for Clinical Laboratory Standards

were used in determining if an isolate was resistant or susceptible to each antibiotic. The standards have been previously reported by Madigan and Martinko.[5]

The data generated were subjected to simple descriptive statistical analysis using frequencies, percentages, and prevalence rates. Chi-squared statistics were also employed. Statistical significance was set at $p<0.05$.

Results

Of the 71 (26% of all diabetic admissions) foot ulcer cases, 40 (56%) were male and 31 (44%) female (see Table 1). The male excess was particularly significant in the 50–60years and 70–90years age groups.

Four bacterial pathogens were isolated from the diabetic foot ulcers studied. Prevalence rates of the bacterial pathogens were: *Clostridium* species 51%; *Staphylococcus aureus* 16%; *Escherichia coli* 20%; and *Klebsiella aerogenes* 12% (see Table 2). Table 3 shows the isolation of more than one bacterial pathogen from a particular diabetic ulcer. Out of 49 isolates obtained from the diabetic wound ulcers, 26 were isolated from more than one wound, giving a polymicrobial infection rate of 53%. *Staphylococcus aureus* and *Escherichia coli* were obtained in ulcers from 12 patients (24.5%). In addition, *Escherichia coli* and *Klebsiella aerogenes* were obtained in 14 patients.

Staphylococcus aureus, *Klebsiella aerogenes*, *Escherichia coli*, and *Clostridium* species had resistance rates of 50%, 16%, 40%, and 48%, respectively, against norfloxacin. *Staphylococcus aureus*, *Klebsiella aerugenes*, *Escherichia coli*, and *Clostridium* species had resistance rates of 62.5%, 50%, 60%, and 36% respectively against gentamicin.

Among the beta-lactam group of antibiotics studied, *Staphylococcus aureus*, *Klebsiella aerogenes, Escherichia coli,* and *Clostridium* species had resistance rates of 50%, 33%, 60%, and 60%, respectively, against ampicillin. *Staphylococcus aureus, Klebsiella aerugenes*, *Escherichia coli,* and *Clostridium* speices had resistance rates of 75%, 33%, 80%, and 60%, respectively, against nitrofurantoin (see Table 4)

Table 1 Age and sex distribution of diabetic patients with diabetic foot ulcers (n=71)

Age (years)	Male (%)	Female (%)	Signifance
10–29	–(0)	–(0)	pNS
30–49	17 (24%)	18 (25%)	pNS
50–69	10 (14%)	6 (8%)	p=0.02
70–89	13 (18%)	7(10%)	p=0.002
Total	**40 (56%)**	**31 (44%)**	**p=0.03**

Table 2 Percentage prevalence of bacterial isolates in diabetic ulcers (n=49 isolates in 71 patients)

Organisms	Prevalence, n (%)
Clostridium species	25 (51.0%)
Staphylococcus aureus	8 (16.3%)
Escherichia coli	10 (20.4%)
Klebsiella aerugenes	6 (12.3%)
Total	**49**

Table 3 Prevalence of polymicrobial interactions (n=26)

Interacting organisms	Prevalence, n (%)
Staphylococcus aureus, Escherichia coli	12 (24.5%)
Escherichia coli, Klebsiella aerogenes	14 (28.6%)

Discussion

Among the complications affecting diabetic patients, foot ulceration is one of the most devastating. Bacteriological infection of foot ulcers has increased the burden of foot ulceration considerably. In Botswana, reports have shown that foot ulceration accounts for 4% of all diabetic hospitalisation,[6] and in Nigeria it has been reported that the prevalence rate of diabetic foot ulceration in Lagos was 59% in males and 48% in females.[7] The most common risk factors for diabetic foot ulceration include neuropathy, poor glycaemic control, ischaemia, and infection.[8–9]

The frequent isolation of *Clostridium* species from the ulcers calls for strict adherence by physicians to sensitivity reports, and also the use of painstaking antiseptic procedures in cleaning wounds. The aspect of antibiotic susceptibility is very important, as the bacterial isolates showed different levels of susceptibility to different antibiotics, which is of epidemiological importance. Some strains of *Clostridium* species were resistant to norfloxacin, streptomycin, gentamicin, erythromycin, and chloramphenicol, while other strains of *Clostridium* species were highly susceptible to norfloxacin, gentamycin, erythromycin, chloramphenicol, ofloxacin, and ciproflaxacin. Some strains of *Klebsiella, aerugenes*, *Escherichia coli,* and *Staphylocuccus aureus* showed much resistance to older beta-lactams such as ampicillin. Some strains of the latter organisms also showed resistance to some aminoglycosides such as chloramphenicol, erythromycin, and streptomycin. Mostly, these isolates showed relatively high susceptibility to the first-generation fluoroquinolones such as ofoxacin, ciprofloxacin, and norfloxacin.

The resistance to antibiotics of *Clostridium* species, *Escherichia coli*, *Klebsiella aerogenes*, and *Staphylococcus aureus* is not surprising because of drug misuse, which is common in Nigeria. For example, in Enugu, Lagos, and Onitsha (large cities in Nigeria), antibiotics are marketed even in commercial motor parks, buses, and supermarkets. Some of the strains of bacterial isolates that colonised the diabetic ulcers in this study may have acquired genes for drug resistance through antibiotic misuse.

In Port Harcourt, Nigeria, other researchers have reported that aerobes and anaerobes constituted 95% and 5% of the total bacterial isolates from diabetic foot ulcers, respectively. This report documented that the aerobic isolates showed significant sensitivity to ciprofloxacin (78%),

pefloxacine (71%), and cefuroxime (70%).[10] As in our own study, this report from Port Harcourt, Nigeria recorded that the infection of diabetic foot ulcers is often polymicrobial. Also in the United Arab Emirates (UAE), the polymicrobial nature of diabetic foot ulcers was documented.[11] In Pakistan a study of 32 patients with diabetic foot ulcers revealed polymicrobial infection in 16 (50%), and single organism infection in only 10 (31%). This research report also showed that all bacterial isolates had high resistance to the antibiotics used.[12] Reports from India also record frequent polymicrobial infections.[13,14]

In conclusion, bacterial infection of diabetic foot ulcers is common. Polymicrobial infection is also fequently seen. Laboratory bacterial isolation, and antibiotic sensitivity results, can greatly help in choosing appropriate antibiotic therapy.

Table 4 Antibiotic susceptibility patterns of bacterial pathogens isolated from diabetic ulcers

Microorganisms	Antibiotics and percentage susceptibility or resistance of the bacterial pathogens																	
	NB		S		GN		E		CHL		PN		OF		NIT		CPX	
	%Sus	%Res	%Sus	%Res	%Sus	%Res	%Sus	%Res	%Sus	%Res	%Sus	%Res	%Sus	%Res	%Sus	%Res	%Sus	%Res
Staphylococcus aureus (n=8)	3(37.5)	4(50)	1(12.5)	7(87.5)	1(12.5)	5(62.5)	4(50)	4(50)	2(25)	5(2.5)	3(37.5)	4(50)	4(50)	2(25)	2(25)	6(75)	7(87.5)	1(12.5)
Klebsiella aerogenes (n=6)	4(66.6)	1(16.56)	0(0)	6(100)	1(16.6)	3(50)	2(33.3)	5(83)	6(100)	2(33.3)	2(33.3)	3(50)	3(50)	3(50)	4(66.6)	2(33.3)	3(50)	3(50)
Escherichia coli (n=100)	3(30)	4(40)	0(0)	5(50)	2(20)	6(60)	3(30)	7(70)	7(70)	4(40)	6(60)	8(80)	5(80)	2(20)	2(20)	8(80)	7(70)	2(20)
Clostridium sp (n=25)	8(32)	2(48)	5(20)	14(56)	6(24)	9(36)	12(48)	8(32)	10(40)	15(60)	15(60)	12(48)	12(48)	9(36)	6(24)	15(60)	24(96)	1(04)

Note
NB=norfloxacin, S=streptomycins, GN=gentamicin, E=erythromycin, CHL=chloramphenicol, PN=ampicillin, OF=ofloxacin, NIT=nitrofurantoin, CPX=ciprofloxacin.
Numbers in parentheses are percentages.
'% Sus' represents percentage of susceptible/sensitive organisms.
'% Res' represents percentage of antibiotic-resistant organisms.

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