Biomedical Dermatology

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Aerobic bacteria associated with diabetic foot ulcers and their susceptibility pattern

Ofonime M. Ogba[1*], Emmanuel Nsan[1] and Eyam S. Eyam[2]

Abstract

Background: Foot ulcers in diabetes mellitus subjects cause morbidity and mortality and lead to non-traumatic amputations worldwide. Knowledge of the microbial burden in the ulcers may improve patients' care and management.

Objectives: This prospective study was designed to isolate, identify and carry out antibiotic susceptibility testing on bacterial isolates associated with diabetic foot ulcers among subjects in University of Calabar Teaching Hospital.

Methods: Subjects with diabetic foot ulcer were recruited after obtaining ethical clearance from the Research Committee and informed consent from the subjects. Samples were obtained from subjects using sterile swabs and subjected to microscopy and culture. Isolates were identified using standard bacteriological techniques. Kirby-Bauer method was used for susceptibility testing.

Results: Out of the 50 subjects recruited, 19 (38.1%) were males and 31 (62.0%) were females with mean age of 55.4 ± 10.1 and a minimum age of 40.0 years. All the subjects had grade 4 wounds. The study recorded 100% infection rates among subjects with 70.0% polymicrobial infections. A total of 97 isolates were obtained from the 50 subjects accounting for the average of 1.94 isolates per subject. The most prevalent isolate was *Staphylococcus aureus* (32 (32.9%)), while the least isolated pathogen was *Klebsiella pneumonia* (10 (20.4%)). Females harboured more isolates (61 (62.9%)) than males (36 (37.1%)), but infection rates were not significantly associated with gender ($\chi^2 = 15.0$, $p \geq 0.05$). Erythromycin was the most effective antibiotic agent (65.6%) against *S. aureus* while gram-negative bacteria were more susceptible to augmentin (87.5%) and ciprofloxacin (75.0%).

Conclusion: The multiple antibiotic resistance of the bacterial isolates calls for the need to monitor resistance. The best practice is to perform antibiotic susceptibility testing before treatment. Wounds should be evaluated for bacterial agents before treatment is instituted. Information on the mi.uction of morbidity and amputation rates on the patients.

Keywords: Bacteria, Diabetic foot, Susceptibility pattern

Background

Diabetic foot ulcer presents as open sore in about 15% of diabetics. They are commonly located at the bottom of the foot. Most diabetics' hospital admission are results of foot ulcer (American Podiatric Medical Association, 2016). Diabetes is a metabolic disorder characterised by hyperglycaemia which results from defects in insulin action, secretion or both. Foot ulcers are complications of uncontrolled diabetes. It could also be due to muscle atrophy, foot deformity, peripheral neuropathy and neuropathic fractures (Raja, 2007).

The prevalence of diabetic foot ulcer is about 13% in North America, 3% in Europe, 7.2% in Africa, 18.1% in Khartoum, Sudan, 15% in Tanzania, 13% in Cameroon and 9.5% in Nigeria (Almobarak et al. 2017).

These result in two thirds of all non-traumatic amputations. Disease presentations include Charcot joint, ulcerations, fracture or gangrene (Krishman et al. 2008; Spichler et al. 2015).

The bacteriology of diabetic foot infection is highly complicated and mostly polymicrobial. It involves both aerobes and anaerobes. Many researchers have presented a picture of mixed infection with aerobic and anaerobic

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bacteria (Chin, 2013). Some of the aerobic bacteria associated with diabetic foot infection include *Staphylococcus aureus*, *S. saprophyticus*, *S. epididermis*, *Streptococcus pyogenes*, *S. mutans*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus species*, *Escherichia coli* and *Klebsiella pneumoniae*. The anaerobic bacteria include *Peptostreptococcus* species, Anaerobic *Streptococci*, *Bacteriodes fragilis* and *Clostridium* species (Lipsky et al. 2012; Richard et al. 2012).

Diagnosis is based on combination of signs such as erythema around lesion, local tenderness, local warmth, pus, swelling and indurations. The risk factors are middle or old age, diabetic neuropathy, infection, cigarette smoking, poor glycaemic control, previous foot ulcerations, amputations and ischemia of small and large blood vessels (Scott, 2013; Wu et al. 2007).

Diabetic foot infection can be either superficial or deep. The deep infection involves the muscles, bones, superficial fascia and joints. This includes cellulitis, necrotizing cellulitis and wet gangrene. Once the foot ulcer gets to this stage, the patient is advised on the option of amputation.

The severity of a diabetic foot infection is grouped into four grades depending on their symptoms (Raja, 2007). There are no signs and symptoms in grade 1 wounds. Grade 2 ulcers have lesions on the skin without systemic involvement nor the deep tissues but with any two of the following: erythema > 0.5–2 cm around the ulcer, local indurations, swelling, tenderness, warmth, pain and purulent discharge. Grade 3 ulcers have erythema > 2.0 cm with infection involving structures deeper than the skin and subcutaneous tissues such as osteomyelitis, septic arthritis, or deep abscess. Grade 4 ulcers present with systemic inflammatory respond with at least two of the following signs: temperature > 38 °C, pulse > 90 bpm, $PaCO_2$ < 32 mmHg, leucocytes > 12,000 or < 4000 per mm^3 and 10% of immature (band forms) leucocytes (Raja, 2007).

Pal and Gupta (2016) in their study in Kolkata, India, reported that grade 4 foot ulcers on diabetic patients were commonly infected with mixed variety of bacteria including ESBL *Klebsiella* species, Methicillin-resistant *Staphylococcus aureus* (MRSA) and so on. They also reported that patients with very long hospital stay had negative culture reports.

In cases where the foot ulcers have developed, infection may be avoided by maintaining good hygiene in and around the foot ulcer and covering the purulent lesions with a waterproof dressing (Singh et al. 2015). Diabetic foot infections are usually inadequately managed due to poor knowledge on the microbial agents associated with the ulcers. There is a need to investigate the pathogens infecting these ulcers and their susceptibility pattern which may improve the patient's management and reduce the frequency of amputation. This study was carried out to characterise aerobic bacteria associated with the foot ulcers, determine antimicrobial susceptibility pattern of isolates to commonly used antibiotics and assess the severity of the diabetic foot among subjects in the locality.

Methods

Study setting and population

The study was carried out in Calabar Municipality, Nigeria. The study setting was Diabetic Clinic, UCTH, Calabar. The UCTH is a 2000-bed space tertiary health institution attended by Cross River State inhabitants as well as people from neighbouring states of Akwa Ibom, Abia and Ebonyi. Calabar is the capital of Cross River State and located at (4°57 N, 8°19E) South-Southern Nigeria. Calabar has an area of 406 km^2 with a population of 371,022. The major occupation of the people is civil service, farming and fishing (National Population Commission, 2006).

The study population were diabetics with foot ulcer attending the diabetic clinic in UCTH. Subjects were enrolled upon approval by the Ethical Research Committee of the UCTH, and written or oral informed consent was obtained. Diabetic subjects without foot ulcer were excluded from the study.

Study design

The study was a prospective hospital-based cohort study that ran for 6 months, from April to September 2017.

Subjects' description

All the subjects were in-patients upon admission to the hospital. In this study, any ulcer below the ankle with full thickness among our subjects was considered as diabetic foot ulcer. All the subjects had grade 4 ulcers, and most of the subjects had the ulcers on both feet which made them ambulatory. Subjects presented with ulcers on the planter, the ankle and some on the entire foot (Fig. 1a–c). It was

Table 1 Demographic profile of diabetic foot ulcer subjects attending Diabetic Clinic in UCTH, Nigeria

Characteristic	No. (%) of subjects		Total	Statistics
	Female	Male		
Age (years)				
40–49	9 (64.3)	5 (35.7)	14 (28.0)	
50–59	13 (61.9)	8 (38.1)	21 (42.0)	
60–69	7 (87.5)	1 (12.5)	8 (16.0)	
70–79	2 (28.6)	5 (71.4)	7 (14.0)	
Total	31 (62.0)	19 (38.0)	50	
Marital status				
Married	24 (77.4)	8 (42.1)	32 (64.0)	
Widow/widower	5 (16.1)	8 (42.1)	13 (26.0)	($\chi^2 = 6.38$, $p = 0.04$)
Divorced	2 (6.5)	3 (15.8)	5 (10.0)	

Fig. 1 **a** Diabetic foot ulcer at the planter of the foot. **b** Diabetic foot ulcer at the heel of the foot. **c** Diabetic foot ulcer of the entire foot

not intentional to enrol only subjects with grade 4 ulcers, but on assessment all our subjects fell into this category.

Data and sample collection

Fifty subjects were enrolled for the study. Data on demographic information, history of antibiotic chemotherapy and duration of diabetes were obtained with a structured questionnaire. Subjects who could read and write completed the forms while semi-literates and the illiterates were assisted following verbal response to questions. Specimens were obtained from the foot ulcers using sterile swab sticks. The specimens were transported for analysis within 1 h of collection.

Processing of specimens

Specimens were inoculated on blood agar, chocolate agar and cysteine lactose electrolyte-deficient (CLED) agar media. Incubation was done aerobically and in carbon dioxide jar for 24 to 48 h at 37 °C (Viswanathan et al. 2012; Carvalho et al. 2014). Anaerobic incubation was not carried out due to lack of equipment and materials. Bacterial isolates were identified based on gross morphology, microscopy and biochemical tests (Ogba et al. 2014). Antibiotic susceptibility testing was done using the Kirby-Bauer disc diffusion method (CLSI, 2011). The antibiotics tested were erythromycin 15 μg; ceftazidime 30 μg; ceftriaxone 30 μg; augmentin 30 μg; rocephin 30 μg; ofloxacin 5 μg; 5 μg, levofloxacin 5 μg and gentamicin 10 μg; ampicillin 10 μg, and cotrimoxazole 25 μg.

Data analysis

Data was analysed using Epi Info 2012 (CDC, Atlanta, GA, USA) Statistical Software. Categorical variables were calculated using frequencies. Interactions between specific categorical clinical variables were tested for significance using the χ^2 test. A p value of ≤ 0.05 was considered statistically significant.

Results

Table 1 shows the demography of diabetic foot ulcer subjects. Of the 50 subjects enrolled for the study, 19(38.0%) were males and 31 (62.0%) were females, with a male to female ratio of 1:1.6. The age range was 40–77 years with mean age 55 ± 10.2 years. Most subjects 21/50 (42.0%) were aged 50–59 years. With regard to marital status, 32/50 (64.0%) were married, 13/50 (26.0%) were widowed and 5/50 (10.0%) were divorced. The effect of marital status on foot ulcer infection was significant ($\chi^2 = 6.38$, $p = 0.04$.). Most of the subjects were civil servants 20 (40.0%) while others were involved in one line of

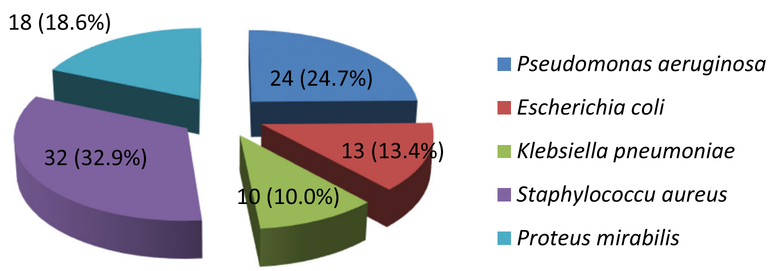

Fig. 2 Distribution of bacterial isolates among subjects

Table 2 Distribution of isolates by age of subjects

Age (years)	No. (%) of subjects examined	No. (%) of isolates					Total
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Escherichia coli*	*Klebsiella pneumoniae*	
40–49	14 (28.0)	8 (25.8)	7 (29.2)	3 (17.6)	6 (46.2)	3 (25.0)	28 (28.9)
50–59	21 (42.0)	13 (41.9)	11 (45.8)	4 (23.5)	3 (23.1)	7 (58.3)	37 (38.1)
60–69	8 (16.0)	6 (19.4)	1 (4.2)	8 (47.1)	0 (0.0)	2 (16.7)	17 (17.5)
70–79	7 (14.0)	4 (12.9)	5 (20.8)	2 (11.8)	4 (30.8)	0 (6.0)	15 (15.5)
Total	50	31 (31.9)	24 (24.7)	17 (17.5)	13 (13.4)	12 (12.4)	97

business or the other. There was a statistically significant association between subjects' occupation and diabetic foot ulcers ($\chi^2 = 22.2$, $p = 0.01$) (Table 1).

Figure 2 shows the distribution of bacterial isolates among subjects. *Staphylococcus aureus* had the highest degree of occurrence (32 (32.9%)) followed by *Pseudomonas aeruginosa* (24 (24.7%)).

A 100% infection rate was recorded in the study with a total of 97 isolates. Mono-microbial infection occurred among 14 (28.0%) of the subjects while polymicrobial infection occurred among 36 (72.0%) of the subjects (Fig. 3).

Table 2 shows the distribution of pathogens by age of subjects. Subjects aged 50–59 years had the highest number of isolates (37 (38.1%)) while subjects aged 70–79 years had the least number of isolates (15 (15.5%)).

Table 3 shows distribution of bacterial isolates by duration of diabetic foot ulcer. Subjects with ulcers ≤ 2 years had the highest number of isolates (56 (57.7%)) while subjects with ulcers ≥ 10 years old had the least number of isolates (6 (6.2%)). There was no statistically significant relationship between the duration of diabetic foot ulcer and the infection rates ($\chi^2 = 11.07$, $p \geq 0.05$).

Table 4 shows the susceptibility rate of bacterial isolates to antibiotics. The susceptibility rate of isolates to commonly used antibiotics was 11.7 to 75.0%%. *Staphylococcus aureus* susceptibility profile was 16.1 to 48.4% for quinolones, 32.3 to 48.4% for cephalosporins, 19.4 to 61.2% for the beta-lactam antibiotics and 67.7% for erythromycin. *Pseudomonas aeruginosa* susceptibility profile was 45.8 to 75.0% for quinolones, 20.8 to 25.0% for cephalosporins and 25.0% for gentamycin.

The gram-positive isolates showed higher susceptibility to erythromycin (67.7%), followed by amoxicillin (61.2%) (Table 4). The gram-negative isolates were more susceptible to ciprofloxacin (80.4–100%), followed by zinaceff (25.0–66.7%) and amoxicillin (50.0–64.7%). They exhibited a high resistance to ampicillin (76.5–84.6%), cotrimoxazole (41.2–84.6%) and gentamycin (58.3–75.0%).

Discussion

In this study, subjects aged 50–59 years were more susceptible to diabetic foot infection. This is in agreement with the report of Karmaker et al. (2016) in Dhaka, Bangladesh with most of their subjects with average age of 58 years and older than 40 years. The mean age of the subjects in this study was 55 ± 10.2 years. The study revealed that diabetics in their fourth and fifth decade of life were more prone to DFU.

A total of 97 aerobic bacterial isolates were encountered in the 50 subjects accounting for an average of 1.94 isolates per subject. This pathogen isolation rate per lesion is lower than the 2.51 reported by Viswanathan et al. (2012) and 2.37 reported by Carvalho et al. (2014) respectively. The lower isolation rate in this study may be due to the incubation method used. Viswanathan et al. (2012) and Carvalho et al. (2014) used both aerobic and anaerobic methods, but only aerobic incubation method was carried out in this study due to lack of equipment and materials.

Table 3 Distribution of bacteria isolates by duration of diabetic foot ulcer

Duration of DFU (years)	No. (%) of subjects examined	No. (%) of isolates					
		S. aureus	*P. aeruginosa*	*P. mirabilis*	*E. coli*	*K. pneumoniae*	Total
≤ 1 years	29 (58.0)	18 (58.1)	15 (62.5)	9 (52.9)	6 (46.1)	8 (66.7)	56 (57.7)
2–5 years	13 (26.0)	9 (29.0)	5 (20.8)	3 (17.6)	6 (46.1)	3 (25.0)	27 (27.8)
6–9 years	6 (12.0)	3 (9.7)	2 (8.3)	3 (17.6)	0 (0.0)	1 (8.3)	9 (9.3)
≥ 10 years	2 (4.0)	1 (3.2)	2 (8.3)	2 (11.8)	1 (7.7)	0 (0.0)	6 (6.2)
Total	50	31 (31.9)	24 (24.7)	17 (17.5)	13 (13.4)	12 (12.4)	97

Table 4 Susceptibility rate of bacterial isolates to antibiotics

Organism	No. of isolates tested	AMX	PN	AUG	E	COT	GEN	OFX	CIP	PEF	CRO	CXM
		No. (%) of isolates susceptible										
S. aureus	31	19 (61.2)	6 (19.4)	NT	21 (67.7)	16 (51.6)	9 (29.0)	8 (25.8)	15 (48.4)	5 (16.1)	10 (32.3)	15 (48.4)
Pseudomonas aeruginosa	24	NT	NT	NT	NT	NT	6 (25.0)	13 (54.2)	18 (75.0)	11 (45.8)	5 (20.8)	6 (25.0)
E. coli	13	7 (53.8)	2 (15.4)	6 (46.1)	NT	2 (15.4)	4 (30.8)	3 (23.0)	7 (53.8)	8 (61.5)	5 (38.5)	7 (53.8)
Proteus mirabilis	17	11 (64.7)	4 (23.5)	9 (52.9)	NT	10 (58.8)	7 (41.2)	6 (35.2)	10 (58.8)	2 (11.7)	6 (35.3)	8 (47.0)
Klebsiella pneumoniae	12	6 (50.0)	NT	10 (83.3)	NT	4 (33.3)	5 (41.7)	7 (58.3)	9 (75.0)	3 (25.0)	5 (41.7)	8 (66.7)

KEY
AUG augmentin; *CIP* ciprofloxacin; *CRO* rocephin; *OFX* ofloxacin; *E* erythromycin; *PN* ampicillin; *COT* cotrimoxazole: *GEN* gentamycin; *CXM* zinaceff; *PEF* pefloxacin; *AMX* amoxicillin

Staphylococcus aureus was the only gram-positive bacterial isolate 31 (31.9%) while *Pseudomonas aeruginosa* 24 (24.7%) was the most common gram-negative isolate. Ramakant et al. (2011) in Lucknow, India, also reported *P. aeruginosa* as the most common gram-negative isolate in diabetic foot ulcer patients in their study.

The polymicrobial infection rate in this study was 36 (72.0%) while the monomicrobial rate was 14 (28.0%). Our findings is higher than the 67.2% reported by Shanmugam et al. (2013) but lower than the 80% and 87.2% polymicrobial infection rates reported by Wright et al. (2011) and Altrichter et al. (2015) respectively. The high polymicrobial infection rate reported in this study point to the fact that most of our subjects suffered severe diabetic foot infections with grade 4 wounds.

Our study reveals an indirect relationship between the duration of diabetic foot ulcer (DFU) and bacterial infections even though there was no statistically significant relationship between DFU and bacterial infections ($\chi^2 = 21.1$, $p \geq 0.05$). Subjects with duration of ulcer ≤ 1 year 29 (58.0%) had the highest burden of bacterial infection 56 (57.7%) while subjects with DFU ≥ 10 years had the lowest bacterial infections. This study did not investigate the reason for the skew in the number of subjects with DFU as the duration of infection increased. The reduction may point to the fact that diabetic subjects with foot ulcers ≥ 10 years may not survive complications of the diabetes which include amputation in our locality.

Antibiotic susceptibility of isolates to commonly used antibiotics was low. None of the isolates showed 100% susceptibility to any of the antibiotic tested. The multiple antibiotic resistance of isolates to quinolones and cephalosporins which are commonly used in our locality calls for an immediate action on the controlled use of antimicrobials in the hospitals and the need to monitor resistance. Good antimicrobial use is necessary for effective wound management. The low susceptibility of isolates observed in this study agrees with the reports of Anguzu and Olila (2007) in Uganda and Ogba et al. (2017) in Nigeria.

A low level of susceptibility of isolates tested with ampicillin and cotrimoxazole was observed. This may be attributed to the fact that ampicillin and septrin have been widely abused and frequently implicated in self-medication in Nigeria. Ogbera et al. (2006) also reported that high level of antibiotic abuse in Nigeria arise from self-medication which is associated with inadequate dosage and failure to

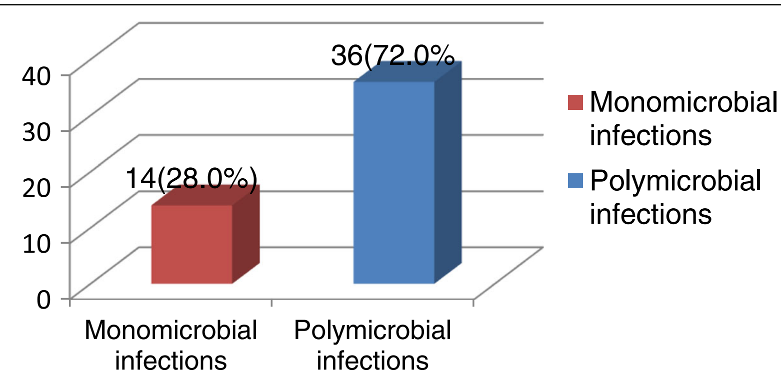

Fig. 3 Distribution of infection types among subjects

comply with treatment regimen. These antibiotics are being sold over the counter with or without prescription.

Conclusion

The study recorded 100% infection rate among the diabetic foot ulcer subjects. All the subjects had grade 4 wounds. The infections were both monomicrobial and polymicrobial. This study showed a dominance of gram-negative bacteria among the isolates. The multiple antibiotic resistance of the bacterial isolates calls for the need to monitor resistance. Best practice is to perform antibiotic susceptibility testing before treatment.

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Availability of data and materials
The dataset(s) supporting the conclusions of this article are included within the article.

Authors' contributions
OO conceived the study. OO, NE and ESE contributed to the design of the study. OO and NE performed the laboratory studies, analysed the data and drafted the manuscript. OO is the guarantor of the paper. All authors read and approved the final version.

Ethics approval and consent to participate
Ethical approval was obtained from the Ethics and Research Committee of the University of Calabar Teaching Hospital, Nigeria, before collection of samples. Informed consent was obtained from the subjects before recruiting them for the study.

Consent for publication
Written informed consent was obtained from the participants for publication of their individual details and accompanying images in this manuscript. The consent form is held by the authors and is available for review by the Editor-in-Chief.

Competing interests
The authors declare that they have no competing interests.

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