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Drug Susceptibility Pattern of Bacterial Pathogens associated with Diabetic Foot Ulcers of Patients in Kano North -Western Nigeria

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Abstract

Diabetic foot ulcer has become the major cause of amputation in people with diabetes. Infected foot ulcers are a cause of morbidity and hospitalization in diabetic patients. The aim of the current study was to isolate and ascertain antimicrobial susceptibility pattern of bacterial pathogens associated with diabetic foot infection in Kano North - West, Nigeria. Samples were collected from 100 patients with diabetic foot ulcers using sterile cotton swab and transported to the laboratory for analysis. The male: female ratio was 3:1. Mono bacterial nature of diabetic foot infection was observed in 68% of the samples while mixed bacterial infection was observed in 21% of the samples. The study showed that Gram negative bacteria (84%) was the most frequently isolated pathogen and *E. coli* was found to be predominant (27%) followed by *Klebsiella* species (21%) and *Proteus* species (19%). Among the Gram positive bacteria, *S. aureus* (16%) was the predominant pathogen. The result also showed that most of the isolated bacteria were sensitive to Ciprofloxacin, Ofloxacin, Nitrofurantoin and Gentamicin. However, they showed resistance to Augmentin, Cefixime, Ceftaxidime and Cefuroxime. Further studies needs to be carried out to explore anaerobic pathogens related to diabetic wound and to ascertain the causes of resistance to antibiotics for proper management of diabetic foot ulcer in the study area.

Key Words: Antibiotics, Bacterial pathogens, Diabetic foot ulcer, Susceptibilities

INTRODUCTION

Diabetes is a chronic metabolic condition that occurs when the body cannot produce or effectively use insulin which may be due to hereditary or environmental factors. Globally, around 370million people have diabetes and this number is increasing in every country (Bakker *et al.*, 2012). As the incidence of diabetes mellitus is increasing dramatically, so are the risks of developing diabetic complications such as heart diseases, kidney failure, sexual dysfunction neuropathy, retinopathy and foot ulceration (Mukhtar *et al.*, 2016).

Diabetic foot complications such as neuropathy, peripheral arterial disease are the most common complications among diabetic patients which when worsened results in foot ulceration that may lead to the amputation of the foot. The life time risk of diabetic patient developing foot ulcers was reported to be 15-25% (Jassim, 2014). Other risk factors of developing foot ulcers include a previous history of foot

ulceration, poor glycemic control, poor education and poor access to health care services.

In Nigeria, an estimated 10% people with diabetes suffer foot complications, and the incidence is in the rise. Presently, Diabetic foot ulcer (DFU) account for the majority of non-traumatic amputations performed in almost all Nigerian tertiary hospitals (Mukhtar *et al.*, 2016). Basher and co-workers (2015) reported that the overall commonest cause of amputation in Kano State Northern-Nigeria was diabetic foot complications.

Infected foot ulcers are a cause of morbidity and hospitalization in diabetic patients, ultimately leading to dreaded complications such as gangrene and amputation (Jassim, 2014). Lipsky *et al.* (2004) defined diabetic foot infection as any inframalleolar infection in patients with diabetes mellitus.

These include paronchia cellulitis, myositis, abscesses, necrotizing fasciitis and osteomyelitis. Once the protective layer of skin is breached, underlying tissues are exposed to bacterial colonization.

Many studies have reported on the bacteriology of diabetic foot infection. Iyanar *et al.*, (2014) stated that the bacteriology of Diabetic foot infection (DFI) is highly complicated. It involves both aerobes and anaerobes and mixed infection. The antibiotic susceptibility pattern also shows lot of variations among different geographical places and also with various periods of time. The multidrug resistant bacteria have been reported in diabetic foot infection (Mohanasoundaram, 2012). Hence it is important to investigate different bacteria involved in Diabetic foot ulcer and also to know about its antibiotic sensitivity pattern in the study area.

MATERIALS AND METHODS

The study was carried out at Murtala Muhammad Specialist Hospital (MMSH) and Aminu Kano Teaching Hospital (AKTH) during the period of April to December, 2018. They are the largest tertiary hospitals in Kano State, Nigeria that provide a high level medical care to a large population of people in the state and are located within the metropolis. Ethical clearance was obtained from Aminu Kano Teaching Hospital (AKTH/MAC/SUB/12A/P-3/VI/2159) and Kano State Hospital Management Board (MMSHZ/0324/III/167) before the commencement of the research. Diabetic patients with sign and symptoms of foot ulcers were included in the study. The sample size was determined using the formula: $\text{sample size} = Z^2 p(1-p)/d^2$

Where Z is a standard normal variate at (5%) type 1 error ($P < 0.05$) = 1.96,

P is the expected proportion in the population based on previous studies = 6% (Mukhtar *et al.*, 2016), d = absolute error or precision (5%)

Sample size = $1.96^2 \times 0.06 (1-0.06)/0.05^2 = 86.7 \approx 87$

Approximately 100 to the nearest 100

Discharges from wound of diabetic foot ulcer patients were collected using sterile cotton swabs (Tamalli *et al.*, 2015). The swabs were labeled and transported immediately to the laboratory for analysis. Oral informed consent was obtained from the patients before sample collection. Patient personal details, antibiotic regime and other relevant information were collected using questionnaire.

Culture and identification

The samples were streaked on chocolate agar and Mac-Conkey agar and incubated at 37°C for 24hrs aerobically. Identification of the isolates was done based on colony morphology, Gram staining and biochemical test such as Triple sugar ion, Citrate, Urease, Indole, Coagulase, Catalase (Tamalli, 2015).

Antibiotic sensitivity test

The organisms isolated were subjected to antibiotic susceptibility testing on Mueller-Hinton agar using Kirby-Bauer disc diffusion method according to CLSI guidelines (Mohanasoundaram, 2012). A sterile swab stick was used to inoculate the standard inoculums (1.5×10^8 CFU/ml) of the test organism evenly on the surface of the Mueller Hinton agar and was allowed to stand for three minutes to dry. Commercially prepared discs of antibiotics were placed on the media (Olowo-okere *et al.*, 2017). The plates were then incubated at 35°C for 18-24hrs after which the zone of inhibition for each of the antibiotics were observed and recorded (Olowo-okere *et al.*, 2017). The antibiotics tested were; Ceftaxidime (30µg), Ciprofloxacin (5µg), Cefuroxime (30µg), Gentamicin (10µg), Ofloxacin (5µg), Augmentin (30µg), Nitrofurantoin (300µg), Cloxacillin (5µg), Cefixime (5µg).

The data collected were analyzed and presented using descriptive statistics of frequency, percentages and tables.

RESULTS AND DISCUSSION

Demographic features of the patients

The demographic profile of the Diabetic foot ulcer patients showed that males (84%) were commonly more affected than females (16%). The male:female ratio was 3:1 which is in consistence with the findings of other studies (Mathangi and Prabhakaran 2013; Iyanar *et al.*, 2014; Yerat and Rangasamy, 2015;). This may be due to higher level of outdoor activities among males compared to females (Zubair *et al.*, 2010). Studies in southern Nigeria and other countries shows almost equal gender predisposition to DFU (Jeber and Saeed, 2013; Akaa *et al.*, 2017) which may be because of the engagement of females in more outdoor activities now due to global economic challenges in order to raise money to cater for the family (Akaa *et al.*, 2017). The highest number of patients with DFU belonged to the age group of 51-60 years followed by 41-50 and 61-70 years. On the other hand lowest number was seen among 20-30 and 31-40 age group which is similar to the findings of other researchers (Yerat and Rangasamy 2015; Akwahet *et al.*, 2015).

This may be due to aging and long exposure to diabetes mellitus because most of the patients investigated had diabetes for up to 10years and above. Table 1. Shahi *et al.*, (2012) found in their study in Iran that risk factor for development of DFU was above 50years of age.

A person with diabetes foot ulcer is more prone to infection which may become severe and lead to amputation. In this study, of the 100 DFU patients analyzed, 18% had undergone amputation while 85% had not. (Table 1).

Table 1: Socio-demographic characteristics of the patients suffering from DFU in Kano, 2018

Parameter	Specificity	frequency
Gender	Male	84
	Female	16
Age	20-30	2
	31-40	4
	41-50	28
	51-60	37
	61-70	19
	71-80	10
Duration of diabetes	≤10	58
	≥11	42
History of amputation	present	18
	absent	82

The findings also showed that there were more outpatients (63%) than the inpatients in the study area. This may be because of the highest cost of managements of DFU in the hospitals and most of the patients cannot afford the cost. Desalu and co-workers (2011) reported that in Nigeria the mean costs for successfully treating a patient with Diabetis Mellitus Foot ulceration was 181,581.00 Nigerian Naira (NGN). This amount is unaffordable by most hospital patients as about 60% of the population live below the poverty line

Bacterial pathogens isolated

Over the past 25years, many studies (Ramani *et al.*, 1991; Ekeh *et al.*,2003) have reported the mixed bacterial nature of diabetic foot infection involving both gram positive and gram negative bacteria with the predominance of gram positive bacteria, usually *S.aureus* in the infection. But nowadays, ongoing studies reported the monobacterial nature of DFU and

the predominance of gram negative bacteria. This kind of inconsistency could be because of time, geographical variation, or the type and severity of the infection included in the study(Jassim, 2014).

In this study, a mono bacterial nature of diabetic foot ulcer was observed in 68% of the samples and mixed bacteria in 21% of the samples while the remaining 11 samples yielded no bacterial growth. This agreed with the findings of a number of studies((Mathangi and Prabhakaran 2013; Tamalli *et al.*, 2015).

While studying the predominant pathogens in diabetic foot infection, it was observed that gram negative bacteria (84%) account for the most frequently isolated pathogen and *E.coli*(27%) was found to be predominant followed by *Klebsiella* species(21%) and *Proteus*(19%). *S.aureus*(16%) was found to be the predominant gram positive bacteria isolated. Table 2.

Table 2: Percentage prevalence of bacterial isolates in diabetic foot ulcer

Isolate	Frequency	Percentage
<i>Escherichia coli</i>	28	27
<i>Staphylococcus aureus</i>	16	16
<i>Klebsiella</i> species	22	21
<i>Pseudomonas aeruginosa</i>	8	8
<i>Proteus</i> species	20	19
<i>Citrobacter</i> species	6	6
<i>Streptococcus</i> specie	3	3
Total	103	100

With regards to the susceptibility pattern, the result showed that most of the isolated bacteria showed good sensitivity against Ciprofloxacin, Ofloxacin, Nitrofurantoin and Gentamicin and less sensitivity to Cefixime,

Ceftaxidime, and Cefuroxime. Moreover, all the isolates showed 100% resistance to Augmenting. The resistance to most of the antibiotics may be because the patients were on antibiotics for a long period of time.

Table 3: Antibiotic Resistance pattern of isolated bacterial species from DFU Patient

Antibiotics Used	Resistance pattern of the Isolated Organisms						
	<i>Citrobacter</i> spp (*6)	<i>E. coli</i> (*28)	<i>Klebsiella</i> spp (*22)	<i>Proteus</i> spp (*20)	<i>Pseudomonas</i> spp (*8)	<i>Staphylococcus</i> spp (*16)	<i>Streptococcus</i> spp (*3)
OFL	1(17%)	12(43%)	11(50%)	10(50%)	6(75%)	5(31%)	2(67%)
GEN	4(67%)	13(46%)	12(55%)	11(55%)	4(50%)	7(44%)	2(67%)
CPR	1(17%)	12(43%)	10(45%)	7(35%)	4(50%)	5(31%)	1(33%)
NIT	4(67%)	15(54%)	10(45%)	13(65%)	7(88%)	7(44%)	2(67%)
CXM	5(83%)	25(89%)	20(90%)	20(100%)	8(100%)	13(81%)	3(100%)
CRX	6(100%)	28(100%)	20(91%)	20(100%)	8(100%)	14(87%)	3(100%)
CAZ	6(100%)	27(96%)	21(96%)	20(100%)	8(100%)	15(94%)	2(67%)
AUG	6(100%)	28(100%)	22(100%)	20(100%)	8(100%)	16(100%)	3(100%)

OFL=Ofloxacin, NIT=Nitrofurantoin, GEN=gentamicin, CPR=Ciprofloxacin, CXM=Cefixime, CRX=Cefuroxime, CAZ=Ceftaxidime, AUG=Augmenting, spp=specie
 *= indicate number of specie isolated

CONCLUSION

It can be concluded that males are more affected with diabetic foot ulcer than females in the study area with the predominance of gram negative bacteria usually *E. coli* in most of the infected ulcers. Antibiotic susceptibility pattern showed more resistance of the investigated bacterial pathogens to

augmentin, cefixime, ceftaxidime and cefuroxime.

Further study needs to be carried out to ascertain the causes of resistance to antibiotics and the predisposition factors to diabetic foot ulcer in people with diabetes in the study area in order to minimize the risks of infection and amputation in people with diabetes in the area.

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