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Risk Factors for Methicillin Resistant *Staphylococcus aureus* (MRSA) from Diabetes Patients with Foot Ulcers (DFU) in a Tertiary Hospital

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Abstract

Diabetic foot ulcer (DFU) is a major complication of diabetes mellitus. Risk of MRSA should be considered in selecting empirical antibiotics. This study was aimed to determine the Risk factors of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Diabetes patients with foot ulcers (DFU). A total of 204 patients with diabetic foot were included in the study. Data collected using a pre-designed questionnaire included patient characteristics and medical history to determine risk factors for developing an MRSA infection in the foot. Specimens were obtained by scraping the ulcer base or the deep portion of the wound edge with a sterile curette, and were promptly sent to the laboratory for culture and identification. The prevalence of MRSA in DFU patients was 22.1%. Male patients with DFU were more infected with MRSA 26(12.7%) than females 19(9.3%) with statistical significance ($p < 0.04$). The age group 41 – 60 years had the highest prevalence of 27(13.2%) with statistically significance ($p < 0.03$). Farmers and DFU patients of 6 – 10 years had the highest prevalence of 20(9.8%) and 22(10.8%) without statistical significance. Risk factors were all significantly associated to MRSA colonization of diabetic foot ulcers and includes patients who had Type 2 diabetes for 1 – 5 years, and fasting blood sugar greater than >126, patients who smoked and drank for more than 10 years, and patients with body mass >30kg/m2. The prevalence of MRSA in DFU patients was 22.1% which was high. Risk factors were all statiatically associated to MRSA colonization of diabetic foot ulcers. The results from this study will guide healthcare workers on how to educate the patients in the study environment on the possible risk factors contributing to the colonization of MRSA in diabetic foot ulcers and the use of antibiotic therapy to eliminate MRSA from colonized wounds.

Keywords: Methicillin resistant *staphylococcus aureus*; Diabetic foot ulcers; Prevalence; Wagner classification; Abia State

Abbreviations: DFU: Diabetic Foot Ulcer; MRSA: Methicillin Resistant Staphylococcus Aureus; DM: Diabetes Mellitus; DF: Diabetic Foot; IDSA: Infectious Diseases Society of America; IWGDF: International Working Group on the Diabetic Foot; IFU: Ischemic Foot Ulcer; NFU: Neuropathic Foot Ulcer; N-IFU: Neuro-Ischemic Foot Ulcer; MSA: Mannitol Salt Agar; NIMR: Nigerian Institute of Medical Research; SPSS: Statistical Package for Social Sciences; KFH: King Fahad Hospital; LGAs: Local Government Areas.

Introduction

Diabetic foot ulcer (DFU) is a major complication of Diabetes mellitus (DM). Diabetic foot ulcers can affect people with both type 1 and type II diabetes [1]. When blood sugar levels are high or fluctuate regularly, skin that would normally heal may not properly repair itself because of nerve damage. Even a mild injury can therefore start a foot ulcer [1].

People with diabetes are more likely to get foot ulcers because of reduced nerve functioning due to peripheral diabetic neuropathy [2]. The narrowed arteries reduce blood flow to the feet and this impairs the foot's ability to heal properly. When the foot cannot heal, a foot ulcer develops [2].

People who also have diabetes for longer periods or manage their diabetes less effectively are more likely to develop foot ulcers [3]. Smoking, lack of exercise, being overweight, and having high cholesterol or blood pressure can all predispose to diabetic foot [3].

Diabetes and foot ulcers are almost synchronous [3]. As many as 25 % of diabetic individuals are expected to develop severe foot problems at some point in their lifetime, which often leads to amputation [4].

Different classification systems are used to assess the severity of diabetic foot (DF), the most often used of which are the Wagner-Meggit classification system that takes into consideration the depth of ulcer, presence of gangrene, and level of tissue necrosis [5]; and the Infectiuos Diseases Society of America/International Working Group on the Diabetic Foot (IDSA/IWGDF) classification system for defining the presence and severity of an infection of DF [5]. Besides, diabetic foot (DF) can also be classified into three types according to whether with or without peripheral arterial or nerve diseases [6], named ischemic foot ulcer (IFU), neuropathic foot ulcer (NFU), and neuro-ischemic foot ulcer (N-IFU), respectively

Standard treatment of diabetic foot according to Wagner classification

Grade	Foot Risk	Prevention
Grade – I	Localized, superficial ulcer	Antibiotics & glycemic control
Grade II	Deep Ulcer to bone, ligament, or joint	Debridement, Antibiotics and glycemic control
Grade III	Deep abscess, osteomyelitis	Debridement, some form
Grade IV	Gangrene of toes, forefoot	Wide debridement and amputation
Grade V	Gangrene of entire foot	Below knee amputation

Source: Mugambi-Nturibi, et al. [7].

Staphylococcus aureus is the most common pathogen among Gram-positive bacteria isolated from ulcers [4]. Almost 50% of *S. aureus* isolates are MRSA [4].

MRSA has been increasingly isolated from diabetic foot ulcers, and several studies have found its emergence in as many as 15–30% of diabetic wounds [5-7]. Infection/colonisation with MRSA may result in prolonged hospital stay and excessive direct economic cost [8-10].

Therefore, the knowledge of risk factors of MRSA becomes necessary in the selection of appropriate empirical treatment of diabetic foot infections.

The objective and significance of this study was to determine the Risk factors of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Diabetes patients with foot ulcers (DFU) in a tertiary hospital in Abia State. This results from this study will guide healthcare workers on how to eliminate MRSA from colonized wounds with the empirical use of antibiotic therapy.

Material and Methods

Study area: This study was carried out at Abia State University Teaching Hospital Aba which is located in Aba South Local Government Area of Abia State, Nigeria.

Study Duration

This study was carried out between April 2016 to May 2017.

Inclusion Criteria

All patients' who gave their consent and who presented with symptoms of diabetic foot ulcers, and who returned their questionaires within the study timeframe were included.

Exclusion Criteria

Diabetic patients who had traumatic ulcer due to car accident, all patients who declined consent and who didn't

present symptoms of diabetic foot ulcers within the study timeframe were excluded.

Ethical Clearance

Ethical permission was obtained from the hospital authorities and patients consented to participate in the study.

Study Subject

A total of two hundred and four (204) patients with diabetic foot ulcers, who returned their questionaaires, were included in this study.

Sample Size Determination

The sample size was determined using the standard formula for calculation of minimum sample size [11]
n=minimum sample size
z=standard normal deviation and probability.
p=prevalence or proportion of value to be estimated from previous studies [12].
q=Proportion of failure (=1-P)
d=precision, tolerance limit, or margin of error, the minimum is 0.05.
Therefore, $n = Z^2pq \div d^2$
Where Z=95% (1.96)
P= 17% (0.17) [12]
q=1-0.17 (=0.83)
d=5% (0.05)
Therefore $n= (1.96)^2 (0.17) (1-0.17)/ (0.05)^2$
n=217

Patients Information

Each participant was requested to complete a questionnaire regarding potential risk factors for MRSA colonization and diabetic profile. The information collected included demographic data (gender, age, and occupation), personal history and the condition of diabetes mellitus, antibiotics usage, smoking habits, drinking habits, fasting blood sugar and body mass index.

Clinical Examination

Once the patient arrive the operating room, ulcers were assessed for signs of infection (swelling, exudates, surrounding, cellulitis, odor, tissue necrosis and crepitation). The lesions were then categorized into 3 main clinical groups: (I) skin ulcer (Wagner 1 and 2); (II) deep tissue ulcer with suspected osteomyelitis (Wagner 3); and (III) gangrenous lesion (Wagner 4 and 5). The diagnosis of infection was based on criteria from the Infectious Disease Society of America and the International Working Group on the Diabetic Foot [5]. All cases were monitored until discharged from the hospital. The affected foot was then prepared using aseptic technique [13]. Once the infected area was addressed surgically, infected tissue was acquired and sent in their respective container to microbiology laboratory.

Microbiological Methods

Direct microscopic examination of ulcer sample was performed. Standard methods for isolation and identification of *S. aureus* were used [14]. The samplings were cultured in blood agar plates. Isolates that showed beta-hemolytic and coagulase-positive reaction were identified as *S. aureus*.

Biochemical Confirmation of *Staphylococcus aureus*

The *S. aureus* was placed on Mannitol Salt Agar (MSA) for 24 hours. Smooth circular colonies with yellow colour indicate a positive result for *S. aureus* [15].

Detection of Methicillin Resistance

MRSA identification was carried out using oxacillin screen plates following the guidelines of NCCLS. Briefly, a suspension equivalent to 0.5 McFarland standards, prepared from each strain, was inoculated homogenously on the entire surface of the Mueller-Hinton agar plate (Oxoid-UK) containing 4% NaCl and 6µg/mL oxacillin, with the help of sterile swabs. All the plates were incubated at 35°C for 24 hours. Indication of growth (>1 colony) identified the isolates as oxacillin/methicillin-resistant [16].

Quality Control

For quality control *Staphylococcus aureus* ATCC 29213 was used as a reference strain for the standardization of antibiotic susceptibility testing obtained from Nigerian Institute of Medical Research (NIMR) Yaba, Lagos State. The qualities of biochemical testing procedures were checked by this reference strain. Lastly, thirteen (13) Diabetic patients without foot ulcer admitted for treatment with various causes were invited as control.

Statistical Analysis

Data generated were analyzed using the statistical package for social sciences (SPSS) software, version 21.0. Qualitative variables were presented as frequencies and percentage. In order to identify risk factors associated with *S. aureus* or MRSA colonization, Student's ttest was performed for continuous variables, and Chisquare test test were performed for categorical variables. A p-value of $P < 0.05$ was considered statistically significant.

Results

Socio-Demographic Characteristics of Study Population

A total of 217 patients were recruited in the study, 204 questionnaires were completed and returned giving a response rate 94%. Among 204 wound swabs specimens processed during the study, the prevalence of MRSA in DFU patients was 22.1%. Males were more infected with MRSA 26(12.7%) than females 19(9.3%) and this was statistically significant ($p < 0.04$). The age group 41 – 60 years had the highest frequency of MRSA 27(13.2%) while the lowest frequency was found in age group 0 – 20 years with statistically significance ($p < 0.03$). Farmers had the highest prevalence of patients with MRSA 20(9.8%) while the least was seen housewives 1(0.5%) and this was not statistically significant ($p < 0.07$). For the duration of foot ulcers infected with MRSA, ulcers that were between 6 - 10 years had the highest prevalence of 22(10.8%) while the least was found in the ulcers greater than 10 years 1(0.5%) and this was not statistically significant ($p < 0.13$).

Characteristics P-value	No. with foot Ulcers (%)	No. of infected foot Ulcers (%)	No. infected with S. aureus	No. Infected +with MRSA (%)	
SEX					
Male	154(75.5)	100(49.1)	71(34.8)	26(12.7)	0.04
Female	50(24.5)	34(16.7)	28(13.7)	19(9.3)	
Total	204(100)	134(65.7)	99(48.5)	45(22.1)	
AGE IN YEARS					
0 – 20	0	0	0	0	0.03
21 – 40	6(2.9)	3(1.5)	2(1.0)	1(0.5)	
41 – 60	103(50.5)	77(37.7)	60(29.4)	27(13.2)	
61 – 80	84(41.2)	48(23.5)	33(16.2)	27(13.2)	
>80	11(5.4)	6(2.9)	4(2.0)	2(1.0)	
Total	204(5.4)	134(65.7)	99(48.5)	45(22.1)	
OCCUPATIONAL STATUS					
Farmers	84(41.2)	60(29.4)	54(26.5)	20(9.8)	0.07
Traders	18(8.8)	9(4.4)	5(2.5)	2(1.0)	
Civil servants	29(14.2)	16(7.8)	7(3.4)	6(2.9)	
Housewives	8(3.9)	5(2.5)	3(1.5)	1(0.5)	
Artisans	65(31.9)	44(21.6)	30(14.7)	16(7.8)	
Total	204(5.4)	134(65.7)	99(48.5)	45(22.1)	
DURATION OF ULCERS					
<1	28(13.7)	25(12.3)	14(6.9)	6(2.9)	0.13
1 – 5	65(31.9)	40(19.6)	36(17.6)	16(7.8)	
6 – 10	96(47.1)	64(31.4)	47(23.0)	22(10.8)	
>10	15(7.4)	5(2.5)	2(1.0)	1(0.5)	
Total	204(5.4)	134(65.7)	99(48.5)	45(22.1)	

Table 1: Demographic characteristics of patients with MRSA infected diabetic foot ulcers.

Table 2 shows the Wagner's classification of foot ulcers in relation to sex. The highest frequency of foot ulcers patients was seen in patients with Grade II 78(38.2%) followed by patients with Grade III foot ulcer 58(28.4%). The least was seen in Grade V patient 8(3.9%). There was no statistical significance among them ($P < 0.07$).

Wagner's Classification	Male (%)	Female (%)	No (%)	P-Value
Grade - I	23(11.3)	12(5.9)	35(17.2)	0.07
Grade - II	44(21.6)	34(16.7)	78(38.2)	
Grade – III	32(15.7)	26(12.7)	58(28.4)	
Grade – IV	17(8.3)	8(3.9)	25(12.3)	
Grade – V	5(2.5)	3(1.5)	8(3.9)	
Total	121(59.3)	83(40.7)	204(100)	

Table 2: Wagner's Classification of Diabetic foot of the patients in relation to sex.

Table 3 shows the Prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) among diabetic foot patients. There was no statistical significance between in-patients and out-patients ($p < 0.16$).

Patients	Number of patients (%)	*S. aureus* (%)	MRSA (%)	P - Value
In- patients	123(60.1)	57(27.9)	30(14.7)	0.16
Out-patients	81(39.7)	42(20.6)	15(7.4)	
Total	204(100)	99(48.5)	45(22.1)	

Table 3: Prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) among diabetic foot patients.

Table 4 shows the Potential risk factors associated with methicillin-resistant *S. aureus* in DFU patients. The risk factors namely types of diabetes, history of drug use, fasting blood sugar, drinking and smoking habit and body mass index were all significantly associated with MRSA in DFU ($P < 0.05$).

	Patients No (%)	MRSA (%) 45(45.5)	MSSA (%) 54(54.6)	Total 99(100)	P-Value
Types of Diabetes					
Type 1	31(15.2)	14(6.9)	17(8.3)	31(15.2)	0.02
Type 2	173(84.3)	31(15.2)	37(18.1)	68(33.3)	
History of antibiotics use					
1 – 10	41(20.1)	8(3.9)	9(4.4)	17(8.3)	0.03
11 – 20	64(31.4)	13(6.4)	18(8.8)	31(15.2)	
>20	99(48.5)	24(11.8)	27(13.2)	51(25.0)	
Fasting Blood sugar (mg/dL)					
<100	1(0.5)	0	0	0	0.01
100 – 125	21(10.3)	7(3.4)	10(4.9)	17(8.3)	
>126	182(89.2)	38(18.6)	44(21.6)	82(40.2)	
Smoking Habit					
<5	23(11.3)	8(3.9)	7(3.4)	15(7.4)	0.04
6 – 10	66(32.4)	13(6.4)	12(5.9)	25(12.3)	
>10	115(56.4)	24(11.8)	35(17.2)	59(28.9)	
Drinking History					
<5	33(16.2)	6(2.9)	5(2.5)	11(5.4)	0.03
6 – 10	53(26.0)	16(7.8)	13(6.4)	29(14.2)	
>10	118(57.8)	23(11.3)	36(17.6)	59(28.9)	
Body mass Index(kg/m2)					
<18.5	5(2.5)	0	0	0	0.02
18.5 – 24.9	20(9.8)	0	0	0	
25 – 29.9	64(31.4)	7(3.4)	14(6.7)	21(10.3)	
>30	115(56.4)	38(18.6)	40(19.6)	78(38.2)	

Table 4: Potential risk factors associated with methicillin-resistant *S. aureus* in DFU patients

Discussion

The present study shows that the prevalence of MRSA in DFU patients was 22.1% and this was similar to that reported from iran (24.7%) [17]. But it was higher than that reported from United Kingdom (3.6%) [18] and Turkey (9.9%) [19]. These differences could be attributed to the differences in age/gender composition, ulcer grades and study settings.

The prevalence of diabetic foot ulcer and MRSA is higher in males than females with maximum infection occurring in 41-60 years of age group who are active working group with statistical significance ($p<0.05$). Awadh and Al-Anazi, reported a higher prevalence in males 43 (74.1%) to females 15(25.9%) in Riyadh, Saudi [20] Arabia. Omuse, et al. also reported a greater percentage of MRSA in males (58%) than in females (42%) in Kenya [21]. The reasons for such differences are not entirely clear, but it appears biologic factors may play important roles like increased rates of peripheral vascular disease and peripheral neuropathy in men [21]. More so, this might also be due to the fact that males within that age group are breadwinners of their families and therefore, do more activities that predispose them to sustaining injuries like farming and technical works [21].

The high prevalence of DFU and MRSA seen in farmers may be due to their living in rural areas surrounding the teaching hospital were this study was carried out. Diabetic patients who live in rural areas of Abia State often spent most of their time in farm area or outdoors and may be subjected to hoe or cutlass cut or rodent bites of their feet. Cuts or bites to the feet of patients with diabetes can lead to the development of ulceration due to poor wound healing process and less opportunity for health care service for it [22-24]. Previously, Ikeh had reported an MRSA prevalence of 43.5% in Jos, Nigeria, of which 81% was from farmers [25]. Nwakwo, reported 28.6% prevalence in kano Nigeria, with 62% of these from traders [15].

Duration of ulcer did not played a significant role in the spread of MRSA in infected diabetic foot ulcers with statistical significance ($p<0.13$). The high prevalence observed from patients whose foot ulcers were between 6-10 years, and also within the age bracket of 41 – 60 years indicates that MRSA infected diabetic foot ulcers is the disease of the middle and old age. This is in agreement with Madani et al., who reported 36% at King Fahad Hospital (KFH) in Jeddah [26].

About 38.2% and 28.4% presented with Wagner's grade II and III respectively. This was consistent with the result of previous study [27]. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers [27].

The prevalence of MRSA in DFU patients was higher in in-patients 14.7% than out-patients 7.4% but this was not statistically significant ($p < 0.16$). MRSA which plays a significant role continues to be a menace in Nigerian hospitals and that the spread is no doubt hospital-aided. This may not be unconnected with the hospital environment, for example, arrangement of people in rooms and wards which makes transfer of these organisms among in-patients easier. Poor hygienic conditions and non-adherence to or even the lack of a relevant antibiotic policy have been suggested as possible reasons for these high prevalence rates [28]. These suggestions continue to remain relevant.

Type of diabetes mellitus was one of the strongest predictors of diabetic foot ulcer occurrence and was statistically significant ($p < 0.02$). Those diabetic patients who had type II diabetes mellitus were more likely to develop diabetic foot ulcer than those who had type I DM. This finding is consistent with the studies conducted in Nigeria and Egypt which indicated type II diabetes mellitus was significantly associated with the occurrence of diabetic foot ulcer [29-30]. The possible explanation could be in type II diabetic patients; there are related complications of the disease, such as mechanical changes in the conformation of the bony architecture of the foot, peripheral neuropathy, and atherosclerotic peripheral arterial disease; as a result, the patient may have less tissue epithelisation, consumption of oxygen, nutrient transportation, and cell detoxification resulting in ulceration in the extremities [30].

The history of use of antibiotics was statistically significant with MRSA in DFU ($p < 0.03$). Patients who used antibiotics for more than 20years had the highest number of isolated MRSA of 11.8%. This shows that long period of antibiotic usage was a risk factor in MRSA in DFU patients. The reason for this could be due to antibiotic resistance as long period of some specific antibiotic use from self medication could have resulted in the resistance to those drugs. Similar results were found in previous studies [19,31].

High glucose level was statistically significant ($p < 0.01$) with MRSA in DFU. Diabetic patients with high blood glucose level are exposed to microvascular complication and neuropathy, and the occurrence of neuropathy may increase the risk for foot ulceration due to increased pressure load and shearing forces [32].

Smoking and drinking for more than 10 years contributed immensely to risk factors of MRSA in DFU of 11.8% and 11.3% respectively with statistical significance ($P < 0.05$). This is also consistent with previous result [27].

The findings in this study showed that overweight diabetic patients which was statistically significant ($p <$

0.02) with MRSA in DFU were more likely to develop diabetic foot ulcer as compared with those who had a normal weight. This is consistent with the studies conducted in Kenya and Ethiopia [22,33]. The possible reason could be due to the presence of higher foot pressure in those heavily weighed and with higher body mass index (BMI) diabetic patients. Obesity and overweight might decrease intensively the normal blood circulation pattern at the lower extremities; as a result, this might lead them to develop diabetic foot ulcer [33].

Conclusion

The prevalence of MRSA in DFU patients was relatively high. Risk factors were all statistically significant to MRSA colonization of diabetic foot ulcers. The results from this study will guide healthcare workers on how to educate the patients in the study environment on the possible risk factors contributing to the colonization of MRSA in diabetic foot ulcers and the use of antibiotic therapy to eliminate MRSA from colonized wounds.

Limitation of the study

Due to inadequate funds, this study was carried out in just one (1) hospital out of the four (4) major tertiary hospitals serving the citizens of Abia State Nigeria. Therefore this result may not give an overall accurate result of prevalence of MRSA from DFU patients in the seventeen (17) Local Government Areas (LGAs) making up Abia State. The results from this study will guide future researchers who may wish to carry out research in the seventeen LGAs of Abia State on the Risk factors for Methicillin Resistant *Staphylococcus aureus* (MRSA) from Diabetes patients with foot ulcers (DFU).

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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