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Prevalence and Antibiotic Susceptibility Profile of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates in Diabetes Patients with Foot Ulcers

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ABSTRACT

Introduction: methicillin-resistant *Staphylococcus aureus* (MRSA) infection is common among diabetes patients with foot ulcers. This study aimed to determine MRSA isolates prevalence and antibiotic susceptibility profile in diabetic foot ulcers (DFU) patients. **Methods:** A total of 204 patients with diabetic foot ulcers admitted to a tertiary hospital in Abia State, Nigeria, were included in the assay. Specimens were obtained by scraping the ulcer base or the deep portion of the wound edge using a sterile curette and were promptly sent to the laboratory for culture, identification, and antibiotic susceptibility test. **Results:** The MRSA prevalence in DFU patients was 22.1% (n=45). Male patients with DFU were more infected with MRSA (n= 26, 12.7%) than females (n=19, 9.3 %), but the difference was not statistically significant ($P < 0.14$). The age group 41-60 years had the highest prevalence (n=27, 13.2%), statistically significant ($P < 0.02$). Farmers had the highest prevalence of 9.8% (n=20) while the least (0.5%) was seen in housewives (n=1) with no statistical significance ($P < 0.07$). The antibiotics sensitivity pattern of MRSA showed 100% sensitivity to vancomycin and chloramphenicol but 100% resistance to penicillin, ceftriaxone, and oxacillin. The multidrug-resistant index was all > 0.2. **Conclusions:** The prevalence of MRSA in DFU patients in a tertiary hospital in Abia State was very high, with an alarming rate of drug-resistant bacteria due to the possibility of misuse and abuse of antibiotics among the populace, which requires collaborations from all stakeholders to prevent drug resistance in the study settings.

INTRODUCTION

Diabetic foot ulcer (DFU) is a significant complication of diabetes mellitus (DM). Diabetic foot ulcers can affect people with both type 1 and type 2 diabetes [1]. When blood sugar levels are high or fluctuate regularly, skin that would naturally heal may not correctly repair itself because of nerve damage. Therefore, even a mild injury can result in a foot ulcer [1]. Diabetes and foot ulcers are almost synchronous; as many as 25% of diabetic individuals develop severe foot problems at some point in their lifetime, leading to amputation [2]. *Staphylococcus aureus* is the most common isolated pathogen from ulcers among Gram-positive bacteria [3]. Almost 41.9% of isolates are methicillin-resistant *staphylococcus aureus* (MRSA) [4], which are resistant to entire classes of β-lactams, including cephalosporins and carbapenems posing a higher risk of developing resistance to quinolones, aminoglycosides, and macrolides [4]. Hence, the infections due to MRSA strains are very challenging to treat [4].

Methicillin resistance in *S. aureus* is mediated through an altered protein called low-affinity penicillin-binding protein (PBP2a) [5], encoded by the *mecA* gene on the chromosomal mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec) [5]. Due to the possible association of MRSA with multiple antibiotic resistance that might result in relatively challenging and higher cost of treatment, the accurate and rapid identification of MRSA isolates is crucial for timely management of the infections caused by this superbug [6].

MRSA is frequently isolated from diabetic foot ulcers, and several studies from Iran and Saudi Arabia have detected 24.7% and 29.1% respectively from diabetic wounds [4, 7]. Infection/colonization with MRSA may result in prolonged hospitalizations and higher costs [7].

Overuse of antibiotics and selecting broad rather than narrow-spectrum agents have contributed to the high

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prevalence of MRSA colonization in diabetic foot wounds [8]. Many MRSA isolates are becoming multidrug-resistant; and are only susceptible to glycopeptide antibiotics such as vancomycin [8]. At present, there are reports of emerging low-level vancomycin resistance among MRSA strains [8]. MRSA has become a significant public health issue during the past decade, including diabetic foot infections of varying severity [9]. Therefore, knowledge of the prevalence of MRSA and its current antimicrobial profile has become necessary to select the appropriate empirical treatment for diabetic foot infections.

In this study, we aimed to determine the prevalence of MRSA in diabetic patients with foot ulcers. We also examined *in vitro* susceptibility pattern of isolates to various antibacterial agents.

MATERIALS AND METHODS

Study area. This study was performed at Department of Medical Laboratory Science in the College of Medicine and Health Sciences (CMHS), Abia State University, Uturu, Nigeria, from April 2016 to May 2017. A convenient sampling method was used to include patients who gave their consent and presented with diabetic foot ulcers. Diabetic patients with traumatic ulcers due to car accidents and other incidents were included in the study. Finally, 204 patients with diabetic foot ulcers, who filled out the questionnaires, were included in this study.

Sample size determination. The minimum sample size (n=217) for MRSA prevalence in diabetic patients with foot ulcers was calculated as described elsewhere [10].

Ethical clearance. Ethical permission (code No. ABSUTH/CS/66/VOL 4/58) was obtained from the hospital authorities, and patients consented to participate in the study.

Isolation and identification of *Staphylococcus aureus*. The patients' demographic data were obtained using a predesigned questionnaire. Samples were collected from the ulcers using a sterile swab stick and inoculated on blood agar and Mannitol salt agar (HiMedia, India) and DNase agar media (Oxoid Limited, UK), and incubated aerobically 48 h at 37 ºC. According to standard bacteriological procedures, characteristic *S. aureus* colonies were further identified by Gram staining, catalase, and coagulase testing [11].

Biochemical identification of *Staphylococcus aureus.* The isolates were culture on Mannitol Salt Agar (MSA) for 24 h. Smooth circular colonies with the yellow color indicated a positive result for *S. aureus* [11].

Detection of MRSA. MRSA identification was performed using oxacillin screen plates following the guidelines of CLSI. Briefly, a suspension equivalent to 0.5 McFarland standards, prepared from each isolate, was inoculated homogenously on the entire surface of the Mueller-Hinton agar plate (Oxoid-UK) containing 4% NaCl and 6µg/mL oxacillin using sterile swabs, and incubated at 35 ºC for 24 h. The appearance of >1 colony indicated oxacillin/methicillin-resistant isolates [11].

Antimicrobial susceptibility testing. The antimicrobial susceptibility testing was performed by the modified Kirby-Baver disc diffusion method using Mueller Hinton agar (HiMedia, India) following the Clinical Laboratory Standard Institute (CLSI) standards [11]. Antibiotic discs including gentamicin (10µg), chloramphenicol (30µg), cotrimoxazole (5µg), tetracycline (30µg), penicillin (25µg), ceftriaxone (5µg), ciprofloxacin (5µg), erythromycin (15µg), vancomycin (10µg), oxacillin (5µg) and augmentin (5 µg) (Oxoid Ltd., Basingstoke, Hampshire, UK) were used in the assay.

Identification of multidrug resistance (MDR) isolates. In this study, the multidrug resistance (MDR) isolates were identified by observing the resistance pattern to at least three antibiotics belonging to at least two antibiotics classes, namely β-lactams, aminoglycosides, and quinolones [11].

Determination of multiple antibiotics resistance (MAR) index. Multiple antibiotic resistance (MAR) index was determined for each selected isolate by dividing the number of antibiotics to which the isolate showed resistance to the total number of antibiotics to which the isolates were exposed. Thus, MAR index = a/b, where (a) represents the number of antibiotics to which the isolates were resistant, and (b) the number of antibiotics to which the isolates were exposed [11].

Quality control. For quality control, *S. aureus* ATCC 29213, obtained from the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos State, was used as a reference strain for the standardization of antibiotic susceptibility testing. The qualities of biochemical test procedures were checked by this reference strain.

Statistical analysis

The generated data were analyzed using the statistical package for social sciences (SPSS) software, version 21.0. Proportions of categorical variables were compared by Chi-square, and $P < 0.05$ was considered statistically significant.

RESULTS

Socio-demographic characteristics of the study population. Two hundred seventeen patients were recruited in the study, and 204 (94%) questionnaires were completed and returned. Among 204 wound swab specimens processed during the study, 134 (65.7%) had a bacterial infection, while 70 (34.3%) were negative.

MSRA prevalence. The prevalence of MRSA in DFU patients was 45 (22.1%). Males were more infected (12.7%, n=26) with MRSA than females (9.3%, n=19), which was not statistically significant (P=0.14). The age

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group 41-60 years exhibited the highest MRSA rate (13.2%, n=27), while the lowest frequency was in the age group 0-20 years (0%, n=0), statistically significant (P = 0.02). Farmers had the highest MRSA prevalence (9.8%, n=20), while homemakers showed the least prevalence (0.5%, n=1), which was not statistically significant (P = 0.07).

Table 1. Demographic of DEU patients with MRSA infections.

Characteristics	No. of samples from foot Ulcers (%)	No. of negative samples (%)	No. of positive samples (%)	No. infected with *S. aureus* (%)	No. infected with MRSA (%)	*P*-Value
SEX						
Male	154 (75.5)	54 (26.5)	100 (49.1)	71 (34.8)	26 (12.7)	P = 0.14
Female	50 (24.5)	16 (7.8)	34 (16.7)	28 (13.7)	19 (9.3)	
Total	204 (100)	70 (34.3)	134 (65.7)	99 (48.5)	45 (22.1)	
AGE IN YEARS						
0-20	0	0	0	0	0	P =0.02
21-40	6 (2.9)	3 (1.5)	3 (1.5)	2 (1.0)	1 (0.5)	
41-60	103 (50.5)	26 (12.7)	77 (37.7)	60 (29.4)	27 (13.2)	
61-80	84 (41.2)	36 (17.6)	48 (23.5)	33 (16.2)	27 (13.2)	
> 80	11 (5.4)	5 (2.5)	6 (2.9)	4 (2.0)	2 (1.0)	
Total	204 (5.4)	70 (34.3)	134 (65.7)	99 (48.5)	45 (22.1)	
OCCUPATIONAL STATUS						
Farmers	84 (41.2)	24 (11.8)	60 (29.4)	54 (26.5)	20 (9.8)	P = 0.07
Traders	18 (8.8)	9 (4.4)	9 (4.4)	5 (2.5)	2 (1.0)	
Civil servants	29 (14.2)	13 (6.4)	16 (7.8)	7 (3.4)	6 (2.9)	
Housewives	8 (3.9)	3 (1.5)	5 (2.5)	3 (1.5)	1(0.5)	
Artisans	65 (31.9)	21 (10.3)	44 (21.6)	30 (14.7)	16 (7.8)	
Total	204 (5.4)	70 (34.3)	134 (65.7)	99 (48.5)	45 (22.1)	

Antibacterial sensitivity pattern of MRSA. The MRSA isolates were 100% sensitive to vancomycin and chloramphenicol and 90.9% sensitive to gentamicin. They were 100% resistant to penicillin, ceftriaxone, oxacillin, 81.8% to ciprofloxacin, and 68.2% to cotrimoxazole (Table 2).

Table 2. Antibiotic sensitivity profile of MRSA isolated from patients with foot **ulcers**

Antibiotics	Sensitive	Resistant
	n (%)	n (%)
Co-trimoxazole (COT)	14 (31.8)	30 (68.2)
Gentamicin (GEN)	40 (90.9)	4 (9.1)
Augumentin (AUG)	37 (84.1)	7 (15.9)
Ciprofloxacin (CIP)	8 (18.2)	36 (81.8)
Penicillin (P)	0	44 (100)
Tetracycline (TET)	16 (36.4)	28 (63.6)
Ceftriaxone (CEF)	0	44 (100)
Oxacillin (OX)	0	44 (100)
Chloramphenicol (CHL)	44 (100)	0
Erythromycin (ERY)	10 (22.7)	14 (31.8)
Vancomycin (VAN)	44 (100)	00

KEY: MRSA= Methicillin-Resistant *Staphylococcus aureus*

DISCUSSION

The present study shows that the prevalence of MRSA in DFU patients was 22.1%, and MRSA was higher in males than females, with maximum infection among the age group 41-60, who are actively working groups. A similar study in Riyadh, Saudi Arabia, reported a higher prevalence of MRSA in males (74.1%) than females, 25.9% [7]. Omuse *et al.* (2012) also reported a higher percentage of MRSA in males (58%) than in females (42%) in Kenya [9]. The reason might be because men within that age group 41-60 years are breadwinners and are involved in more activities like farming and technical works that predispose them to injuries [9].

The high prevalence of DFU and MRSA in farmers may be due to the high sampling values of patients who were farmers [12-13]. Previously, an MRSA prevalence of 15-30% in DFU patients was reported, of which 45% belonged to farmers [14]. Another study reported a 42.86% prevalence of MRSA from Coimbatore, Tamil Nadu, India [21].

In our study, age played a significant role in MRSA prevalence in DFU patients (P<0.02). The high prevalence in patients within the age group 41-60, primarily male, indicates that MRSA-infected diabetic foot ulcers are the disease of middle and old age. In Abia State, where this study was performed, the 41-60 years age group comprises active working groups, primarily farmers. These farmers are prone to machete and hoe cut on the feet, which quickly develops into foot ulcers, as seen in this study. This finding agrees with a similar result in Korea, where males ≤ 65 years had the highest prevalence of MRSA of 81(15.0%) [16].

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Interestingly, higher sensitivity observed for gentamicin (90.9%) has also been reported by other authors [9, 19]. However, other authors have reported a considerably higher frequency of gentamicin resistance in Northeast Ethiopia [20]. The explanation for the observed variability may be linked to the variations in antibiotic prescription practices between countries and regions [20].

In our study, resistance to cotrimoxazole was (68.2%); this antibiotic is inexpensive and available in Nigeria, and because of its broad activity spectrum, it is prescribed for different infections [17]. This finding was almost similar to that in Coimbatore, Tamil Nadu, India, where MRSA isolates showed 82.76% resistance to cotrimoxazole [21]; the increased resistance to cotrimoxazole was attributed to the indiscriminate usage of this drug. Likewise, the high resistance rate of MRSA isolates to ciprofloxacin (81.8%) in our study is consistent with a similar study in Coimbatore, Tamil Nadu, India [21].

The MRSA isolates in our study were 100% sensitive to vancomycin and chloramphenicol and 90.9% to gentamicin but 100% resistant to penicillin, ceftriaxone, and oxacillin, consistent with results in other studies [17-18]. As indicated in this study, vancomycin has been the drug of choice for MRSA infections for the past two decades [18]. Recent reports have described MRSA in glycopeptide-intermediate *Staphylococcus aureus* (GISA) strains associated with vancomycin therapeutic failure. Such MSRA strains have also been reported in India and Ethiopia [8, 19]. The MRSA isolates that were intermediately resistant to glycopeptides from India and Ethiopia appear to have developed from preexisting MRSA infections [20].

The isolation of such strains from several parts of the world suggests that GISA is emerging worldwide, portending MRSA strains emergence for which no effective therapy is available, a situation similar to the pre-antibiotic era [18]. In this study, none of the isolates proved to be vancomycin-resistant. Similar to the study in Ido-Ekiti, Nigeria [17], the high multidrug-resistant index (MAR) in our study shows a possibility of misuse and abuse of antibiotics among the populace even before they come to the hospital.

In conclusion, the high prevalence of MRSA among DFU patients in a tertiary hospital in Abia State and the alarming rate of drug-resistant bacteria calls for collaborative efforts from all stakeholders to prevent drug resistance in similar settings. In addition, these findings will help assess the appropriate empirical antibiotics regimen for diabetic foot ulcers infected with MRSA to shorten hospital stays and reduce cost.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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