IBOM MEDICAL JOURNAL
Vol.17 No.1 | January - April, 2024 | Pages 56 - 61
www.ibommedicaljournal.org

Occurrence of high level methicillin resistance *Staphylococcus aureus* in patients from health facilities in Akwa Ibom State, Nigeria

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Abstract

Background: Methicillin resistant *S. aureus* (MRSA) has become a major public health predicament worldwide. This is owing to its involvement in the evolution of MDR strains and difficulty in therapeutic management of infected patients. This study was conducted to investigate the prevalence of methicillin resistance *Staphylococcus aureus* among patients in two health facilities in Akwa Ibom State, Nigeria.
Materials and Methods: Clinical isolates of patients from University of Uyo Teaching Hospital (UUTH), Uyo and General Hospital, Ikot Abasi (GHIA) were investigated based on the strategic location of the hospitals. The study design was a descriptive cross-sectional study. Three hundred clinical samples were collected from male and female in and out-patients of all ages and processed using standard bacteriological methods. Detection of *Staphylococcus aureus* and MRSA strains were done according to standard protocols while antibiotic susceptibility testing of MRSA isolates was conducted using Kirby-Bauer disc diffusion method and interpreted following the CLSI 2021 guidelines.
Results: The prevalence of MRSA strains in this study was 42.9%. Majority of patients with MRSA were from UUTH (44%) closely followed by patients from GHIA (40%). High antibiotics resistant rates of MRSA were recorded for ampicillin (96.6%), ciprofloxacin (73.3%), erythromycin (63.3%) and cotrimoxazole (60%). Gentamicin and ceftriaxone sensitivity rates were 53.3% and 63.4%, respectively.
Conclusion: Health facilities in the state should institute effective antimicrobial stewardship, intensify surveillance and screening of *Staphylococcus aureus* for MRSA strains to guard against dissemination of multidrug resistant strains in both hospital and community settings because of the clinical implications.

Keywords: Methicillin resistance, S. aureus, clinical samples, beta-lactams

Introduction

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) constitutes a serious public health challenge with concomitant increase in morbidity and mortality rates.[1] It is recognized as one of the most important causes of both acute and chronic cases of community-acquired and hospital-associated infections including urinary tract infections, blood stream infections, soft tissue infections and pneumonia.[2] The evolution and dissemination of MRSA is traceable to the introduction of methicillin in clinical world to treat infections caused by penicillinase-producing *Staphylococcus aureus* in 1959.[3] The high rate of methicillin resistance among *Staphylococcus aureus* isolates in clinical samples is associated with high rate of treatment and management failure.[4] This resulted in a renewed interest in the use of clindamycin for treatment of

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DOI: 10.61386/imj.v17i1.378

infections caused by *S. aureus*.[5] Due to several reports of very high number of *S. aureus* strains harboring resistance genes to clindamycin, there was urgent need to replace clindamycin with vancomycin as the drug of choice in the treatment of infections caused by MRSA.[6] Unfortunately, the report of emergence of vancomycin intermediate sensitive *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) in clinical samples impacted negatively on the efficacy of vancomycin leading to treatment failures.[6] MRSA does not only resist beta-lactam classes of antibiotics such as cephalosporins and carbapenems alone but also pose a higher risk of developing resistance to other non beta-lactams such as quinolones, aminoglycosides, and macrolides.[7,8] Methicillin-resistant *Staphylococcus aureus* has been reported with alarming frequencies worldwide, and the reported strains exhibit multidrug resistance.[8]

Methicillin-resistant *Staphylococcus aureus* produces a variety of virulent factors that cause diseases of varying severity in patients treated in hospitals.[9] It is implicated in skin infections, osteoarthritis and respiratory tract infections. Methicillin-resistant *Staphylococcus aureus* causes abscess in deep organs and is responsible for toxin - mediated infections. Infections caused by methicillin-resistant *Staphylococcus aureus* have been reported to be responsible for increased length of hospital stays, rising health care costs and a high mortality rate. MRSA carriers are also prone to septicaemia, wound infections and occasional toxic shock syndrome.[10,11] Methicillin-resistant *Staphylococcus aureus* is highly prevalent in hospitals worldwide and prevalence rates >50% have been reported in Asia, Malta, North and South America and Scotland[12,13] while prevalence rates 20% - 50% have been reported in Japan, 44.4%; Singapore, 35% and Malaysia, 26% by Matheson et al.[12] The prevalence of MRSA in Awka, Anambra State, Nigeria was reported to be 22.6%,[14] 19.2% in Ekiti State, Nigeria[12] and Ohagim et al.[15] reported 11% in Uyo, Akwa Ibom State Nigeria. In Akwa Ibom State, the situation in tertiary and secondary health facilities has not been reported. This study was carried out to investigate the prevalence of methicillin resistance *Staphylococcus aureus* among patients attending two health facilities (tertiary and secondary) in Akwa Ibom State, Nigeria.

Materials and Methods

Study design: This was a cross sectional hospital-based study involving 300 patients of all ages with suspected cases of ear, wound and urinary tract infections at both tertiary (UUTH) and secondary (GHIA) healthcare facilities in Akwa Ibom State. The study was approved by the Health Ethics Committee of the State Ministry of Health and the University of Uyo Teaching Hospital, Uyo.

Sample collection and processing: The midstream urine, HVS, aspirate, throat swab, sputum, semen/urethral swab, pus, blood, eye swab, ear swab, and wound swab samples were aseptically collected and processed using standard microbiological methods. Gram staining reactions and biochemical tests such as catalase, coagulase, and DNase tests were conducted to identify the isolates.

Detection of methicillin resistant *Staphylococcus aureus* (MRSA) strains using disc diffusion method: The 24 hr cultured colonies of *S. aureus* isolates were subcultured onto peptone water and incubated for 3 hr. at 37°C. The broth culture was diluted to 0.5 McFarland turbidity standard equivalents. Sterile swab stick was used to inoculate the test organisms unto Mueller Hinton Agar plates. Sterile forceps were used to carefully distribute oxacillin (1 µg) and cefoxitin (30µg) discs evenly on to the inoculated plates at a distance of 30 mm. The plates were kept on the bench for 10 minutes to allow pre-diffusion of the antibiotics, these was inverted and incubated aerobically at 37°C for 18 – 24 hr. The zones of inhibition were measured using a meter rule and compared with Clinical and Laboratory Standard Institute guidelines (CLSI) guidelines. An inhibition zone of <11 mm to oxacillin and ≤21 mm to cefoxitin indicated methicillin-resistance.[16]

Antimicrobial susceptibility testing: Antibiotic susceptibility testing for MRSA isolates was carried out using Kirby- Bauer disc diffusion method on Mueller-Hinton agar (MHA) plates. The isolates were grown in nutrient broth at 37°C for 24 hr. Two loopful (0.08 ml) of the suspension of each isolate, standardized by matching with 0.5 McFarland turbidity standard was inoculated onto a 90 mm diameter Petri dish containing already prepared MHA. The plates were allowed to set before

aseptically placing on their surfaces the following antibiotic sensitivity discs: ceftriaxone (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (1.25 µg/23.75 µg) and gentamicin (10 µg) (Oxoid, UK). The plates were incubated at 30°C for 24 hr. and resultant inhibition zone diameter was interpreted in accordance with the CLSI guidelines.[16] The reference strain *Staphylococcus aureus* ATCC 25923 was used for quality control. Isolates that were resistant to three or more antimicrobial classes were regarded as multi-drug resistant *S. aureus*.

Table 1: Frequency distribution of *S. aureus* and MRSA isolates in clinical samples from UUTH and GHIA

Facility	No. screened	*S. aureus* (%)	MRSA (%
UUTH	200	50(25.0%)	22(44.0%)
GHIA	100	20(20.0%)	8(40.0%)
Total	300	70(23.3%)	30(42.9%)

Key: UUTH – University of Uyo Teaching Hospital; GHIA – General Hospital IkotAbasi

Table 2: Distribution of MRSA and MSSA according to type of clinical samples in the selected Health facilities in AkwaIbom State

Type of Specimen	*S. aureus*	MSSA (%)	MRSA (%)
Wound Swab	21	9(42.9)	12(57.1)
Urine	22	17(77.3)	5(22.7)
Blood	6	4(66.6)	2(33.3)
Eye Swab	1	1(100.0)	-
Ear Swab	2	1(50.0)	1(50.0)
Aspirate	3	1(33.3)	2(66.7)
Throat Swab	3	1(33.3)	2(66.7)
Sputum	4	3(75.0)	1(25.0)
Semen/Urethral swab	2	1(50)	1(50.0)
Pus	4	1(25.0)	3(75.0)
HVS	2	1(50.0)	1(50.0)
Total	70	40(57.1)	30(42.9)

Table 3: Antimicrobial susceptibility pattern of MRSA and MSSA strains

Antibiotic	MRSA (n = 30)			MSSA (n = 40)		
(µg/ml)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
Ceftriaxone (30)	11(36.6)	14(46.7)	5(16.7)	2(5.0)	15(37.5)	20(50.0)
Ampicillin (10)	-	29(96.6)	2(6.70	25(62.5)	-	1(2.5)
Gentamicin (10)	14(46.7)	1(3.3)	12(40.0)	5(12.5)	2(5.0)	20(50.0)
Erythromycin (15)	5(1.7)	19(63.3)	6(20.0)	15(37.5)	7(17.5)	5(37.5)
Sulfamethoxazole/ Trimethoprim (1.25/23.75)	5(16.7)	18(60)	11(36.7)	12(30.0)	5(12.5)	20(50.0)
Ciprofloxacin (5)	5(16.7)	22(73.3)	3(10.0	17(42.5)	5(12.5)	17(4.5)

Results

The distribution of *S. aureus* and MRSA infections among patients in the two health facilities is presented in Table 1. Generally, out of the 300 clinical samples screened, 70 (23.3%) yielded *S. aureus* of which 30 (42.9%) were MRSA strains. For facility specific results, 50 (25%) out of the 200 patient samples from University of Uyo Teaching Hospital (UUTH) were *S. aureus* of which MRSA strain prevalence was 22 (44%). At General Hospital Ikot Abasi (GHIA), 20 (20.0%) of the 100 samples were *S. aureus* of which MRSA prevalence was 8 (40%). However, the overall prevalence of MRSA infection in the study population was 10% (30/300).

The distribution of MRSA and MSSA isolates by clinical samples are indicated in Table 2. A total of 30 (42.9%) MRSA and 40 (57.1%) MSSA strains were detected in the study. The major sources of MRSA were from pus (75%), throat swab and aspirates (67.7%) each, and wound swab (57.1%) compared to urine and sputum samples (22.7%) and (25.0%), respectively. No MRSA was isolated from the eye swab samples. Hence, majority of MSSA strains were from eye swab (100%), urine (77.3%) and sputum (75%).

The antimicrobial resistance pattern of the MRSA and MSSA strains from the health facilities are shown in Table 3. MRSA strains exhibited high antimicrobial resistance to ampicillin (96.6%), ciprofloxacin (73.3%), erythromycin (63.3%), cotrimoxazole (60%), while low resistance was observed with gentamicin (46.7%) and ceftriaxone (36.6%). Gentamicin (40%) and trimethoprim/sulfamethoxazole (36.7%) were intermediate sensitive to MRSA strains. MSSA strains were mostly sensitive to ampicillin (62.5%) while ceftriaxone was the least sensitive (5.0%).

Discussion

The MRSA strains exhibit multidrug resistance to commonly used antibiotic regimens in Nigeria and continues to remain an important factor contributing to failure of therapeutic management of infected patients.[17] Understanding the epidemiology of MRSA and its resistance profile is imperative for selection of the appropriate empirical antimicrobial treatment of infections. In this study, the prevalence of *S. aureus* in patients from University of Uyo

Teaching Hospital (UUTH) and General Hospital Ikot Abasi (GHIA) were 20% and 25%, respectively, giving overall prevalence of 23.3%. This result is a little higher than the 15.3% prevalence of *S. aureus* isolates obtained from clinical samples collected from secondary and tertiary health facilities in Ibadan, Southwestern Nigeria by Adetayo et al.[18] The observed difference in the prevalence rate may be attributed to variation in study samples and location.

In this study the overall prevalence of MRSA from patients was 42.9%. Patients from the tertiary health facility (UUTH, Uyo) had a higher prevalence rate (44%) than those the secondary health facility in Ikot Abasi, Akwa Ibom State (40%). This result falls in line with the range reported by Alli et al.[19] in Ibadan, Ille-Ife and Osogbo cities, South west Nigeria with a prevalence of 42.3%, Yusuf et al.[20] in Benin City South South Nigeria with a prevalence of 42.7% and Ike et al.[21] in Jos City, North central Nigeria with a prevalence of 43%. About half a decade ago, Moses et al. (2017) reported MRSA prevalence of 40% among surgical patients in UUTH, Uyo.[22] This indicates an increase in methicillin resistance *S. aureus* among patients at UUTH, and a cause for concern to healthcare practitioners in therapeutic management of patients. The result is slightly low compared to the report of 53.7% by Ugwu et al.[23] as well as the 79% recorded in Benin South South Nigeria by Okwu et al.[24] However, much lower prevalence rates have been reported in different parts of the country. For instance, Okon et al.[25] reported a prevalence of 12.5% in North East, Shittu et al.[26] in Maiduguri, North East and (Ado-Ekiti, Ille Ife, Oshogbo, Lagos and Ibadan), South west reported 16.5% prevalence MRSA. The observed difference may not be unconnected with the fact that MRSA prevalence rates are known to vary with geographical location, the population of study as well as the method of detection used.

Among the MRSA strains isolated from various clinical samples in this study, the highest (75%) was obtained from pus samples. This finding is in line with the reports of Eyob et al.[27] who reported (71.9%) from pus samples, the highest among other clinical isolates in a multi-centered study by Nsofor et al.[28] in Aba, Southeast Nigeria who reported a prevalence of 61.5% from pus. The high prevalence of MRSA in pus could be attributed to poor personal hygiene and exposure of wound to contamination and infection. People in the rural communities of Akwa Ibom State tend to treat their wounds with local herbs, self-medication or employ services of unqualified poorly trained personnel. They only seek medical attention when situation becomes critical. These practices could expose them to the vulnerability of acquiring MRSA infection.

Antibiotic resistance has become a major health concern in the 21st Century. In this study, a high rate of MRSA resistance to ampicillin (96.9%), ciprofloxacin (73.3%), erythromycin (63.3%), sulphamethaxazole/trimethoprim (60%), was recorded which makes these antibiotics unreliable for the treatment of infections caused by MRSA. This necessitates immediate institution and review of antimicrobial stewardship and surveillance policies for effective control of MRSA resistance in clinical settings. However, gentamicin and ceftriaxone were promising alternatives with increased susceptibility rate efficacy of 46.7% and 36.6%, respectively. This is consistent with reports from previous studies.[15,29] The possible reason for high resistance of MRSA isolates to ampicillin, ciprofloxacin, erythromycin and sulphamethaxazole/trimethoprim in this study may be due to the prevailing practice of indiscriminate use of antibiotics and inadequate laboratory guidance before prescribing or administering antimicrobial therapy.[30] It is very common to find people in our environment, purchasing over-the-counter mostly from patent medicine shops without prescription by a physician. Some of these antibiotics may be substandard despite efforts by National Agency for Food and Drug Administration and Control (NAFDAC) to regulate and control drugs and allied products in Nigeria. These acts need to be checked to avoid undesirable consequences of widespread of pan-resistance genes among persons in Akwa Ibom State.

Conclusion

The prevalence of MRSA among clinical isolates of infected patients from Uyo and Ikot Abasi is relatively high, particularly among patients in UUTH, being a referral hospital. Drugs such as erythromycin, ampicillin, ciprofloxacin and sulphamethaxazole/trimethoprim (cotrimoxazole)

commonly prescribed by clinicians were observed to have reduced therapeutic value against MRSA isolates except for drugs such as gentamicin and ceftriaxone that could be used as alternative therapy for the treatment of MRSA infections in Akwa Ibom State. Both phenotypic (using oxacillin, 1 µg and cefoxitin, 30 µg combined disc diffusion method) and sensitivity methods or either could be used for routine diagnosis of MRSA, if standard phenotypic procedure is strictly followed. The high resistance of MRSA isolates to erythromycin, ciprofloxacin and ceftriaxone portrays the compelling rationale of application and adherence to infection control measures that aim to reduce spread of infection by MRSA especially among susceptible individuals. In view of this, early screening for MRSA using a combination of phenotypic and sensitivity methods, is recommended for the diagnosis of MRSA in colonized patients. However, the use of ceftriaxone and gentamicin antibiotics by clinicians for empirical treatment of patients with MRSA infections in Akwa Ibom State is recommended.

Acknowledgement
The authors wish to acknowledge the technical assistance of Dr. Nseobong G. Akpan of Institute for Biomedical Research and Innovations, University of Uyo in the course of conducting this study.

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