Research Article

The role of methicillin-resistant Staphylococcus aureus in clinical infections in Obafemi Awolowo university teaching hospitals complex, Ile-Ife, South Western Nigeria

Abstract

This study determined the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) from 246 clinical samples collected at a tertiary care hospital, its antimicrobial susceptibility, spectrum of infections and the associated risk factors. Standard procedures were used for isolation, screening, and susceptibility testing. The result showed that 41 (40.2%) out of 102 *S. aureus* isolated were methicillin-resistant while 61 (59.8%) were methicillin-sensitive. The prevalence rate of MRSA for male and female group was 37.3% and 43.1% respectively. Thirty (73.2%) methicillin-resistant *S. aureus* isolates were obtained from inpatients while 11 (26.8%) were from outpatients. MRSA were significantly isolated from the Orthopaedic ward (OR=3.36; P=0.031) and the antenatal ward (OR=8.33; P=0.037). High resistance rates were exhibited by MRSA isolates to cefotaxime (102, 79.4%) and clindamycin (102, 49%) but had low resistance rates to gentamicin (102, 27.5%) and chloramphenicol (n=102, 28.4%). MRSA were more significantly resistant to gentamicin (χ^2=12.284; p=0.0001), clindamycin (χ^2=20.234; p=0.0001) and cefotaxime (χ^2=13.812; p=0.0002) than MSSA. Thirty-two (78%) out of 41 MRSA isolated were multidrug resistant. All isolates (MRSA and MSSA) were susceptible to vancomycin with MIC values ranging from 2.1 to 0.12µg/ml. This study showed a high prevalence of MRSA in clinical infections that were resistant to treatment options in OAUTHC, Ile-Ife. Being in the antenatal and orthopaedic wards is a predictor of MRSA infections in the hospital. In spite of reports that vancomycin resistant *Staphylococcus aureus* is increasing, it is yet to be a problem in the hospital and vancomycin remains a drug of choice for the treatment of MRSA and multidrug resistant *Staphylococcus aureus* infections.

Keywords: *Staphylococcus aureus*, methicillin resistance, nosocomial infections, vancomycin

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Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus;* CDC, center for disease control and prevention; OAUTHC, Obafemi Awolowo university teaching hospitals complex; STI, sexually transmitted infections; GOPD, general out-patient department; ENT, ear, nose and throat; TSA, tryptone soya agar; CLSI, clinical laboratory and standard institute; MIC, minimum inhibitory concentration; VRSA, vancomycin-resistant *Staphylococcus aureus*

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major problem worldwide causing hospital acquired infections and infections in the community.[1] Healthy people may carry MRSA asymptomatically for long periods of time but patients with compromised immune system are at a significant greater risk of symptomatic infections. The spectrum of infections due to MRSA are manifold[2,3] and are associated with worse outcome in addition to prolonged hospital stay, higher cost of treatment and increased mortality.[4,5] MRSA prevalence varies with geographical location, type of hospital and the population studied. The Center for Disease Control and Prevention (CDC) reported that MRSA is the most frequently identified antimicrobial drug-resistant pathogen in the US causing about 10% of all *Staphylococcus* infections and more than 50% of health-care associated *Staphylococcus* infections.[6] By 2012, the percentage has increased to more than 34% of clinical isolates of *S. aureus* while in Europe 26% was reported, and 45% in the western Pacific.[7]

In Nigeria and many developing countries of Africa, studies have shown that the incidence of MRSA has been on the increase[8–13] thereby increasing concerns because of the linked increase in morbidity and mortality rates. A previous study on the carriage of MRSA in Ile-Ife reported a 50% carrier rate of MRSA in the nasal cavity and hands of hospital Staff.[14] This raises serious concerns about the possibility of transmission of MRSA outside the health care system and risk to patients. There is still paucity of current information on MRSA in patients in Africa and treatment options available compared to the developed world. Therefore, this study was designed to focus on patients to determine the prevalence of MRSA, associated risk factors and antimicrobial susceptibility profiles of MRSA isolates.

Materials and methods

Study population

This study was carried out at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, south west of Nigeria. Ethical approval for the study was sought and obtained from the Ethics and Research Committee of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife.

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Sample and data collection

Two hundred and forty-six (246) clinical samples were collected between the months of August 2012 and February 2013 from both the outpatient clinics and the in-patient wards of the hospital. The various wards/clinics included in the study were the Medical (male and female), Surgical (male and female), Intensive Care Unit (I.C.U.), Orthopaedic, Children, Accident/Emergency, Antenatal, Surgical outpatient, Neonatal, Sexually Transmitted Infections (STI) clinic, General Out-Patient Department (GOPD) and Ear, Nose and Throat (ENT) clinic. The clinical samples included urine, blood, semen, urethral swab, high vaginal swab, endocervical swab, wound swabs and wound biopsy, pus and wound exudate, ascitic fluid, catheter tips, sputum and stool. All were transported to the medical microbiology and parasitology laboratory in sterile containers for processing. Patients data consisted of age, gender, ward and diagnosis were collected using patients data card.

Isolation and Identification of Staphylococcus aureus strains

Each specimen was inoculated on Mannitol Salt Agar (Oxoid, England) and incubated aerobically at 37°C for 24hours. Tiny yellowish colonies were picked and subcultured to nutrient agar (Oxoid, England), incubated aerobically at 37°C overnight and observed for morphological and cultural characteristics. All colonies typical of *S. aureus* morphologically were Gram stained. Additional confirmatory tests that included catalase test, coagulase tests and DNase test were done for all the isolates using standard microbiological methods. All isolates that were Gram-positive cocci, catalase, coagulase and DNase positive were considered as *S. aureus*. Representative and well separated colonies confirmed to be *S. aureus* were picked and stored in Tryptone Soya Agar (TSA) (Oxoid, England) for further investigation.

Screening for methicillin resistance

All isolates of *S. aureus* were screened for methicillin resistance using cefoxitin by disc diffusion method according to CLSI guidelines. The diameter of zone of inhibition of each isolate was measured and resistance was estimated by comparing with Clinical Laboratory and Standard Institute (CLSI) interpretative chart.[15]

Antibiotic susceptibility testing

Antimicrobial susceptibility testing for the isolates was performed by the Kirby-Bauer disc diffusion technique on Mueller Hinton agar (CM0337) (Oxoid, England) according to the guidelines set by CLSI (CLSI, 2009). Six antimicrobial agents which included oxacillin (OX) (1μg), linezolid (LZD) (30μg), chloramphenicol (C) (30μg), gentamicin (CN) (30μg), clindamycin (DA) (2μg) and cefotaxime (30μg) were tested against each isolate of *S. aureus*. The zones produced by each antibiotic were measured using a ruler to the nearest millimeter according to Clinical Laboratory and Standard Institute reference values.[15]

Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration (MIC) of the ten multi drug resistant MRSA isolates that showed intermediate results for vancomycin by disc diffusion tests was determined using the MIC-Evaluation test kit according to CLSI guidelines.[15]

Statistical analysis

The Chi square (χ^2) test, Fisher's exact test and odd ratio (OR) (two tailed) were performed using SPSS statistical software (version 15) (Chicago, IL, USA) to determine the statistical significance of the data. All reported p-values were two-sided and a p-value of less than or equal to 0.05 was considered to be statistically significant

Results

Staphylococcus aureus isolated from different wards and specimens

A total of 102 *S. aureus* strains were isolated and characterised from 246 clinical specimens. The highest number of *S. aureus* isolated was from the GOPD (23, 22.5%), followed by the surgical outpatient clinic (19, 18.6%) and the orthopaedic ward (17, 16.7%). Specimens from the neonatal ward and sexually transmitted infection (STI) clinic yielded the lowest frequency of *S. aureus* with 1 (0.9%) and 2 (2%) respectively (Table 1). Of all the 246 clinical specimens examined, wound swab yielded the highest frequency of *S. aureus* isolated 58 (56.9%) followed by 28 (27.5%). The lowest frequency of *S. aureus* isolated 1 (0.98%) was found in stool samples (Table 2).

Table 1 Distribution of S. aureus and MRSA from patients at the different wards/units

Wards	No of specimen (%)	S. aureus isolated (%)	MRSA (%)
	n=246	n=102	n=41
In-patient wards	183 (74.4)	73 (71.6)	30 (73.2)
Accident/ emergency	13 (5.6)	6 (5.9)	0 (0)
Medical wards	20 (8)	6 (5.9)	1 (2.4)
Surgical wards	9 (3.6)	6 (5.9)	0 (0)
Orthopaedic ward	18 (7.2)	17 (16.7)	11 (26.8)
Antenatal ward	31 (12.4)	6 (5.9)	5 (12.2)
Surgical outpatient clinic	61 (25)	19 (18.6)	9 (21.9)
Intensive care unit	12 (4.8)	8 (7.8)	3 (7.3)
Neonatal wards	5 (2)	1 (0.98)	1 (2.4)
Children ward	14 (5.7)	4 (3.9)	0 (0)
Outpatient clinics	**63 (25.6%)**	**29 (28.4)**	**11 (26.8)**
GOPD	55 (22)	23 (22.5)	10 (24.9)
STI	4 (1.6)	2 (1.96)	0 (0)
ENT	4 (1.6)	4 (3.9)	1 (2.4)

GOPD, general outpatient department; STI, sexually-transmitted infection clinic; ENT, ear nose and throat clinic

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Table 2 The Frequency of *S. aureus* and MRSA from different clinical specimens

Clinical Specimens	No of specimen (%)	*Staphylococcus aureus* (%)	MRSA (%)
	n=246	n=102	n=41
Urine	117 (47.6)	28 (27.5)	10 (24.4)
Stool	1 (0.41)	1 (0.98)	0 (0)
Wound swab	69 (28.0)	58 (56.9)	26 (63.4)
Aspirates/pus	12 (4.9)	4 (3.9)	2 (4.9)
Urogenital swabs	37 (15.0)	8 (7.8)	2 (4.9)
Sputum	1 (0.41)	1 (0.98)	0 (0)
Blood	9 (3.7)	2 (1.96)	1 (1.8)

Methicillin-resistant Staphylococcus aureus (MRSA) isolated

A total of 41 (40.2%) methicillin resistant *S. aureus* (MRSA) were detected from 102 *S. aureus* isolates tested. Thirty (73.2%) of the *S. aureus* that were methicillin-resistant were obtained from inpatients and considered hospital-associated while eleven (26.8%) were from outpatients (GOPD) and considered community-associated. The highest prevalence of MRSA was found in the orthopaedic ward 11 (26.8%) followed by the general outpatient department 10 (24.4%) and the surgical outpatient clinic 9 (22%) while low prevalence were found in the neonatal ward 1 (2.5%), medical ward 1 (2.5%) and ENT ward 1 (2.5%). No MRSA was isolated from the accident and emergency ward, children ward and the STI clinic (Table 1). As shown in Table 2, 26 (63.4%) MRSA was isolated from wound swabs, 10 (24.4%) from urine and two (4.9%) from aspirates/pus and urogenital swabs.

Risk factors associated with MRSA infections

Different risk factors were associated with MRSA infections. These included, age, gender, ward and diagnosis as shown in Table 3. Thirty-nine (41.5%) MRSA was isolated from adults and two (25%) from children. There was no significant difference in MRSA isolation between adults and children (χ^2=0.834; P=0.361). All the patients from whom MRSA were isolated were divided into six age categories. The highest isolation rate of MRSA was found in patients that were in the age range of 21-40 years while the lowest rate was seen in the patients that are below 20 years of age (Figure 1). Of a total of 41 MRSA isolated, nineteen (37.3%) were isolated from male and twenty-two (43.1%) from female. Thirty (41.1%) isolates were hospital-associated MRSA while eleven (37.9%) were community-associated methicillin-resistant *Staphylococcus aureus*. There was no significant difference in the isolation of MRSA between male and female (χ^2=0.367; P=0.545), and between samples from the hospital and the community (χ^2=0.086; P=0.769) (Table 3). Of all the wards from which MRSA was isolated in this study, MRSA was eight times likely to be isolated in antenatal ward (OR=8.33; CI=0.8-400.53; p=0.037) and three times likely to be isolated from orthopaedic ward (OR=3.36; CI=1.01-12.09; p=0.031) compared to the rest of the wards. No clinical diagnosis was significantly associated with MRSA infection (P>0.05).
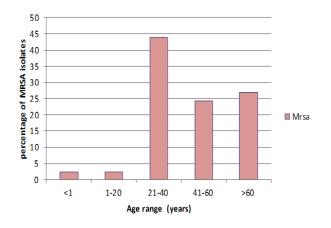

Figure 1 Age distribution of patients in whom MRSA were detected.

Table 3 Risk factors associated with MRSA infections

Risk factor	*Staphylococcus aureus* isolates (n=102)		
	MRSA (%)	MSSA (%)	P value
	n=41	n=61	
Age			
Adult	39 (41.5)	55 (58.5)	P= 0.20762*
Children	2 (25)	6 (75)	
Sex			
Male	19 (37.3)	32 (62.7)	χ^2 = 0.367; P = 0.545
Female	22 (43.1)	29 (56.9)	
Location			
Community	11 (37.9)	18 (62.1)	χ^2 = 0.086; P = 0.769
Hospital	30 (41.1)	43 (58.9)	
Wards			
Orthopaedic ward	11 (26.8)	6 (9.8)	O.R.=3.36; P=0.031; C.I.=1.01 to 12.09
Intensive care unit	3 (7.3)	5 (8.2)	O.R.= 0.88: P = 1.000; C.I. =0.13 to 4.86
Antenatal	5 (12.2)	1 (1.6)	O.R.=8.33; P = 0.037; C.I. = 0.8 to 400.53
SOP	9 (22)	10 (16.4)	O.R = 1.43: P = 0.605; C.I. = 0.46 to 4.40
GOPD	10 (24.4)	13 (21.3)	O.R.= 1.19; P = 0.810; C.I. = 0.41 to 3.36
Diagnosis			
Burn wounds	2 (4.9)	5 (8.2)	O.R.= 0.57; P = 0.699; C.I. = 0.05 to 3.75
Wounds/ ulcers/abcesses	7 (17.1)	13 (21.3)	O.R.= 0.76; P = 0.800;

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Table continued

Risk factor	*Staphylococcus aureus* isolates (n=102)		
	MRSA (%)	MSSA (%)	P value
	n=41	n=61	
Sepsis	5 (12.1)	2 (3.3)	C.I. = 0.23 to 2.32
			O.R.= 4.10; P = 0.114;
			C.I. = 0.62 to 44.53
Osteomyelitis	4 (9.8)	4 (6.6)	O.R = 1.54; P = 0.711;
			C.I. = 0.27 to 8.78
Surgical wound	8 (19.5)	6 (9.8)	O.R.= 2.22; P = 0.240;
			C.I. = 0.61 to 8.4

*Fisher's exact test; P≤0.05 is significant; χ^2, chi square; O.R, odd ratio; C.I, confidence interval

Antimicrobial resistance profiles of S. aureus and MRSA isolates

Among the 102 *S. aureus* isolated, high rates of resistance were recorded for cefotaxime (81, 79.4 %) and oxacillin (67, 65.7%). However, few isolates were resistant to linezolid 9 (8.8%) (Table 4). A comparison of antimicrobial resistance profiles of MRSA and MSSA isolates showed that MRSA isolates were more commonly resistant to gentamicin (p=0.0001), oxacillin (p=0.0001), and cefotaxime (p=0.0002) compared to MSSA isolates. Eleven (10.8%) isolates of *S. aureus* were resistant to one type of antibiotics, 27 (26.5%) were resistant to two types of antibiotics and 53 (52%) were multi-drug resistant (resistant to three or more different types of antimicrobial drugs). In addition, MRSA isolates were more commonly resistant to three or more types of antibiotics than MSSA isolates (p=0.0001) (Table 5).

Table 4 Comparison of antimicrobial resistance profiles of MRSA and MSSA isolates

Antimicrobial agents	*Staphylococcus aureus* isolates		Total	P value
	MRSA (%)	MSSA (%)		
	n=41	n=61	n=102	
Cefoxitin	41(100)	0 (0)	41 (40.2)	0.0001*
Oxacillin	40 (97.5)	27 (44.3)	67 (65.7)	0.0001
Linezolid	3 (7.31)	6 (9.84)	9 (8.8)	0.66
Chloramphenicol	16 (39)	13 (21.3)	29 (28.4)	0.052
Gentamicin	19 (46.3)	9 (14.8)	28 (27.5)	0.0001
Clindamycin	22 (53.7)	28 (45.9)	50 (49.0)	0.442
Cefotaxime	40 (97.5)	41 (67.2)	81 (79.4)	0.0002

*Pearson's chi square: P≤0.05 is significant

Table 5 Multi-drug resistance profiles of MRSA and MSSA to different number of antibiotics

No of antibiotics	No of resistant Isolates		Total	P value
	MRSA (%)	MSSA (%)		
	n=41	n= 61	n=102	
1	0 (0)	11 (18)	11 (10.8)	0.011*
2	9 (22)	18 (29.5)	27 (26.5)	0.396
3	13 (31.7)	10 (16.4)	23 (22.5)	0.07
4	9 (22)	6 (9.8)	15 (14.7)	0.09
5	5 (12.1)	2 (3.3)	7 (6.7)	0.081
6	4 (9.8)	2 (3.3)	6 (5.9)	0.35
7	1 (2.4)	2 (1.6)	3 (2.9)	1
>3	32 (78)	21 (34.4)	53 (52.0)	0.0001

*Pearson's chi square: P≤0.05 is significant.

Minimum inhibitory concentration

All the 10 MRSA isolates were susceptible to vancomycin (256-0.015µg/ml) with MIC values ranging from 2.1 to 0.12µg/ml.

Discussion

Results of this study indicated that the prevalence of hospital-associated MRSA isolates was 73.2%. Though the prevalence obtained in this study was higher than the prevalence of 11% reported in earlier studies in Nigeria by Shittu et al.,[16] in Ile-Ife, 28.6% by Nwankwo et al.,[17] in Kano, 34.7% by Taiwo et al.,[18] in Ilorin and 11% by Obasuyi[19] in Benin-city, this observed differences may be due to different population studied, the antibiotic consumption pattern and the periods when the studies were conducted. The same reasons can also be advanced for the studies reported by Jacobus et al.,[20] in USA 58%; Kateete et al.,[21] in Uganda 46% and Olowe et al.,[11] in Oshogbo 47.8% that reported higher prevalence value than we reported. Other plausible reasons for the observed differences in these studies include differences in the laboratory methods employed to screen MRSA as well as differences in sampling and culture techniques.[22]

MRSA were significantly isolated from patients in the orthopaedic wards. This may be because orthopaedic patients usually stay longer in the hospital while waiting for their fractures to heal before they are discharged. Although, a limitation of the present study is the inability to use molecular typing techniques to establish transmission of strain between patients and staff on one hand and the community on the other. However, we can safely speculate that such a transmission is likely ongoing as hospital staff has shown to be carriers of MRSA in significant numbers[14] and efficient transmitters through the hands when infection control principles are not strictly adhered to. Other authors who have reported different rates of MRSA in institutionalized settings in support of our findings include Fadeyi et al.,[23] that reported MRSA rate of 52.5% in a similar orthopaedic ward in a Nigerian hospital and Onanuga et al.,[10] who reported a high prevalence rate of MRSA among pregnant women in Abuja as we observed in the antenatal clinic in this study.

The assessment of the antimicrobial susceptibility profile of the MRSA and MSSA isolates showed high rates of resistance to clindamycin and cefotaxime. The rates of resistance were lower to

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chloramphenicol and gentamicin which was contrary to the high resistance of 58% reported by Kalsoom and Hameed in 2006.[24] A comparison of the multi-drug resistance profiles of MRSA and MSSA to different number of antibiotics showed no significant difference except to three or more antibiotics (p=0.0001) suggesting that MRSA isolates are more likely to be resistant to three or more antimicrobial agents than methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates. The presence of the *mec*A gene in MRSA is responsible for making MRSA isolates resistant to more antibiotics as compared to MSSA isolates.

All the ten multidrug *S. aureus* isolates were sensitive to vancomycin with values ranging from 0.12-2.1μg/ml which is comparable to findings of Kan.[25] This indicates that though reduced susceptibility of *Staphylococcus aureus* to vancomycin have been reported in Japan and USA, cases are still rare in Nigeria.[26] This study showed that MRSA and multidrug resistant strains of *S. aureus* in this setting are susceptible to vancomycin (which is the first line treatment of choice for MRSA). Decreasing vancomycin susceptibilities, increasing incidence of MRSA infections and emergence of vancomycin-intermediate and vancomycin-resistant strains leading to clinical failures have made it necessary to re-evaluate the options for the treatment of serious MRSA infections.[27] Consideration of alternative drugs for the treatment of MRSA infections is warranted when deciding upon appropriate antimicrobial therapy. Despite increasing reports of vancomycin-resistant *Staphylococcus aureus* (VRSA) worldwide, they are still very few reports in Nigeria.[26]

Conclusion

This study has established that MRSA is a major pathogen in both hospital based and community acquired infection in our setting. The implication is that it will be a major consideration of the decision process when therapeutic options are considered for a patient. More studies are clearly needed particularly in all regions of Africa particularly Nigeria and other developing poor-resourced areas to determine the current epidemiology outlook of this increasingly important nosocomial and community-acquired pathogen and the various risk factors that might be associated with MRSA infections.

Acknowledgments

Conflicts of interest

Authors declare that there is no conflict of interest.

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