# Methicillin Resistant Staphylococcus aureus (MRSA) in Hospitals of Sokoto Metropolis - A Multicentre Surveillance and Review of Literature

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# ABSTRACT

**Background**: Epidemiologic surveillance is an indispensible tool in understanding the clonal nature and evolution of pathogens. From a previously unstudied region we conducted a study on methicillin-resistant *Staphylococcus aureus* (MRSA) from Sokoto, Nigeria. This research was conducted to study the prevalence of methicillin-resistance in clinical isolates of *Staphylococcus aureus* among patients attending some selected hospitals in Sokoto metropolis.

**Methods**: A total of 936 non-repetitive clinical specimens from patients attending three selected hospitals were processed during the study period. Specimens were cultured and isolates identified using standard bacteriologic methods. Methicillin resistance was determined in parallel using  $30\mu g$  cefoxitin disk and Brilliance<sup>TM</sup> MRSA 2 Agar.

**Results**: From the 936 specimens analysed, 367(39.2%) staphylococci were isolated. Of these isolates, 234(25%) were identified as *S. aureus* using standard bacteriologic techniques. The prevalence of MRSA was found to be 43.2% (101/234). The distribution of MRSA by study centre shows that there was no significant difference (p=0.282) in the prevalence of MRSA among the 3 hospitals. Ear swabs had the highest prevalence of MRSA of 75\%. The prevalence of MRSA was significantly higher in females, similarly, the prevalence was found to be significantly higher in in-patients (55.8% vs. 25%) than out-patients (p=0.000).

**Conclusions**: The study has established, as a baseline study, the prevalence of MRSA in healthcare centres within the study area at 43.2%. The female gender and hospitalisation were found to be significant risk factors for infection with MRSA.

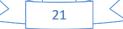
Key Words: Staphylococcus aureus, MRSA, Cefoxitin, Brilliance™ MRSA 2

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**Author's contributions**: This work was carried out and approved in collaboration between all the authors. AMG designed the study; AMG sourced for funding; SBM wrote the protocol; SBM contributed in literature search; AMG and SBM did the experiments; SBM did statistical analysis; AMG drafted the manuscript; SBM supervised the study; AMG and SBM wrote the final manuscript; SBM proofread the manuscript.

Received: June/23, 2019; Accepted: August/04, 2019; Published: August/31, 2019.

**Citation**: Ganau AM and Manga SB. Methicillin Resistant Staphylococcus aureus (MRSA) in Hospitals of Sokoto Metropolis - A Multicentre Surveillance and Review of Literature J Med Lab Sci, 2019; 29 (2): 21-36.



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## INTRODUCTION

Staphylococci are ubiquitous colonisers of human epithelial surfaces that can become opportunistic pathogens. Virulent strains of these bacteria are responsible for the Healthcare majority of Associated Infections (HAIs) and can cause severe disease that can be fatal (1). Methicillin resistant Staphylococcus aureus (MRSA) was first isolated in the early 1960s, until recently infections have been associated with the hospital environments and referred to as hospital-acquired MRSA (HA-MRSA) (2). However, in the early half of the 1990s, community-acquired MRSA (CA-MRSA) infections began to appear in otherwise healthy people with no known risk factors for the hospital associated infections (3). Moreover, CA-MRSA clones have been spreading rapidly and infiltrating healthcare facilities in many continents worldwide. Livestock associated MRSA is almost only restricted to certain high-risk groups of workers in direct contact with live animals (4). Continuous drive to understand the changing epidemiology of S. aureus infections will not only guide appropriate antimicrobial treatment and effective infection control but will also shed light on the dynamics in the evolution of localized strains and species (5).

Literature on antibiotic surveillance, which is supposed to serve as the foundation for infection control programmes and regimen prescription in this part of the country is very scarce (6). To our knowledge, there has been no report on MRSA in human populations from Sokoto, Kebbi and Zamfara States. However, studies that have reported varying prevalence rates from other parts of the country abound (7,8,9). The aim of the study is to determine the prevalence and associated risk factors of MRSA among in- and outpatients attending three selected hospitals in Sokoto metropolis. It is the intent of this work to bridge some of the gap and bring to light the challenges posed by antibiotic resistant bacteria, in particular, MRSA.

#### MATERIALS AND METHODS

## **Study Design**

We carried out a cross-sectional descriptive study on MRSA isolated from clinical specimens over an eight month period, between February and October 2015.

#### **Sampling Technique**

Systemic random sampling was employed in collecting specimen from study subjects. Specimen was collected from every other study subject for whom culture has already been indicated by the attending physician.

#### Study Area

Sokoto State is located in the North-West geopolitical zone of Nigeria between longitudes  $11^{0}3'$  to  $13^{0}50'$  East and latitudes  $4^{0}$  to  $6^{0}40'$  North (10). It covers a total land area of 33,776.89 square kilometres in the Iullemmeden Basin surrounded to the East and South by the Precambrian basement complex (11). Population size is 3,7026,76 persons spread over an area of 33,776.89 square kilometres of land (11). The population mainly consists of the Hausa/Fulani ethnic groups that engage in farming and animal husbandry as their major occupations (12).



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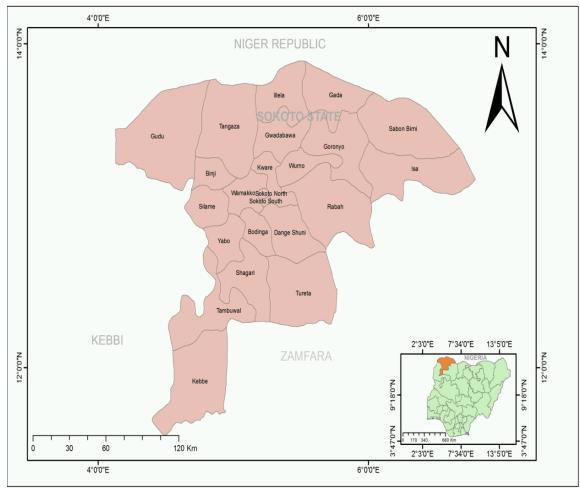


Figure 1. Map of Sokoto State and surrounding State and international boundaries (12).

#### **Study Population**

The study was carried out amongst patients attending three selected hospitals located within Sokoto metropolis; Usmanu Danfodiyo University Teaching Hospital (UDUTH), Specialists Hospital Sokoto and Maryam Abacha Women and Children hospital Sokoto. These hospitals span three Local Government Areas of the state; Sokoto North, Sokoto South and Wamakko Local Government Areas.

#### **Ethical Considerations**

Medical research approval was received from the Sokoto State Ministry of Health (SMH/1150/V.III). We also obtained approval from the ethical committees of the three hospitals in which the research was carried out; the ethical committees of Usmanu Danfodiyo University Teaching Hospital (UDUTH/HREC/2015/No.307), Specialists Hospital and that of Maryam Abacha Women and Children Hospital Sokoto (SHS/SUB/133/Vol.I).

# Isolation of Staphylococci from Clinical Specimens

Specimens (surgical and traumatic wound swabs, ear Swabs, urogenital Swabs, seminal fluids, urine, blood, nasal swabs and cerebrospinal fluids) were collected from patients for whom culture had already been indicated by the attending physicians, they were then transported to the laboratory in cold chain for processing. Each specimen was inoculated on blood agar (5% sheep blood) and mannitol salt agar (MSA) plates for primary culture. The cultured agar plates were then incubated at 37°C in ambient air for 24 hours. Morphologic differentiation by Gram stain was used as the initial presumptive identification method.

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#### **Biochemical Identification of** *Staphylococcus aureus*

Morphologic differentiation by Gram stain was used as the initial presumptive identification method. Staphylococci and similar bacteria were distinguished from the family Streptococcaceae by performing a catalase test, for which only the Streptococcaceae test negative. Two drops of 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were placed at the ends of a clean grease-free glass slide, one is labelled test and the other as control. A wooden applicator stick was used to collect a few colonies of the growth from a non-blood containing media and emulsified in the drop labelled as 'Test' and observed for effervescence due to oxygen production. The control drop was used to emulsify a control strain known to be catalase positive for validation (13).

Staphylococci were then differentiated from Macrococci and Micrococci with the modified oxidase test microdase (Remel Inc., Lenexa, Kansas), sensitivity to bacitracin (0.04U) and 100µg furazolidone (14). Microdase was performed as per manufacturer's instruction. Briefly, the microdase disk was aseptically placed in a sterile petri dish, using a wooden applicator stick the disk was then smeared with several colonies of an 18 hours culture of the test organism. The smeared disk was then observed for a purple-blue color (positive test) development within 2 minutes. No change in color denotes a negative test result. Sensitivity to bacitracin (0.04U) and 100µg furazolidone was determined by inoculating a 0.5 MacFarland suspension of the test bacteria on a plate of Mueller Hinton agar. Disks impregnated with the antibiotics were then placed on the plate and incubated at 37°C overnight, the zones of inhibition were then determined the following day using a metre rule. Gram positive cocci that were negative for the microdase test, resistant to 0.04U bacitracin (<10mm) but sensitive to 100µg furazolidone (>15mm) were

considered to be species of Staphylococci (15).

## **Coagulase Tests**

Slide and tube coagulase tests were then performed, serially, to differentiate S. aureus from other coagulase-negative Staphylococci. Bound coagulase (clumping factor) was detected using the rapid slide coagulase test. Two thick suspensions of the test organism were made on a clean grease-free glass slide. One of these was marked as the 'Test' and the other as the 'Negative control'. A loopful of pooled ethylenediaminetetraacetic acid (EDTA) anticogulated rabbit plasma was then added to the suspension marked 'Test' and mixed, no plasma was added to the control suspension. The slide was then rocked for 10 seconds and observed for clumping in the 'Test' suspension. A positive slide coagulase test required no further investigations, a negative result, however, necessitates the conduct of the tube coagulase test (15).

The tube coagulase test was performed by adding 200 $\mu$ L of rabbit plasma in each of three separate tubes marked as 'Test', 'Positive control' and 'Negative control'. Then 800 $\mu$ L litre of an overnight culture of the test isolate was added to the tube marked 'Test', the same volume of an overnight culture of a known *S. aureus* isolate was added to the 'Positive control' while sterile nutrient broth was added to the 'Negative control' tube and all tubes were then incubated at 37°C for 4 hours with observations for any clot formation made at an hourly interval (16).

# **Other Biochemical Tests**

To differentiate *S. aureus* from other coagulase-positive staphylococci (CoPS)  $\beta$ -galactosidase (ONPG) (Oxoid<sup>TM</sup>, UK) and pyrrolidonyl arylamidase (Lpyrrolidonyl- $\beta$ -Naphthylamide, PYR) (Oxoid<sup>TM</sup>, UK) tests were performed (17). PYR was performed with the Oxoid Remel<sup>TM</sup> PYR Disc with Reagent. A PYR

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disk was place on a clean glass slide and moistened with  $10\mu$ L of distilled water. three well isolated colonies were then removed with an applicator stick and smeared onto the PYR disk and incubated at room temperature for 2 minutes. One drop of Color Developer was then applied to the disk and observed for 1 minute for a pink-red color (positive test) development. No color change within a minute of adding Color Developer indicated a negative test result.

The only known CoPS that are negative to both tests are *S. aureus* and *S. hyicus* (18). *S. aureus* isolates were finally confirmed by their growth on Modified Baird-parker agar (Oxoid<sup>TM</sup>, UK), formed grey-black colonies with a clear halo around the margins (17,18).

## Detection of Methicillin Resistance Cefoxitin Disc Diffusion Test

Bacterial suspensions were prepared for each isolate by making a direct broth suspension of the isolates; 3 well isolated colonies from an overnight culture on nutrient agar plates were suspended in 5mL of sterile water. The suspension was then adjusted to achieve a turbidity that is equivalent to 0.5 McFarland standard. Within 15 minutes after adjusting the turbidity a sterile cotton swab was dipped into the adjusted suspension. The swab was then rotated firmly against the inside wall of the tube above the fluid level to remove excess fluid. A Mueller-Hinton agar (MHA) plate was then inoculated with the swab by streaking the swab over the entire surface of the agar. A 30µg cefoxitin disk (Oxoid<sup>TM</sup>, UK) was then placed on the surface of the inoculated MHA plate and incubated at 37°C in ambient air for 24 hours. The zone of inhibition was measured in millimeter and the results interpreted using the CLSI 2015 guidelines (19).

Each batch of tests run was validated using *mecA* negative *S. aureus* ATCC<sup>®</sup> 25923

(23–29 mm) and *mecA* positive *S. aureus* ATCC<sup>®</sup> 43300 ( $\leq$  21 mm) (LYFO DISK<sup>®</sup>, Microbiologics<sup>®</sup>, USA).

## Brilliance<sup>TM</sup> MRSA 2 Agar

The suspension of each of the isolates used above (0.5 McFarland equivalent) was also spot inoculated on a commercially prepared ready to use poured plates of Brilliance<sup>™</sup> MRSA 2 Agar (Thermo Scientific<sup>™</sup>, USA) and incubated at 35°C for 24 hours (20). Quality control organisms were the same as used for Cefoxitin disk diffusion. Any bacterial growth after 24 hours resulting in intense blue colonies was considered to be indicative of resistance to Methicillin (Figure 2).

## Statistical Analysis

The IBM<sup>TM</sup> Statistical Package for the Social Sciences (SPSS) version 20.0 software was used to determine the prevalence and Chi-square crosstabs to determine any statistical associations. Probability of p<0.05 was used as the criterion of significance.

## RESULTS

A total of 936 non-repetitive clinical specimens were collected during the time of the study (February-October 2015). Of all the specimens examined 234(25%) yielded growth of Staphylococcus aureus, out of which 101(43.2%) were identified as Methicillin-resistant Staphylococcus aureus MRSA. The highest prevalence for MRSA of 49.0% was found in Specialists Hospital, it is followed by Usmanu Danfodiyo University Teaching Hospital with a prevalence of 38.9% while Maryam Abacha Women and Children Hospital had the lowest prevalence of 38.3%. However, variations were found to be these statistically insignificant (p=0.282. $\chi^2$ =2.533) (Table 1).

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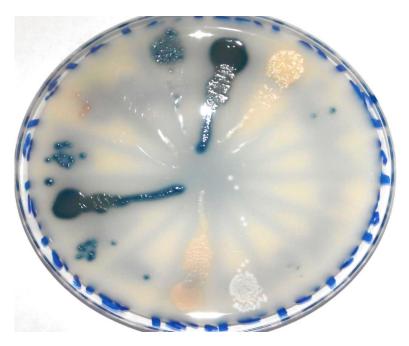
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Centre	Samples Examined	MSSA (%)	MRSA (%)	Total	P value	$\chi^2$
					0.282	2.533
Usmanu Danfodiyo University Teaching Hospital, Sokoto	397	44 (61.1)	28 (38.9)	72		
Specialists Hospital, Sokoto	288	52 (51)	50 (49)	102		
Maryam Abacha Women and Children Hospital, Sokoto	251	37 (61.7)	23 (38.3)	60		
Total	936	133	101	234		

Table 1: Distribution of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) for the study centres

The cultural characteristics of bacteria isolated on the Brilliance<sup>TM</sup> MRSA 2 Agar (Thermo Scientific<sup>TM</sup>, USA) are shown below. It's a ready to use poured plates on which methicillin-resistant *Staphylococcus aureus* (MRSA) appears as denim-blue

colonies, it inhibits the growth of methicillin-susceptible *S. aureus*. Methicillin-resistant coagulase negative staphylococci (MR-CoNS) appear as white-cream colonies on the medium (Fig. 2)



**Figure 2**: An inoculated plate of Brilliance<sup>TM</sup> MRSA 2 Agar (Thermo Scientific<sup>TM</sup>, USA). The denim blue colonies are indicative of MRSA, growth of MSSA is inhibited on this medium. Methicillin resistant coagulase negative staphylococci grow as the white-cream colonies seen in the figure above.

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Of all the specimens analyzed during the course of this study, ear swabs were found to have the highest prevalence rate for MRSA isolation with a 75% prevalence, these were followed by surgical wound, cerebrospinal fluid and urine specimens all of which had a prevalence of 50.0%, traumatic wound specimen had 49.0% MRSA prevalence while urogenital

specimen had a 28.6% prevalence. Nasal swabs had a prevalence of 23.8% while no strain of MRSA was isolated from blood and seminal fluids (0.0%) collected during the study period. These variations among different specimen types were found to be statistically significant (p=0.021,  $\chi^2$ =18.098).

Table 2: Distribution of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) from different specimens analysed in the Study centres.

Specimen	MSSA (%)	MRSA (%)	Total	<i>P</i> value	$\chi^2$
				0.021	18.098
Surgical Wound	22 (50)	22 (50)	44		
Traumatic Wound	51 (51)	49 (49)	100		
Ear Swab	2 (25)	6 (75)	8		
Urogenital Swabs	10 (71.4)	4 (28.6)	14		
Semen	2 (100)	0 (0)	2		
Urine	6 (50)	6 (50)	12		
Blood	4 (100)	0 (0)	4		
Nasal Swab	32 (76.2)	10 (23.8)	42		
CSF	4 (50)	4 (50)	8		
Total	133 (56.8)	101 (43.2)	234		

When isolates were tallied in accordance with the gender of participants in the study, as shown in Table 3, females (48.6%) were found to be more prone to colonization and infection by MRSA than their male counterparts (34.1%). The variation is statistically significant (p=0.030,  $\chi^2$ =4.731).



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Table 3: Distribution of Methicillin Sensitive Staphylococcus aureus (MSSA) and Methicillin
Resistant Staphylococcus aureus (MRSA) by gender in all centres

Gender	MSSA (%)	MRSA (%)	Total	P value	$\chi^2$
				0.030	4.73
Male	58 (65.9)	30 (34.1)	88		
Females	75 (51.4)	71 (48.6)	146		
Total	133 (56.8)	101 (43.2)	234		

The most significant risk factor for infection with MRSA, as found by the study is hospitalization. Inpatients with an MRSA prevalence of 55.8% were found to

be more infected with strains of MRSA then outpatients with a prevalence of 25.0%. The variation is statistically significant (p=0.000,  $\chi^2$ =21.888) (Table 4).

Table 4: Distribution of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) between in-patients and out-patients in all centres

Category	MSSA (%)	MRSA (%)	Total	<i>P</i> value	$\chi^2$
				0.000	21.888
In-patients	61 (44.2)	77 (55.8)	138		
Out-patients	72 (75)	24 (25)	96		
Total	133 (56.8)	101 (43.2)	234		
Total	155 (50.0)	101 (+3.2)	2JT		

#### DISCUSSION

Methicillin-resistant *Staphylococcus* aureus (MRSA) has been recognized worldwide as a major cause of nosocomial and community-acquired infections. The aim of this study was to determine the of prevalence MRSA in Sokoto metropolis. A total of 936 non-repetitive clinical specimens of ear swabs, pus, wound swabs. aspirates, urogenital specimens and cerebrospinal fluids from patients attending three hospitals in Sokoto metropolis were collected and processed over a period of 8 months (March to October 2015).

Out of the total 936 samples examined during this period 367(39.2%) Staphylococci were isolated, out of which 234(25%) were confirmed as coagulasepositive Staphylococci and identified as Staphylococcus aureus subsp. aureus. The isolation rate of 25% for S. aureus is comparable to the 24.5% from a study in South eastern part of Nigeria (21), and the 28.95% reported from the town of Douala, Cameroun (22). It's, however, much lower than the 35.55% reported from the South Indian town of Surat by Mulla et al. (23). In an earlier study, Nilsson and Ripa (24) demonstrated an isolation rate of up to 48% (125/259) in newly admitted patients a routine infection during control surveillance in a Swedish county (24). Out of a total of 234 S. aureus isolated, 101(43.2%) were identified as MRSA by the use of cefoxitin disk and Brilliance TM MRSA 2 Agar (Thermo Scientific™,

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USA), representing a total prevalence of 43.2% for all the centres studied. Our result compares favourably with one of the earliest reports on MRSA from the Northern part of this country, where a prevalence of 43% was reported by Ikeh (7). Similarly a few reports from the Southern part of Nigeria agrees with our finding; Yusuf and Airauhi (25) reported a prevalence of 42.7% in clinical specimens at University of Benin Teaching Hospital (UBTH) Edo State while Alli and colleagues (26) reported 42.3% from tertiary healthcare facilities in the South-West.

The 43.2% prevalence reported herein also compares favourably with other studies from around the world; Sullivan et al. (27) reported 43.0% from the United States and the Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group in an extensive study reported a prevalence of 41% in a study of 26.310 Staphylococcus aureus isolates from across India (28). Moreover, Perwaiz and colleagues reported a prevalence of 43% from Pakistan (29).

However, the prevalence we reported may be considered to be very high when compared to other reports from parts of Nigeria; Kolawole and colleagues (30) from Ile-Ife Osun State reported 11.5% (7/61) prevalence among patients that underwent surgical procedures. The authors equally identified the clonal complexes CC5 and CC15 as the most widespread in the institution (30). In a wide study including tertiary health centres in the North eastern part of Nigeria a prevalence of 12.5% (12/96) was reported (31). Similarly, in a surveillance study involving hospitalized and apparently healthy volunteers conducted in Ekiti and Ondo states a 13.0% prevalence was reported (32). Moreover, two other studies conducted in Abuja and Edo State set the prevalence of MRSA at 13.1 and 13.9% respectively (33, 34).

However, higher prevalence than what we reported herein has been seen in other

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studies conducted across the country. Of note is the nasal colonization rate of 52.5% reported from Kwara (35). In another study Udobi and colleagues (9), reported 63.2% MRSA prevalence from patients and materials in the orthopaedic ward of a tertiary care hospital in Kaduna, Northern Nigeria. During the study the authors recorded an 85.25% (185/217) isolation rate for *S. aureus* (9). An even higher prevalence of 63.3% was found in the anterior nares of apparently healthy volunteers in Plateau State (36).

Nevertheless, the highest reported prevalence we came across, from Nigeria, during our literature search was 79.0% from Edo State. The authors examined a total of 3,612 clinical specimens and isolated 1,315 *S. aureus* out of which 1,039(79.0%) were identified as MRSA (37).

In the countries of the South Africa Development Community (SADC) prevalence varies with 7.0% reported from Zimbabwe and 13.6% in Namibia (38,39). In Botswana, Troung and colleagues (40) reported 22.6% while in the KwaZulu-Natal Province of South Africa a prevalence of 26.9% was reported (40, 41). A pattern can be observed in these studies, even though there appears to be a wide variation in reported prevalence from these SADC countries they are all lower than what we reported in this study.

The pattern of MRSA reported from the literature in East Africa is similar to what is seen in South African countries, variable prevalence that is lower than what we are reporting from North Western Nigeria. In Tanzania a prevalence of 15% was reported by Moremi and colleagues (42), Ethiopia had a prevalence of 23.08% according to a report (43). Kenya had a slightly higher prevalence of 26.3% (44). All lower than what we discovered in our locality.

The prevalence rate of MRSA by study centre differs, Specialists Hospital has the highest MRSA prevalence of 49% (50/102) followed by UDUTH with 38.9%

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(28/72) while Maryam Abacha Women and Children Hospital has the lowest 38.3% prevalence of (23/60).The variations in the prevalence rates between the centres has been shown to be statistically insignificant (*p*>0.05). Probable explanations for the observed variations may be attributed to the effects of demographic population differences, variations in practices and possibly differences in approaches to infection prevention and control measures in the different hospitals.

When the prevalence was analysed based on the type of specimen collected we found that samples that are more likely to yield MRSA include ear swabs (75%), surgical wounds (50%), urine (50%) and traumatic wounds (49%) (p<0.05). The highest prevalence seen in relation to ear swabs may be associated with the relatively lower sample size of this specimen. In a related study in Nigeria, surgical and traumatic wounds were found to be the most infected specimen type (6).

The significance of gender dimorphism as a risk factor in infectious diseases is critical, it helps in identifying individuals with an increased risk of becoming colonized or infected with MRSA. this demography Understanding will invariably help in healthcare planning and be of immense could strategic considerations in designing preventive strategies like targeted screening on admission or prophylaxis. The study demonstrates a relatively higher rate of infection in females (48.6%) by MRSA than males (34.1%). А probable explanation for this variation could be the fact that more women tend to visit hospitals than their male counterparts, for antenatal care and paediatric visitations for example. This assertion is supported by the higher number of females (146 vs. 88). Other studies conducted across the country have reported similar higher rate of infections amongst women (38). However, our finding is at variance with a study in Namibia (39).

Moreover, in other studies from around the world the male gender was identified as a significant risk factor for colonization and infection with MRSA. In an extensive study in the United States the male gender was identified as a significant risk factor for colonization (45). Earlier and contemporary studies, however, in the same country reported a higher prevalence in males but the findings were statistically insignificant (46, 47). Other studies of blood-stream infections in countries across Europe and the United States have reported a significant preponderance in males (48, 49). Being multinational studies, a possible explanation for the variations in findings with our report can be attributed to fundamental differences in surveillance methodology and the fact that the effect of different MRSA epidemic clones and strains may differ in different countries.

One of the main probable cause for the higher prevalence seen in males includes more engagement in contact sports than their female counterparts. Contact sports predisposes particularly to skin colonization and infections by S. aureus Secondly, certain physiologic (47). differences between the male and female sex have been shown to confer a certain level of protection to the female gender. The female hormone estrogen have been shown to have a protective effect against certain infectious diseases like cholera, enterotoxigenic E. coli and streptococcal infections in premenopausal women (50,51,52). Moreover, behavioural studies in communities have shown that males tend to have a lower compliance to handhygiene practices than females (53). The situation is also similar amongst healthcare professionals, where females have been found to show a higher tendency of washing hands following patient contact (54).

However, the variance observed in our findings with higher prevalence in females may be associated with variations in study design and other unidentified

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compounding factors. Most of the cited studies that consider sex distribution are limited in their focus to specific infection types like blood-stream infections or skin colonization only (48, 49). Our sampling technique is more diverse. Another probable explanation for the pattern of infection observed in this study may be associated with gender population behaviour and environmental influences of the female sex in our setting. It's natural for women to visit the hospital more than men do, for the obvious reasons associated with pregnancy (antenatal and postnatal care) and complications that may arise from childbirth. This also explains the sample size effect where more females participated in our study.

As expected the rates of infection between the two patient groups considered in this study (the in-patients and out-patients) showed that inpatients are more prone to colonization by MRSA because they are more at risk than patients who only visit the hospital once in a while and in most cases for only a few hours (Table 4). Inpatients (55.8%) are statistically more out-patients infected than (25%)(p=0.000). Risk factors like indwelling antibiotics medical devices. administration, surgery, parenteral feeding and dialysis are more common amongst patients being treated as inpatients. Most studies involving the two groups have reported similar findings within and outside the country (37, 46).

MRSA was discovered initially in hospitalized patients as a nosocomial infection or associated with contact with healthcare facilities (55). Although the prevalence and risk of acquisition of MRSA in hospitalized patients has been shown to decline in some EU countries, the same cannot be said of third world countries in Asia and Africa in particular (56). Struggling healthcare systems and frequent sporadic epidemics and poor funding makes it difficult to obtain reliable data for comparison.

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The Brilliance<sup>™</sup> MRSA 2 Agar (Thermo Scientific<sup>™</sup>, USA) identified 115 of the 234 S. aureus isolates as being Methicillin resistant, that is 14 isolates more than what was detected by the Cefoxitin disk. All the 14 isolates were then isolated from the Brilliance<sup>™</sup> MRSA 2 Agar (Thermo Scientific<sup>TM</sup>, USA ) in pure culture, subcultured on nutrient agar and retested for Methicillin resistance using Cefoxitin (30µg) disk. All the 14 isolates tested negative, the finding is comparable to the findings of Veenemans and colleagues (57), the researchers demonstrated a sensitivity of 100% and specificity of 99.1% for Brilliance<sup>™</sup> MRSA 2 Agar (Figure 2) when compared to MRSA-ID (bioMérieux, France) (57).

## CONCLUSIONS

The study has established, as a baseline study, the prevalence of MRSA in healthcare centres within the study area at 43.2%. The female gender and hospitalisation were found to be significant risk factors for infection with MRSA. A viable and practical antibiotics stewardship program needs to be put in place.

# **CONFLICT OF INTEREST**

None to declare.

## **SOURCE OF FUNDING**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

#### REFERENCES

- 1. DeLeo FR, Otto M. Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. *PLoS Pathog* 2009;5(7):*e1000533*. doi:10.1371/journal.ppat.1000533.
- Yoshida H, Kawai F, Obayashi E, Akashi S, Roper ID, Tame HRJ, Park YS. Crystal Structures of Penicillin-Binding Protein 3

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(PBP3) from Methicillin-Resistant *Staphylococcus aureus* in the Apo and Cefotaxime-Bound Forms. *J Mol Biol* 2012;423:351-364.

- Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, Mackenzie FM. Methicillinresistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. *Int J Antimicrob Agents* 2012;39(4):273-282.
- 4. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL. Isolation and characterization of methicillinresistant *Staphylococcus aureus* from pork farms and visiting veterinary students. *PLoS ONE* 2013;8(1):e53738.
- Felmingham D, White AR, Jacobs MR, Applebaum PC, Poupard J, Miller LA, Grüneberg N. The Alexander Project: the benefits from a decade of surveillance. J Antimicrob Chemother 2005;56(2):ii3–ii21.
- Ghebremedhin B, Olugbosi MO, Raji AM, Layer F, Bakare RA, König B, König W. Emergence of a Community-Associated Methicillin Resistant *Staphylococcus aureus* Strain with a Unique Resistance Profile in Southwest Nigeria. J Clin Microbiol 2009; 47(9):2975-2980.
- Ikeh IE. Methicillin-Resistant Staphylococcus aureus (MRSA) at Jos University Teaching Hospital. Afr J Clin. Exper Microbiol 2003;4(1):52-55.
- 8. Onanuga A, Awhowho GO. Antimicrobial resistance of *Staphylococcus aureus* strains from patients with urinary tract

infections in Yenagoa, Nigeria. J. Pharm. Bioallied Sci 2012;4(3):226-230.

- Udobi CE, Obajuluwa AF, Onaolapo JA. Prevalence and Antibiotic Resistance Pattern of Methicillin-Resistant Staphylococcus aureus from an Orthopaedic Hospital in Nigeria. Biomed Res Int 2013;1-4.
- 10. Tsoho BA, Salau SA. Profitability and constraints to dry season vegetable fadama in Sudan savannah ecological zone of Sokoto state, Nigeria. J Dev Agric Econ 2012;4(7):214-222.
- National Population Commission. 'Population Distribution by Sex, State, LGA & Senatorial District', NPC 2010, Abuja, Nigeria.
- 12. Christopher OA, TA, Alaga Ogbole OJ. Geological Site in Sokoto Suitability State. Nigeria, Northwestern for Phosphates Prospecting. Greener J Geol Earth Sci 2016; 4(2):040-050.
- 13. Bannerman TL. Staphylococci, Micrococcus and other catalase cocci positive that grow aerobically. In: Murray, P.R., Baron, E.J. and Jorgensen, J.M. (Eds). Manual of Clinical Microbiology, 8th edition. American Society for Microbiology Press 2003, Washington. Pp.384-404.
- 14. Paulis DNA, CS, Predari Chazarreta DC, Santoianni EJ. Five-Test Simple Scheme for Species-Level Identification of Clinically Significant Coagulase-Negative Staphylococci. J Clin Microbiol 2003;4(3):1219-1224.

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http://jomls.org ; info@jomls.org

- 15. Iorio PLN, Ferreira RBR, Schuenck PR, Malvar LK. Brilhante PA, Nunes FPA, Bastos RCC, dos Santos NRK. Simplified and Reliable Scheme for Species-Identification Level of Staphylococcus Clinical Isolates. J Clin Microbiol 2007;45(8):2564-2569.
- 16. David PK, Cyrus NK, Fred AK, Alfred O, Moses SO, Nanteza A, Moses LJ, Florence CN. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Ann Clin Microbiol* 2010;9(23).1-7.
- 17. Gandra EV, Silva JA, de Macedo MRP, de Araújo MR, Mata MM, da Silva WP. Differentiation Between *Staphylococcus aureus*, *S. intermedius* and *S. hyicus* Using Phenotypical Tests and PCR. *Alim. Nutr. Araraquara* 2005;16(2):99-103.
- Roberson JR, Fox LK, Hancock DD, Besser TE. Evaluation of Methods for Differentiation of Coagulase-Positive Staphylococci. J Clin Microbiol 1992;30(12):3217-3219.
- Clinical and Laboratory Standards Institute, CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement (M100-S25) 2015, Wayne, PA 19087 USA.
- 20. McElhinney R, Millar C, Scopes E. Comparative evaluation of chromID **MRSA** agar and Brilliance 2 MRSA agar for detection of MRSA in clinical samples. Br JBiomed Sci 2013;70(1):41-3.

## Ganau and Manga

- 21. Nsofor CA, Nwokenkwo VN, Ohale CU. Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Various Clinical Specimens in South East Nigeria. *MOJ Cell Sci Rep* 2016;3(2):00054
- 22. Bissong MEA, Wirgham T, Enekegbe MA, Niba PTN, Foka (2016). Prevalence FET. and Antibiotic Susceptibility Patterns of Methicillin Resistant Staphylococcus aureus in Patients Attending the Laquintinie Hospital Douala, Cameroon. Eur J Clin Biom Sci;2(6):92-96.
- 23. Mulla S, Patel M, Shah L, Vaghela G. Study of antibiotic sensitivity pattern of methicillin-resistant *Staphylococcus aureus*. *Indian J Crit Care Med* 2007;11(2):99-101.
- 24. Nilsson P, Ripa T. *Staphylococcus aureus* Throat Colonization Is More Frequent than Colonization in the Anterior Nares. *J Clin Microbiol* 2006;44(9):3334-3339.
- 25. Yusuf E, Airauhi L. Prevalence and pattern of methicillin resistant Staphylococcus aureus in a tertiary healthcare facility in Nigeria. *Med J Zambia* 2015;42(1):7–11.
- 26. Alli OA, Ogbolu DO, Shittu AO, Okorie AN, Akinola JO, Daniel JB. Association of virulence genes with mecA gene in Staphylococcus aureus isolates from Tertiary Hospitals in Nigeria. *Indian J Pathol Microbiol* 2015;58(4):464– 71.
- 27. Sullivan KV, Turner NN, Roundtree SS, McGowan KL. Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-

http://jomls.org ; info@jomls.org

Susceptible *Staphylococcus aureus* (MSSA) Using the KeyPath MRSA/MSSA Blood Culture Test and the BacT/ALERT System in a Pediatric Population. *Arch Pathol Lab Med* 2013;137:1103-1105.

- 28. Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V, Goswami P, Gupta V, Harish BN, Kagal A, Kapil A, Rao R, Rodrigues C, Sardana R, Devi KS, Sharma A, Balaji V. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. *Indian J Med Res* 2013;137:363-369.
- 29. Perwaiz S, Barakzi Q, Farooki BJ, Khursheed N, Sabir N. Antimicrobial susceptibility pattern of clinical isolates of Methicillin Resistant *Staphylococcus aureus*. J Pak Med Assoc 2007;57;2-4.
- Kolawole DO, Adeyanju A, Schaumburg F, Akinyoola AL, Lawal OO, Amusa YB, Köck R, Becker K. Characterization of Colonizing *Staphylococcus aureus* Isolated from Surgical Wards' Patients in a Nigerian University Hospital. *PLoS ONE* 2013;8(7):1-6.
- 31. Okon KO, Shittu AO, Usman H, Adamu, N, Balogun ST, Adesina OO. Epidemiology and Antibiotic Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* Recovered from Tertiary Hospitals in Northeastern, Nigeria; J Med Med Sci 2013;4(5):214-220.
- 32. Babafemi EO, David OM, Oluduro AO, Famurewa O. Epidemiology of methicillinresistant *Staphylococcus aureus* among hospitalized patients and

Ganau and Manga

apparently healthy individuals in Ekiti and Ondo States, Nigeria. J Pharm Biomed Sci 2014;4(11):1125-1130.

- 33. Akanbi BO, Mbe JU. Occurrence Of Methicillin And Vancomycin Resistant Staphylococcus aureus In University Of Abuja Teaching Hospital, Abuja, Nigeria. Afr J Clin Exp Microbiol 2013;14(1):10-13.
- 34. Okwu MU, Mitsan O, Okeke OP. Prevalence antimicrobial and susceptibility profiles of community-acquired methicillinresistant *Staphylococcus* aureus (CA-MRSA) isolates among healthy individuals in Okada, South-South, Nigeria. US Open Biol Chem Sci Pharm J2014;1(1):1-9.
- 35. Fadeyi A, Bolaji BO, Oyedepo OO, Adesiyun OO, Adeboye MAN. Olanrewaju TO. Aderibigbe A, Salami AK, Desalu OO, Fowotade A, Nwabuisi C, Akanbi AA, Raheem RA. Olalere Methicilin A. Resistant Staphylococcus aureus Carriage amongst Healthcare Workers of the Critical Care Units in a Nigerian Hospital. Am J Infect Dis 2010;6(1):18-23.
- 36. Ajoke OI, Okeke IO, Odeyemi OA, Okwori AEJ. Prevalence Of Methicillin-Resistant Staphylococcus aureus From Healthy Community Individuals Volunteers In Jos South, Nigeria. J Microbiol Biotechnol Food Sci 2012;1(6):1389-1405.
- 37. Onemu OS, Ophori EA. Prevalence Of Multi-Drug Resistant *Staphylococcus aureus* In Clinical Specimens Obtained From Patients Attending The University Of Benin

http://jomls.org ; info@jomls.org

Ganau and Manga

Teaching Hospital, Benin City Nigeria. J Nat Sci Res 2013;3(5):154-159.

- 38. Mauchaza K, Madzimbamuto FD, Waner S. Methicillin-resistant Staphylococcus aureus in Zimbabwe. Ghana Med J 2016;50(2):68-71.
- 39. Festus T, Mukesi M, Moyo SR. The Distribution Of Methicillin Resistant *Staphylococcus aureus* Isolated At The Namibia Institute Of Pathology In Windhoek, Namibia. *Indian J Med Res Pharm Sci* 2016;3(7):1-8.
- 40. Truong H, Shah SS, Ludmir J, Tawanana EO, Bafana M, Wood SM, Moffat H, Steenhoff AP. *Staphylococcus aureus* skin and soft-tissue infections at a tertiary hospital in Botswana. *S Afr Med J* 2011;101:413-416.
- 41. Shittu AO, Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates *Staphylococcus* of aureus in KwaZulu-Natal province, South Africa. BMC Infect Dis 2006;6(125):1-13.
- 42. Moremi N, Mshana SE. Kamugisha E, Kataraihya JB, Tappe D, Vogel U, Lyamuya EF, Claus H. Predominance of methicillin resistant Staphylococcus aureus -ST88 and ST1797 causing new wound infection and abscesses. J Infect Dev Ctries 2012;6(8):620-625.
- 43. Kejela T, Bacha K. Prevalence and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among primary school children and prisoners in Jimma Town,

Southwest Ethiopia. *Ann Clin Microbiol Antimicrob* 2013;12:1-11.

- 44. Ouko TT, Ngeranwa JN, Orinda GO, Bii CC, Amukoye E, Lucy M, Wamae CN. Oxacillin Resistant *Staphylococcus aureus* Among HIV Infected And Non-Infected Kenyan Patients. *East Afr Med J* 2010;87(5):179-186.
- 45. Robicsek A, Beaumont JL, Wright MO, Thomson RB, Kaul KL, Peterson LR. Electronic prediction rules for methicillin-resistant *Staphylococcus* aureus colonisation. Infect Control Hosp Epidemiol 2011;32(1):9-19.
- 46. Jarvis WR, Jarvis AA, Chinn RY. National prevalence of Methicillin resistant *Staphylococcus aureus* in inpatients at United States health care facilities 2010. *Am J Infect Control* 2012;40(3):194-200.
- 47. Schechter-Perkins EM, Mitchell PM, Murray KA, Rubin-Smith JE, Weir S, Gupta K. Prevalence and predictors of nasal and extranasal staphylococcal colonisation in patients presenting to the emergency department. *Ann Emerg Med* 2011;57:492-499.
- 48. Lessa FC, Mu Y, Davies J, Murray M, Lillie M, Pearson A, Fridkin SK. Comparison of incidence of bloodstream infection with methicillin-resistant *Staphylococcus aureus* between England and United States 2006– 2007. *Clin Infect Dis* 2010;51(8):925-928.
- 49. Laupland KB, Lyytikäinen O, Søggard M, Kennedy KJ, Knudsen JD, Ostergaard C, Galbraith JC, Valiquette L, Jacobsson G,
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#### Ganau and Manga

Collignon P, Schønheyder HC. The changing epidemiology of *Staphylococcus aureus* bloodstream infection: a multinational population-based surveillance study. *Clin Microbiol Infect* 2013;19(5):465-471.

- Nicol T, Bilbey DL, Charles LM, Cordingley JL, Vernon-Roberts B. Oestrogen: the natural stimulant of body defence. *J Endocrinol* 1964; 30:277-291.
- 51. Kamada M, Irahara M, Maegawa M, Yasui T, Takeji T, Yamada M, Tezuka M, Kasai Y, Aono T. Effect of hormone replacement therapy on post-menopausal changes of lymphocytes and T cell subsets. *J Endocrinol Invest* 2000;23(6):376-382.
- 52. Alzamora R, O'Mahony F, Harvey BJ. Estrogen inhibits chloride secretion caused by cholera and *Escherichia coli* enterotoxins in female rat distal colon. *Steroids* 2011;76(9):867-876.
- 53. Garbutt C, Simmons G, Patrick D, Miller T. The public hand hygiene practices of New Zealanders: a national survey. *N Z Med J* 2007;120(1266):U2810.
- 54. van de Mortel T, Bourke R, McLoughlin J, Nonu M, Reis M. Gender influences handwashing rates in the critical care unit. *Am J Infect Control* 2001;29(6):395-399.
- 55. French GL. The continuing crisis in antibiotic resistance. Int J Antimicrob Agents 2010;36(Sup.3):S3-S7.
- 56. European Centre for Disease Prevention and Control, ECDC. Antimicrobial resistance surveillance in Europe 2009. Annual report of the European

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AntimicrobialResistanceSurveillanceNetwork (EARS-Net)2010, Stockholm, Sweden: ECDC.

57. Veenemans J, Verhulst C, Punselie R, van Keulen PHJ, Kluytmans JAJW. Evaluation of Brilliance MRSA 2 Agar for Detection of MethicillinResistant
Staphylococcus aureus in Clinical Samples. J Clin Microbiol 2013;51(3):1026-1027.