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ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND MAR INDEX OF STAPHYLOCOCCUS AUREUS ISOLATED FROM HEALTHCARE WORKERS IN A GENERAL HOSPITAL IN LAGOS STATE, NIGERIA

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Abstract

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing in hospital settings and is a marker for multiple drug resistance to commonly prescribed antibiotics. Forty (40) MRSA and fifteen (15) methicillin-sensitive *Staphylococcus aureus* (MSSA) isolated from healthcare workers (HCWs) at Isolo General Hospital, Lagos State were subjected to antimicrobial susceptibility testing with selected antibiotics and their multiple antibiotic resistance (MAR) index values were calculated. MRSA isolates showed the highest resistance to Ciprofloxacin (70.0%) and Erythromycin (67.5%) and lowest resistance to Mupirocin and Linezolid (17.5%). The MSSA isolates showed high levels of susceptibilities to Linezolid (100%), Gentamicin (93.3%) and Mupirocin (86.7%). Resistances of MSSA ranged from 0.00% (linezolid) to 40.0% (Erythromycin), with other antibiotics having intermediate values. Erythromycin resistance of MSSA (40.0%) is an emerging problem for an antibiotic which currently serves as alternative in penicillin-allergic patients. The MAR index values of MRSA and MSSA isolates ranged from 0 to 1.0 and 0 to 0.5 respectively, an indication that majority of the MRSA isolates and a few of MSSA were hospital acquired. These data show that antimicrobial resistance is increasing among *Staphylococcus aureus* strains in the study area. This calls for implementation of infection control measures including routine MRSA screening of HCWs, MRSA screening for all patients admitted to the hospital, urgent reporting of MRSA laboratory results, improved hygiene, and adoption of antimicrobial policy that minimizes inappropriate or excessive antibiotic therapy and prophylaxisin our hospitals to prevent the spread of MRSA and other resistant microorganisms.

Keywords: *Staphylococcus aureus*, MRSA, MAR index, antibiotics, infection control, antimicrobial stewardship.

Introduction

Staphylococcus aureus is an opportunistic pathogen known to cause both healthcare-associated and community-associated infections [Wang *et al.*, 2019]. The bacterium occurs as a normal microbiota of the human skin and anterior nares. When acting as an opportunistic pathogen, it can cause several skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis, as well as serious infections such as burns and surgical site infections, bloodstream infections, pneumonia, or bone and joint infections (Khan *et al.*, 2018; Hardtstock *et al.*, 2020; Lewis *et al.*, 2018). Antimicrobial resistance among *S. aureus* strains poses an increasing challenge in the medical setting and public health. Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a global and major healthcare associated pathogen (Lee *et al.*, 2018).

It appears that the human carriage of *S. aureus* varies in different settings and geographic locations. Several reports suggest that up to 35% of the human population harbour *S. aureus* in their anterior nares (Etter *et al.*, 2020; Sakr *et al.*, 2018). In the hospital setting, *S. aureus* nasal carriage rate was reported to be 25.5% (27/106) while MRSA was 8.5% (9/106) (Walana *et al.*, 2020).

Among healthcare workers, prevalence of *S. aureus* has been reported. A study in Ethiopia reports the overall prevalence of *S. aureus* and MRSA to be 12% (29/242) and 5.8% (14/242) respectively (Legese *et al*, 2018). Walana *et al.*, (2020) reported nasal carriage of 40% among healthcare workers in Ghana.

Healthcare workers have been reported to be a source of cross contamination and spread of healthcare-associated infections. In a study conducted on the spread of *Staphylococcus aureus* between medical staff and high-frequency contact surfaces in a hospital, MRSA identified on the surface of medical devises were genetically identical to MRSA on the hands of healthcare workers. The result of the study established the possibility of cross-contamination of *S. aureus* or MRSA on medical workers' hands and contact surfaces (Shi *et al.*, 2015). MRSA can be transferred from the hands of healthcare workers to patients where these multiple drug resistant strains have the potentials to establish life-threatening infections.

High MRSA carriage of health care professionals have been reported as the key mechanism of transmission among patients during treatments, patients contact and aerosolization following sneezing (Popovich *et al.*, 2021).

Methicillin resistance is encoded by the *mecA* gene, which is part of a staphylococcal chromosome cassette *mec* (SCC*mec*), a mobile genetic element that may also contain genetic structures such as Tn*554*, pUB110, and pT181 which encode resistance to non-β-lactam antibiotics (Katayama *et al.*, 2000; Ito and Hiramatsu, 1998; Kondo *et al.*, 2022). This means that a high percentage of MRSA strains are likely to be multiple drug resistant by virtue of possession of SCC*mec*.

Multiple antibiotic resistance arises as a result of several factors which include inappropriate use of antimicrobial drugs, over-prescription of antibiotics, overuse of antibiotics in food production, to mention a few. In Nigeria, most people practice self-prescription and over-the-counter purchase of antibiotics with risk of over-dosing or under-dosing, resulting in resistance (Esan *et al.*, 2018).

Inappropriate use of antimicrobials leads to increasing rates of antibiotic resistance, resulting in decreased treatment options for MRSA infection, higher treatment costs, longer hospitalization, and increased morbidity and mortality (Antimicrobial Resistance Collaborators, 2022). The problem of MRSA is observed all over the world, although, the burden of infection is high in developing countries.

Knowledge of MRSA prevalence and recent antimicrobial susceptibility pattern is very important for appropriate selection of the antimicrobial agents (Shibabaw *et al.*, 2014). However, in most hospitals of African countries, there is neither surveillance system nor control policy for MRSA, and this plays significant role in increasing the problem (Fadeyi *et al.*, 2010). The presence of multiple antibiotic resistance among MRSA strains in apparently healthy individuals and among healthcare workers portend serious health risks with potentials for failure of patient management worldwide.

Multiple antibiotic resistance (MAR) index is a valid and useful method of tracking drug resistance. MAR index is calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics against which the organism is tested. MAR indices above the critical limit of 0.2 suggest that the bacteria originate from a high-risk source of contamination where several antibiotics are often used (Afunwa *et al.*, 2020; Sandhu *et al.*, 2016; Osundiya, 2013).

This current study was aimed to determine the antimicrobial susceptibility patterns and MAR index of methicillin resistant *S. aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA) among healthcare workers at Isolo General Hospital in Lagos State.

Materials and methods

Forty (40) Methicillin resistant *Staphylococcus aureus* (MRSA) and 15 Methicillin sensitive *Staphylococcus aureus* (MSSA) isolated from healthcare workers at Isolo General Hospital, Lagos State were subjected to antimicrobial susceptibility testing with selected antibiotics and their MAR index were calculated. Isolo General Hospital, Lagos State is located at Latitude 6.5265° N and Longitude 3.3182° E.

The *S. aureus* were isolated using Mannitol salt agar (MSA). Tiny yellowish colonies on MSA that were Gram positive cocci in clusters were presumptively identified as *S. aureus* and confirmed using coagulase test as previously described (Cheesbrough, 2005). The MRSA were identified using Cefoxitin disk diffusion test according to CLSI standards (CLSI, 2020).

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion technique on Mueller Hinton Agar according to the guidelines set by Clinical and Laboratory Standards Institute (CLSI, 2020). Sensitivity discs of six antimicrobial agents with known concentrations from OXOID® were selected. They included Ciprofloxacin (CIP, 30 µg), Linezolid (LZD, 30 µg), Chloramphenicol (C, 30 µg), Gentamicin (CN, 30µg), Mupirocin (M, 2ug) and Erythromycin (ERY, 2 µg). The zones produced by each antibiotic were measured using a standard calibrated ruler to the nearest millimetre and interpreted according to CLSI standards (CLSI 2020). Quality control of the antimicrobial susceptibility tests was done by testing the *S. aureus* strain (ATCC 25923) alongside the test organisms.

Disc diffusion (Kirby-Bauer) method.

A loopful of the isolated *S. aureus* was inoculated into 10 ml sterile saline solution by using direct colony suspension method. The turbidity of the test suspension was standardized to match the 0.5 MacFarland turbidity standards. This was used within 15 minutes after preparation. The comparison between the inoculum and the 0.5 McFarland turbidity standards was done in adequate light against a white paper with contrasting black lines. A sterile swab was dipped into the standardized inoculum solution. Excess liquid was removed from the swab by pressing it against the side of the tube and used to inoculate the surface of already prepared Mueller-Hinton Agar (Oxoid CM0337). The swab was streaked over the entire surface of the medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum. Finally, the entire edge of the agar was swabbed. Antibiotic discs were applied to the surface of the inoculated agar plate using a disc dispenser (Oxoid 6-place, 90 mm) within 15 minutes of inoculating the MHA plate and incubated overnight at 37°C. The zones of inhibition for each antibiotic were measured in mm and compared with values provided by the Clinical and Laboratory Standards Institute. *S. aureus* (ATCC 25923) was used as control in testing the sensitivity of *S. aureus* respectively (CLSI, 2020).

Determination of multiple antibiotic resistance (MAR) index.

The multiple antibiotics resistance (MAR) index was determined for each of the selected bacterial isolates using a formula MAR = x/y, where x is the number of antibiotics to which the test isolate displayed resistance and y is the total number of antibiotics against which the test organism has been evaluated for sensitivity (Riaz *et al.*, 2011).

Results

Table 1: Antibiotic susceptibility pattern of the isolates

ANTIMICROBIAL AGENTS	*STAPHYLOCOCCUS AUREUS* ISOLATES					
	MRSA (%) n =40			MSSA (%) n =15		
	S	I	R	S	I	R
GENTAMICIN	27(67.5)	5(12.5)	8(20)	14(93.3)	–	1(0.67)
ERYTHROMYCIN	9(22.5)	4(10)	27(67.5)	8(53.3)	1(6.7)	6(40)
CIPROFLOXACIN	8(20)	4(10)	28(70)	10(66.7)	1(6.7)	4(26.7)
CHLORAPHENICOL	15(37.5)	–	25(62.5)	10(66.7)	–	5(33.3)
LINEZOLID	33(82.5)	–	7(17.5)	15(100)	–	0(0)
MUPIROCIN	33(82.5)	–	7(17.5)	13(86.7)	–	2(13.3)

KEY: S: Sensitive, I: Intermediate, R: Resistance, MRSA: Methicillin resistant *Staphylococcus aureus*. MSSA: Methicillin sensitive *Staphylococcus aureus*.

Susceptibility pattern of isolates:

From the results of the antibiotic susceptibility testing, MRSA isolates were susceptible to Gentamicin (67.5%), Erythromycin (22.5%), Ciprofloxacin (20.0%), Chloramphenicol (37.5%), Linezolid (82.5%), and Mupirocin (82.5%). MRSA showed Intermediate resistances to gentamicin (12.5%), Erythromycin (10%), and Ciprofloxacin (10%). MRSA showed resistance to Gentamicin (20.0%), Erythromycin (67.5%), Ciprofloxacin (70%), Chloramphenicol (62.5%), Linezolid (17.5%), and Mupirocin (17.5%), with Ciprofloxacin having highest resistance (Table 1).

MSSA were susceptible to gentamicin (93.3%), Erythromycin (53.3%), Ciprofloxacin (66.7%), Chloramphenicol (66.7%), Linezolid (100%), and Mupirocin (86.7%). Intermediate resistances were observed against Erythromycin (6.7%), and Ciprofloxacin (6.7%). MSSA isolates showed resistance to Gentamicin (0.67%), Erythromycin (40.0%), Ciprofloxacin (26.7%), Chloramphenicol (33.3%), Linezolid (0.00%), and Mupirocin (13.3%), with Erythromycin having highest resistance (Table 1).

Table 2: Multiple Antibiotics Resistance (MAR) Index of MRSA and MSSA Isolates

MAR INDEX	NO OF ISOLATES	
	MRSA (%) n =40	MSSA (%) n =15
0.0	2 (5)	5(12.5)
0.2	7(17.5)	4(10)
0.3	10(25)	5(12.5)
0.5	12(30)	1(2.5)
0.7	5(12.5)	0(0)
0.8	3(7.5)	0(0)
1.0	1(2.5)	0(0)

KEY: MSSA: Methicillin sensitive *Staphylococcus aureus*, MRSA: Methicillin resistant *Staphylococcus aureus*

Chi square: 11.618, p value: 0.00

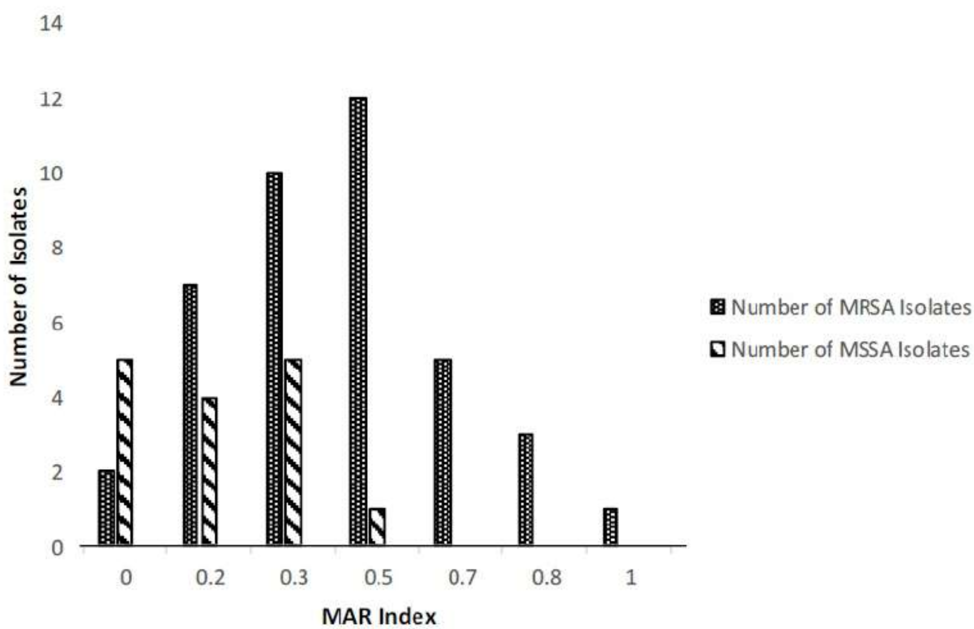

Figure 1 MAR Index values of MRSA and MSSA Isolates

MAR Index analysis of the isolates:

Data from Table 2 show that multiple antibiotic resistance (MAR) index values of the isolates ranged from 0.00 to 1.00. Out of 40 MRSA isolates, 31 (77.5%) had MAR index values above 0.2 (Figure 1). Similarly, six (6) out of 15 MSSA isolates (40.0%) had MAR index values above 0.2.

Discussion

The results of the study reveal high levels of resistance of MRSA to the antibiotics. MRSA isolates showed the highest resistance to Ciprofloxacin (70.0%) and Erythromycin (67.5%) and lowest resistance to Mupirocin and Linezolid (17.5%). However, it is notable that resistances to Mupirocin, a relatively new antibiotic, and Linezolid which is not commonly used in any secondary level hospital in Nigeria were as high as 17.5%.

The MSSA isolates showed higher susceptibilities to the antibiotics than were observed for MRSA isolates. MSSA isolates showed high levels of susceptibilities to Linezolid (100%), Gentamicin (93.3%) and Mupirocin (86.7%). Resistances of MSSA ranged from 0.00% (Linezolid) to 40.0% (Erythromycin), with other antibiotics having intermediate values. Erythromycin resistance of MSSA (40.0%) is an emerging problem for an antibiotic which currently serves as alternative in penicillin-allergic patients.

Multiple antibiotic resistance (MAR) index values of MRSA and MSSA isolates ranged from 0 to 1.0 and 0 to 0.5 respectively. MAR index values above 0.2 found in77.5% of MRSA isolates indicates that they originate from high-risk source of contamination where antibiotics are often used (Osundiya, 2013). One (2.50%) of MRSA isolates had a MAR index of 1.0, an indication that it was resistant to all the antibiotics tested.

Similarly, six (6) out of 15 MSSA isolates (40.0%) had MAR indices above 0.2, indicating that 40.0 % of MSSA isolates originate from potentially dangerous sources where several antibiotics are regularly used, such as a hospital ward. The MAR index values found in this study show that majority of the MRSA and a few MSSA isolates were hospital acquired. Expectedly, a lower percentage of MSSA isolates (40.0%) had MAR indices above the critical limit of 0.2, when compared with MRSA isolates (77.5%). The high MAR index of MRSA isolates is in consonance with the report that the *mecA* gene which confers methicillin resistance is associated with multiple antimicrobial resistance (Katayama *et al.*, 2000; Ito and Hiramatsu, 1998).

In a previous study carried out in Enugu, Udeani *et al.*, (2016), reported higher MAR indices ranging from 0.33 to 0.77. High MAR index may be due to prolonged use of antibiotics either topically or systematically followed by poor infection control mechanism and hygiene (Udeani *et al.*, 2016).

MSSA isolates in the present study showed high sensitivities to new antibiotics such as linezolid and mupirocin, which is consistent with similar studies (Gitau *et al.*, 2018). Erythromycin resistance of MSSA (40.0%) is an emerging problem for an antibiotic which currently serves as alternative in penicillin-allergic patients.

Results of the present study are comparable to those reported by other authors. In a previous study in Enugu, Nigeria, Udeani *et al.* (2016) reported similar resistances of MRSA to erythromycin (81.0%), and ciprofloxacin (71.4%).

In a study conducted in Owerri, South-East Nigeria, Emeakaroha *et al.*, (2017) reported that *S. aureus* isolates were susceptible to Nitrofurantoin and Cefuroxime, but resistant to Penicillin, Chloramphenicol, Cotrimoxazole. The isolates were also 25%, 28.6% and 35.7% susceptible to Ampicillin, Amoxycillin and Erythromycin, respectively.

Gitau *et al.* (2018) reported high rates of susceptibilities of MRSA and MSSA to Linezolid (95.3%) and high rates of resistance to commonly used antibiotics such as Gentamicin (24%), Erythromycin (34%), and Linezolid (7.0%). Other authors reported 100% resistance to Erythromycin in Ethiopia (Dilnessa and Bitew, 2016), Chloramphenicol (73%), Erythromycin (68%), Gentamicin (64%) (Okwu *et al.*, 2012). It is evident from several reports that susceptibility patterns of MRSA vary with time and geographic locations.

In a Tanzanian study in which 379 HCWs were enrolled, a high nasal carriage of MRSA and resistance to commonly prescribed antimicrobial agents were observed. 157/379 (41.4%) were colonized with *S. aureus*, of whom 59 (37.6%) were MRSA carriers giving an overall prevalence of 59/379 (15.6%). MRSA isolates showed high resistance toward Kanamycin (83.7%), Gentamicin (83.1%), Ciprofloxacin (71.2%), and Trimethoprim-Sulfamethoxazole (46.8%) compared to methicillin-sensitive *S. aureus* isolates ($p \leq 0.001$) (Joachim *et al.*, 2018).

In a study in England tracing the transmission of MRSA in a hospital, 605 genetically distinct subtypes were identified. Only 25 instances of transmission to patients (seven from health-care workers, two from the environment, and 16 from other patients) were detected. In the presence of standard infection control measures, healthcare workers were infrequently sources of transmission to patients. *S aureus* epidemiology in the ICU and HDU was characterized by continuous ingress of distinct subtypes rather than transmission of genetically related strains (Price *et al.*, 2017).

The implications of Methicillin-resistant *Staphylococcus aureus* (MRSA) carriage by healthcare workers are manifold. Firstly, MRSA among healthcare workers increases the risk of spreading the organism in hospital settings from HCWs to patients, and from patient to patient. Secondly, MRSA are multidrug resistant (Katayama *et al.*, 2000; Ito and Hiramatsu**,** 1998), and their spread in the hospital setting poses great limitations in the treatment of bacterial infections.

Implementation of infection control measures including contact precautions, urgent reporting of MRSA laboratory results, and routine MRSA screening of HCWs is highly needed to reduce MRSA spreading (Joachim *et al.*, 2018). Standard infection control and antimicrobial stewardship practice should include but not limited to hand hygiene; MRSA screening for all patients admitted to the hospital; *S. aureus* suppression with skin washes for all patients (with aqueous solution of 4% Chlorhexidine gluconate) and nasal Mupirocin (2%) for MRSA positive patients; environmental cleaning and decontamination of surfaces, beddings, curtains; antimicrobial policy that minimizes inappropriate or excessive antibiotic therapy and prophylaxis, and limited use of glycopeptides, third-generation Cephalosporins, and Quinolones (Price *et al.*, 2017).

Conclusions

The results of this study showed that healthcare workers in the hospital carry MRSA with high MAR index and there is a high possibility of transmitting these to patients, thereby posing greater risks of hospital-acquired infections with resistant bacteria while limiting treatment options. There is a need for improved infection control strategies accompanied by a more responsible antimicrobial stewardship in secondary level health institutions in the country.

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