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ASSESSMENT OF ASYMPTOMATIC METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS CARRIAGE AMONG HEALTHCARE WORKERS IN THE UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL, NIGERIA

Easter Godwin Nwokah[1], Oroma Eddeh-Adjugah[2] and Constancy Prisca Aleru[1]

[1]Department of Medical Laboratory Science, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

[2]School of Medical Laboratory Science, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria

All correspondence to +234(0)8036703755

Abstract:

Health-care workers are presumably important in MRSA transmission, although, they more frequently act as vectors than the main sources of MRSA transmission. Asymptomatic methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among Health care workers (HCWs) in the University of Port Harcourt Teaching Hospital, Nigeria was investigated. Hand and nasal swabs were collected from two hundred and twenty-two HCWs (111 males and 111 females). Following standard microbiological protocols, samples were processed for isolation of

Staphylococcus aureus while detection of MRSA was done using the Disk diffusion method-performed with oxacillin(1µg) disk placed on Mueller-Hinton agar with 4% NaCl supplementation. Of the two hundred and twenty-two HCWs (111 males and 111 females) involved, *Staphylococcus aureus* were isolated in 78 subjects (35.1%) whereas 5 (2.3%) of the total subjects yielded MRSA. As regards gender, out of the 5 isolates of MRSA, females HCWs showed higher but non-significant carriage rate (6.67 %) of the methicillin-resistant *Staphylococcus aureus* than in their male counterparts (6.06 %). This study involved age groups 20 to 30, 31 to 40 and 41 to 50 which gave MRSA incidence rates of 2 (6.25%), 2 (8.0%) and 1 (4.76%) respectively suggesting there is no significant relationship between MRSA carriage and the age groups of the study population. Among the antibiotics used for susceptibility testing, vancomycin showed the highest susceptibility rate of 94 %, followed by ciprofloxacin (59 %) and the least being cloxacillin (21 %). Regular screening of HCWs and high hygiene standards are instructive in the prevention and control of MRSA transmission and for a robust infection control programme.

Keywords: MRSA, Healthcare Workers, Nasal Carriage, Antibiotic Resistance.

1. Introduction

Staphylococcus aureus remains one of the most common agents of infections including skin and soft-tissue infections (SSTIs), toxic shock syndrome, pneumonia, arthritis, meningitis, septicemia, osteomyelitis, endocarditis, wound and urinary tract infections amongst others [1-3].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is presently a leading cause of a wide range of both community and hospital-acquired infections in human and animals worldwide [1, 4, 5]. Methicillin-resistant *Staphylococcus aureus* are strains that are resistant to methicillin and essentially all other beta-lactam antibiotics [6]. Some epidemic strains are known to be more prevalent and spread within or between hospitals and countries [6, 7]. Several genes have been found in strains of MRSA which confer on them high virulence and resistance to several antibiotic classes; the genes include, *mec*A (a gene that codes for penicillin resistance), *luk*S-*luk*F (responsible for widespread skin and soft tissue infections) and *tet*M (this gene codes for

tetracycline resistance), *erm* (macrolide resistance) among others [8, 9]. The acquisition of genes such as *mec*A that codes for penicillin binding protein (PBP2A) by the strains, confers almost complete resistance to all beta-lactam antibiotics, including the semi-synthetic penicillin [10-12]. According to reports, the presence of the *mec*A gene defines MRSA; notwithstanding, some studies do not test for the *mec*A gene and as a result, define MRSA by antibiotic susceptibility testing. Virulent factors such as toxic shock syndrome toxin-1 (TSST-1), exfloliative toxins, and enterotoxins have all been reported to be found in MRSA; they allow MRSA adhere to surfaces, damage or avoid the immune system and produce toxic effects [8,13].

Hospital-acquired MRSA (HA-MRSA) strains have been reported to cause different types of infections, ranging from surgical site infections to invasive disease. They are the main cause of nosocomial infections associated with indwelling surgical sites and medical devices [7, 14]. The risk factors of HA-MRSA are said to be both extrinsic and intrinsic. For instance, in extrinsic, the hospital setting could impact transmission, while in intrinsic, the strains could have the ability to be transmitted from nation to nation and internationally [15, 16].

These strains of *Staphylococcus aureus* are known for their ability to asymptomatically colonize both those who are healthy and the hospitalized patients. The asymptomatic hosts are presumed to disseminate *Staphylococcus aureus* to immunocompromised patients in the hospital settings. However, those carriers who are asymptomatic are said to be at higher risks of infection and are assumed to be an important source of the strains that spread from person to person [17, 18].

The reservoir of MRSA are the anterior nares, even if other sites of the body such as, the hands, skin, intestinal tract and axillae are as well frequently colonized. Those who are colonized are generally said to be asymptomatic and nasal carriage of the organism has been associated with an increased risk of infection for the individuals who are colonized [19-21]. Nasal carriage among Health-care workers is one of the important sources of HA-MRSA infection, although patient to patient transmission of the infection, reportedly, occurs primarily through carriage on the hands of healthcare workers [17, 20, 22]. It is also observed that those who are nasal carriers are more than those who carry the bacterium on their hands [22]. The Health-care workers are, therefore, important in MRSA transmission, although, they more frequently act as vectors than the main sources of MRSA transmission [20].

In several places in Nigeria and elsewhere, the incidence of MRSA causing potential infections amongst hospitalized and non-hospitalized patients has been reported. This study is therefore aimed at assessing the asymptomatic methicillin-resistant *Staphylococcus aureus* carrier status of health care workers in the University of Port Harcourt Teaching Hospital, Nigeria.

2. Materials and methods

Study population

Two hundred and twenty-two (222) health care workers (HCWs) in the University of Port Harcourt Teaching Hospital were involved in this study from June to December, 2014. Among them were 111 (50 %) males and 111 (50 %) females and they were categorized into three age groups of 21-30, 31-40 and 41-50. The subjects were also drawn from six (6) different categories/professional groups of laboratory staff, laundry, medical record, canteen, nurses and doctors.

Sample collection

The samples collected for this study were hand swabs and nasal swabs using sterile swab sticks and were taken to the laboratory for immediate processing. Inclusion criteria were staff without signs and symptoms of any disease or infection and who have not been administered with antibiotics in the previous two weeks, among others, based on responses from administered questionnaire.

Specimen processing and Identification of Isolates

The media used for the isolation and identification of *S. aureus* were Blood agar medium, 10 % cooked meat broth, mannitol salt agar medium and nutrient agar medium (all Oxoid, UK) and were aseptically prepared based on the manufacturer's instructions. Following inoculation, plates were incubated aerobically at 37^{o}C for 24 hours. The suspect isolates were confirmed using colonial morphology on DNase agar plate, Mannitol salt agar plate (yellow colonies showing Mannitol fermentation and non-yellow (mannitol negative) colonies), Gram stain, Catalase and coagulase (bound / free) tests following standard protocols [23, 24]. All confirmed isolates were stored at +4 ℃ if susceptibility testing was delayed.

Antimicrobial Susceptibility Testing

All isolates of *Staphylococcus aureus* were subjected to *in-vitro* anti-microbial susceptibility testing method on Muller-Hinton agar (MH), using 2-hour-old nutrient broth culture and OXOID- made antibiotic discs, as per the method described by Kirby and Bauer [25]. Briefly, inocula of bacteria were prepared to 0.5 McFarland standards and tested against the following antibiotics - Ciprofloxacin (5µg), Gentamicin (10µg), Cloxacillin (10µg), Vancomycin (30µg), Erythromycin (15µg), Ofloxacin (5µg), Oxacillin (1µg). Sterile swab stick was dipped into the bacteria suspension and used to streak the MH agar, after which the antibiotic disc was placed on the surface of MH agar plate and incubated at 37 ℃ for 24 hrs. The zones of inhibition around the discs were measured with ruler and interpreted as sensitive, moderately sensitive and resistant using the interpretation chart as prescribed by CLSI [26].

Detection of MRSA

Detection of MRSA was done using the Disk diffusion test. This was performed with 1µg of oxacillin per disk placed on Mueller-Hinton agar with 4% NaCl supplementation. The zone of inhibition was determined after 24 hours of incubation at 37 ℃. Organisms showing inhibition zone diameters equal to or lesser than 10 mm were interpreted as resistant to oxacillin. Organisms with a zone equal to or greater than 12 mm were interpreted as susceptible while those with an inhibition zone of 11-12 mm were interpreted as intermediate. *S. aureus* strains NCTC 6571 and NCTC 12493 were used as susceptible and resistant controls respectively.

Results

Two hundred and twenty-two (222) health care workers (HCWs) were screened for asymptomatic carriage of methicillin-resistant *Staphylococcus aureus*. 78 (35.1 %) of the subjects were colonized with *Staphylococcus aureus whereas* 5 (2.3 %) of the total subjects showed carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) (Figure 1).

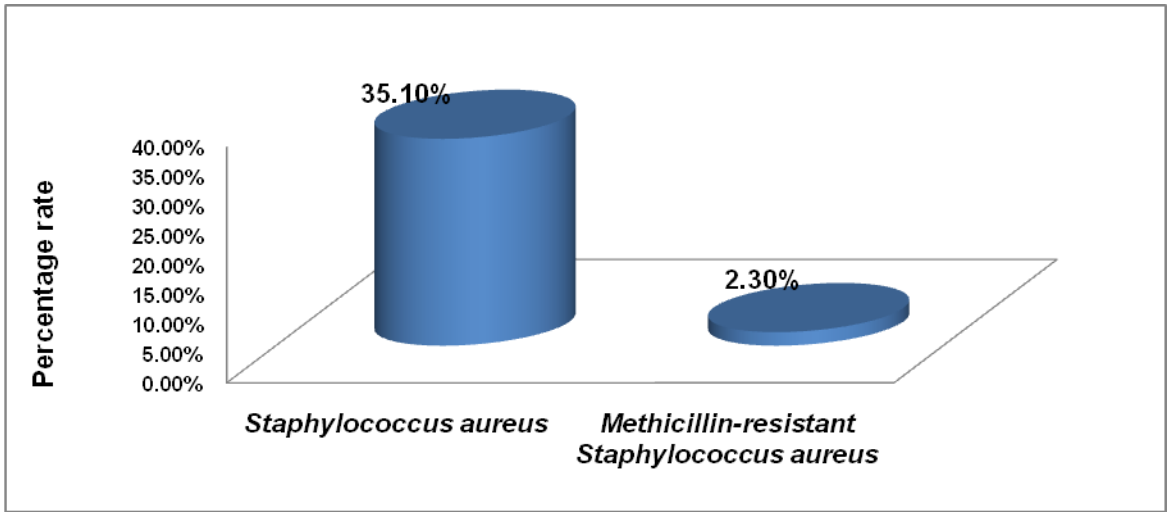

Figure1. Percentage carriage rates of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus among Healthcare workers

Table 1. Gender distribution of MRSA

Gender	No. of Subjects	No. of *S. aureus* isolates (%)	No. of MRSA (%)
Female	**111**	**45 (57.69)**	**3 (6.67)**
Male	**111**	**33 (42.31)**	**2 (6.06)**
Total	**222**	**78 (100)**	**5 (6.41)**

As regards gender, out of the 5 MRSA isolates in table 1, females showed higher but non-significant carriage rate (6.67%) of the methicillin- resistant *Staphylococcus aureus* than in their male counterparts (6.06 %).

Table 2. Age-related carriage of MRSA

Age Bracket (Years)	No. of *S. aureus* isolates (%)	No. of MRSA (%)
(20-30)	**32 (41.03)**	**2 (6.25)**
(31-40)	**25 (32.05)**	**2 (8.0)**
(41-50)	**21 (26.92)**	**1 (4.76)**
Total	**78 (100)**	**5 (6.41)**

As illustrated in table 2, there is no significant relationship between MRSA carriage and the age groups involved in this study.

As shown in figure 2, among the antibiotics used for susceptibility testing, vancomycin showed the highest sensitivity rate of 94 %, followed by ciprofloxacin (59 %) and the least being cloxacillin (21 %).

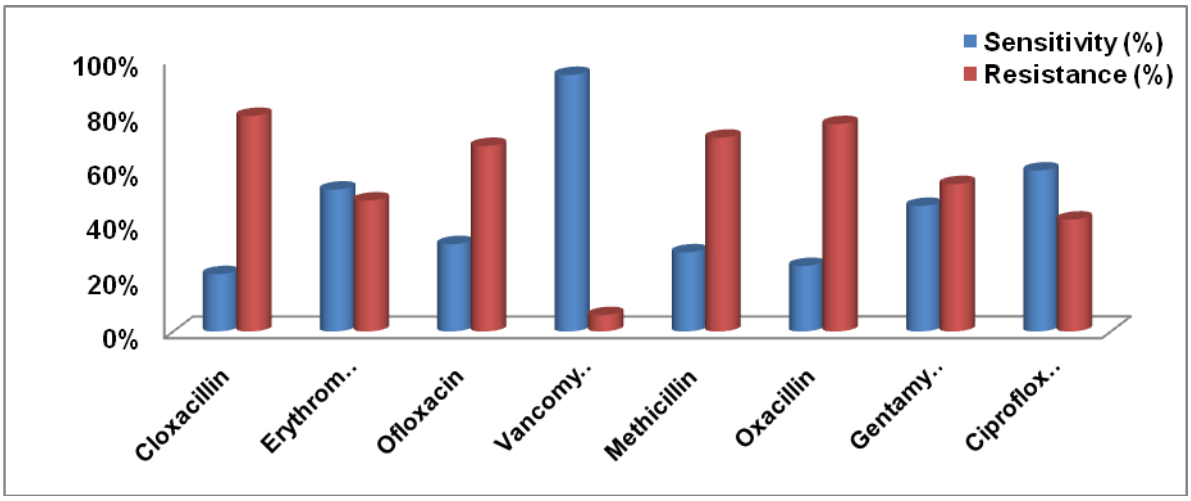

Figure 2. Antibiogram of *Staphylococcus aureus* isolates from HealthCare Workers

3. Discussion

Methicillin-resistant *Staphylococcus aureus* is a major cause of nosocomial infections that are becoming more and more difficult to treat by reason of multiple-drug resistance to hitherto used antibiotics. The presence of asymptomatic methicillin resistant *S. aureus* among health care workers is one great challenge to quality healthcare delivery. This indicates that patients in the hospital might be at risk of the infection caused by the organism and this could be a potential source of hospital and community outbreak.

This study revealed a Methicillin-resistant *Staphylococcus aureus* (MRSA) carriage rate of 2.3% among health care workers in the University of Port Harcourt Teaching Hospital, Nigeria. This is far lower than 52.5 % and 34.7 % reported from some other parts of Nigeria by Taiwo *et al*.,[27] and Fadeyi *et al*., [28] respectively, but in tandem with some reported lower rates such as the 2.0% reported among HCWs from India [29] and 4.0 % in a study in New Zealand [30].

Some researchers have reported fluctuations/variations in MRSA carriage rates. Albrich and Harbarth [20], in a review involving 127 investigations and the screening of 33, 318 health care participants, revealed that 4.6 % of health care personnel were either infected or colonized with

MRSA. Conversely, they also reported 41 studies that involved 10, 589 participants, which revealed an MRSA carriage of 23.7 %. The reasons for the variations in the MRSA carriage rate could be differences in microbiological methods, such as sampling techniques, culture and methods used for the identification of MRSA as well as local infection control standards of MRSA. Some workers have suggested that screening of HCWs for MRSA should be performed before starting work duties aimed at detection of transient, short-term MRSA carriage, which may occur during work shift [31].

Furthermore, according to this study, methicillin-resistant *Staphylococcus aureus* carriage rate was not significantly higher among the females (6.67 %) than in males (6.06 %). Radhakrishna *et al*., [32] reported a higher MRSA carriage rate among female housekeeping staff and nursing staff (13.3% and 2.7%) respectively than their male counterparts in Mangalore, India. These observations were attributed to female workers being more involved with nursing career than males as well as Nurses being the health care workers with highest frequency of contact with the patients and most likely to transmit MRSA. According to Madeleine *et al.,* [33], more attention should be paid to the prevention of MRSA colonization among nursing staff, in as much as this professional group seems to experience the highest risk for MRSA colonization. There is therefore, need to create more awareness among female staff, especially nursing staff, by educating them on prevention and control of MRSA carriage.

This study involved age groups 21-30, 31-40 and 41-50 which gave MRSA incidence rates of 2 (6.25%), 2 (8.0%) and 1 (4.76%) respectively suggesting there is no significant relationship between MRSA carriage and the age groups of the study population.

Staphylococcus aureus from this study exhibited a multi-drug resistance profile, especially to the commonly available agents in our setting, the highest being cloxacillin (79 %). Multi-drug resistance is well reported in study environment and attributed to inappropriate use of agents [34, 3, 35]. Worrisome is the observed vancomycin resistance rate of 6%. Certain reports have revealed the presence of VRSA in Nigeria. Akambi and Mbe, [36], reported vancomycin resistance in 4 isolates out of 213 isolates of *S. aureus* in the University of Abuja Teaching Hospital; a VRSA prevalence rate of 57.7% has also been reported in Zaria, Northern Nigeria [37] and 6.3% among MRSA [38]. However, only the disk diffusion method was employed in the susceptibility testing for VRSA in many of these studies, including the present one, and this

needs further elucidation. Vancomycin-resistant strains are a source of concern because until recently, vancomycin was the only uniformly effective treatment for staphylococcal infections, particularly MRSA. Resistance to vancomycin severely limits therapeutic options.

Findings from the present study center suggest HCWs' awareness and improved local infection control standards. However a 35.1% carriage rate of *Staphylococcus aureus* is a concern and should underscore regular screening of HCWs for a robust infection control programme.

High hygiene standards are instructive in the prevention and control of MRSA transmission. Good hand wash practices by health care workers and patients remain the most important infection control measure. Furthermore, screening patients on admission and during hospitalization and appropriate decontamination of hospital environments are important strategies for prevention and control of MRSA transmission. Adherence to these recommendations would curb the spread of MRSA and other infections.

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