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Nasal Carriage of Methicillin Resistant *Staphylococcus aureus* amongst Students in a Nigerian Tertiary Institution

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to treatment with a common class of antibiotics such as penicillin and cephalosporin most widely used to treat patients. *Staphylococcus aureus* is a common bacterium that colonizes the nose. It may be spread to susceptible people through coughs and sneezes especially among large number of people living close together. MRSA infections can be more difficult to treat than other bacterial infections. There is paucity of reports on the prevalence of MRSA in Nigerian tertiary institution. This study investigated the nasal carriage of MRSA among students in a Nigerian tertiary institution of learning. A total of 138 students comprising of 80 males and 58 females were investigated through culture, isolation and identification for *Staphylococcus aureus*. This isolate were further investigated for methicillin resistance using oxacillin. Sensitivity of the isolates was also compared with that of some orthodox antibiotics. Twenty isolates were identified as *Staphylococcus aureus* (prevalence of 14.5%) out of which nine were MRSA giving a total prevalence of 6.5%. This prevalence comprised of 5.8% males and 0.7% females. Of 80 males, prevalence of MRSA was 10% while out of the 58 females prevalence of MRSA was 1.7%. Resistance of *Staphylococcus aureus* increase in order: gentamicin (0%), vancomycin and ofloxacin (25%), and ceftriaxone (30%), cefuroxime (35%), oxacillin (45%), erythromycin (60%), amoxicillin (75%) and ceftazidime (90). While the usage of vancomycin as alternative to methicillin is upheld in this study gentamicin proved a better option as it inhibited all the methicillin resistant *Staphylococcus aureus*.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to treatment with a common class of antibiotics such as the penicillins and cephalosporins. This class of antibiotics are commonly employed in the management of both in-patients and out-patients. *Staphylococcus aureus* colonises the skin, particularly the nose, skin folds, hairline, perineum and navel.

Resistance to methicillin was rare until the late 1960s, when a multidrug-resistant MRSA was reported in Europe [1]. MRSA has become a cosmopolitan public health quandary, responsible for noteworthy morbidity and mortality resulting to health care costs [2]. The strains of hospital associated MRSA (HA-MRSA) are the most frequent cause of hospital-acquired infections [2]. Methicillin-resistant *S. aureus* has been reported as the leading cause of skin and soft tissue infection in emergency clinics with rate of infection rising in primary care clinics and intensive care units [3].

Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA, have emerged that have a predisposition to infect young individuals without risk factors. It has been estimated that about 50% of adults are either persistent or intermittent *S. aureus* carriers [4]. This microorganism can become an adaptable pathogen responsible for a broad spectrum of infections. *S. aureus* infections vary from common skin infections such as furunculosis and impetigo to severe deep-seated infections such as septicemia pneumonia, osteomyelitis, endocarditis, urinary tract infection and septic bursitis [2].

S. aureus ranks first or second among bacterial pathogens incriminated in bloodstream infections [5] and is the foremost cause of nosocomial pneumonia [6] MRSA has become epidemic not only in nosocomial infections but also in community-associated infections [7]. Invasive infections of MRSA have high morbidity and mortality rates [8]. Most of invasive staphylococcal and CA-MRSA infections are related to the nasal carriage of *Staphylococcus* [9].

Methicillin-resistant *S aureus* is not limited to any particular part of the world; it is worldwide in distribution and therefore a global predicament [10]. Population risk factors connected with *S. aureus* infections include, people who are frequently in crowded places, people with weak immune systems [11], intravenous drug users [12], school children sharing sports and other equipment [13], students living in hostels and people who spend time

together in confined spaces with other people such as occupants of homeless shelters, prison inmates, military recruits in basic training) [14].

MRSA is resistant to penicillin-like beta-lactam antibiotics. β-lactam antibiotics are a class of broad-spectrum antibiotics containing a beta-lactam ring in their molecular structures. This includes penicillin derivatives (penams), cephalosporin (cephems), the monobactams and the carbapenems [15]. Most β-lactam antibiotics work by inhibiting the cell wall biosynthesis in the bacterial organism.

A number of drugs still retain activity against MRSA, including glycopeptides (e.g., vancomycin and teicoplanin), linezolid, tigecycline, daptomycin, and even some new beta-lactams, such as ceftaroline and ceftobiprole [16]. The challenge is that MRSA has shown exceptional adaptability at emerging and spreading in different epidemiological settings over time [16]. This ability complicates the epidemiology of MRSA infections and creates a challenge for infection-control systems that focus only on health care-associated infections [16]. In addition, transfer of resistance to linezolid and glycopeptides antibiotics has been reported [16]. This calls for serious concern. Antimicrobial resistance is genetically based; resistance is mediated by the acquisition of extrachromosomal genetic elements containing resistance genes. Examples include plasmids, transposable genetic elements, and genomic islands, which are transferred between bacteria through horizontal gene transfer [17].

There are two main modes of bacterial resistance to β-lactams: enzymatic hydrolysis of the β-lactam ring through production of the enzyme β-lactamase or the enzyme penicillinase [18] and possession of altered penicillin-binding proteins (PBPs) [19].

Nasal carriers of MRSA may spread the organism to other people through sneezing and coughing. Information about the throat and nasal carriage of MRSA is scanty in tertiary institutions in Nigeria. Determining the prevalence of MRSA will help to estimate the level of risk to the organism and identify preventive and curative treatment methods for MRSA associated infections among students in tertiary institutions. This study aimed to investigate the prevalence of methicillin resistant *Staphylococcus aureus* among the students in a tertiary institution in Nigeria.

MATERIALS AND METHODS

Study area

The research was carried out at Afe Babalola University, Ado- Ekiti, Ekiti State, Nigeria. The institution is on latitude 7.6066 and longitude 5.3066 (2011-2018 distancesFrom.com). The investigation was carried out at the Research Laboratory of Medical Laboratory Science Department of the University.

Study population

The study population is the undergraduate students of the institution living in the school hostel.

Study design

The study was carried out over a period of three months. Selection of participants was by random sampling. Consent forms were administered randomly to students and those who returned the forms were enlisted for the study. Oxacillin was the drug of choice used to determine the bacterial susceptibility to methicillin as methicillin is now commercially unavailable. Oxacillin, a beta lactam antibiotic has similar mechanism of resistance to beta lactam antibiotics as methicillin. Sensitivity of *Staphylococcus aureus* was tested against oxacillin, gentamicin, ceftriaxone, amoxicillin, vancomycin, ofloxacin, ceftazidime, erythromycin and cefuroxime.

Ethical approval

Ethical approval was sought for and received from the Ethical Committee, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State. Informed consent of the students was obtained. The procedure and the purpose of the experiment were clearly explained to each student before samples are collected. Individual results were also issued to the students on request. All procedures and techniques for the study were in accordance with the National Institute of Health Guidelines.

Inclusion and exclusion criteria

Eligible students for this study include apparently healthy students of the institution. Ineligible students consisted of students having any symptom of upper respiratory tract infections.

Sample size

From the mathematical formula [20], for estimation of minimum sample size, the sample size of 138 students was obtained in relation to the estimated prevalence of the carriage of MRSA [21] in the locality. This sample size comprised of 80 males and 58 females.

Sample collection

Nasal swabs were collected from consented students and taken to the laboratory for examination within 30 minutes of collection. A total of 138 samples were collected between May, 2017 and July 2017.

Bacteria culture and isolation on mannitol salt agar (Oxoid Ltd, U.K.)

Test principles and procedure

Sodium chloride, concentration of the medium, 7.5%, is nearly ten times the usual concentration seen in most media serves to inhibit most organisms except staphylococci in mixed flora specimens. The beef extract and peptones supply the essential elements carbon, nitrogen, and sulphur. Acid production by *Staphylococcus aureus* fermentation of mannitol results in the formation of colonies with a yellow zone. Those staphylococci that do not ferment mannitol show a purple or red zone around the colonies [22].

The mannitol salt agar plates were allowed to warm to room temperature and the agar surface was dried in a dryer (Memmert GmbH + Co. KG, Germany) before inoculation. Collected nasal swabs were rolled over a small area of the agar surface and streaked for isolation with a sterile wire loop [22]. Plates were incubated aerobically in incubator (Axion Medical Ltd, U.K.) at 37ºC for 48 hours.

Colonial morphology

Mannitol fermenters (*S. aureus*) were selected and subjected to further tests to confirm their identity.

Gram reaction

Gram staining was used to differentiate between Gram negative and Gram positive organisms

Principle: Gram reaction differentiate between gram positive bacteria and gram negative bacteria based on differences in the permeability of the cell wall to peptidoglycan during staining. Gram positive bacteria have more of peptidoglycan thereby retain the crystal violet whereas Gram negative bacteria takes colour of the counterstain [23].

Catalase test

The principle is based on the ability of an organism to produce the enzyme catalase which splits H_2O_2 to free oxygen (bubbles) and water [23]. Production of active bubbling was taken as positive catalase test indicating the presence of staphylococcus.

Coagulase test

Free coagulase converts fibrinogen to fibrin by activating a coagulase reacting factor present in plasma. This is detected using the tube method [23]. Rapid slide test was done to detect bound coagulase.

Tube test method: 0.2 ml of plasma was put into a test tube and 0.08 ml of test broth culture was added. After mixing gently, the tube was incubated at 37^{o}C. The test was observed for clotting after 1 hour, 2 hours and overnight at room temperature. Clotting confirmed the presence of *S. aureus* [22].

Antibiotic Susceptibility Test

The Clinical Laboratory Standard Institute (CLSI) modified disc agar diffusion technique was used [22].

Preparation of inoculums

Discrete colonies of confirmed *Staphylococcus aureus* isolates growing on nutrient agar plates were emulsified in 3 ml of phosphate buffered solution (PBS) and the turbidity was adjusted to 0.5 McFarland standards through dilution with sterile buffer or further incubation [24].

Inoculation of plates for sensitivity testing

Using a sterile swab stick, the surface of Mueller Hinton agar (Oxoid Ltd, UK) incorporated with 0.5% Sodium chloride in a 90 mm diameter plate was inoculated with the bacterial suspension by streaking the surface of the agar in three directions, rotating the plate approximately 60° to ensure even distribution. The plates were allowed to dry for 10 minutes before antibiotic discs were aseptically applied to the surface of the agar [22]. They were allowed a further drying period of 30 minutes and then incubated at 30°C. The diameter of zones of inhibition produced by each antibiotic disc was measured and sensitivity were read off by comparing with standard interpretative chart of the NCCLS and Fluka zone interpretative chart in accordance with WHO requirement [22].

RESULTS

Of the 138 students' sampled, *S. aureus* were isolated from 20 students giving a total prevalence of 14.5%. 16 (11.6%) isolates were from male samples and 4 (2.9%) from female samples. Nine (6.5%) isolates showed resistance to oxacillin. Thus, the overall prevalence of methicillin resistant *Staphylococcus aureus* in this study was 6.5% (Table 1). MRSA total prevalence among males was 5.8% while among the females it was 0.7% (Table 1). Of the 80 males sampled, prevalence of *S. aureus* and MRSA were 20% and 10% respectively while out of 58 females examined prevalence of *S. aureus* and MRSA were 6.9% and 1.7% respectively. The percentage resistances of *S. aureus* increase in order gentamicin (0%), vancomycin and ofloxacin (25%), ceftriaxone (30%), cefuroxime (35%), oxacillin (45%), erythromycin (60%), amoxicillin (75%), and ceftazidime (90%) (Table 2). No resistance was recorded against gentamicin whereas 25% resistance was recorded against vancomycin and ofloxacin. The highest resistance (90%) was recorded against ceftazidime (Table 2).

Table 1: Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) in relation to gender of students (n=138).

Gender	Number of gender	Number of *S. aureus* (%)	Number of MRSA (%)
Male	80	16 (11.6)	8 (5.8)
Female	58	4 (2.9)	1 (0.7)
Total	138	20 (14.5)	9 (6.5)

Table 2: Mean inhibition diameter, percentage sensitivity and resistance of *Staphylococcus aureus* against the various antibiotics

Antibiotics	No. of *S. aureus* sensitive to the various antibiotics (No. resistance)	Mean zone of inhibition (mm)	Percentage resistance	Percentage sensitive
Oxacillin 5µg	11(9)	19.5 ± 2.40	45	55
Gentamicin 10µg	20 (0)	18.65 ± 1.62	0	100
Ceftriaxone 30µg	14 (6)	21.64 ± 1.41	30	70
Amoxicillin 30µg	5 (15)	20 ± 2.21	75	25
Vancomycin 30µg	15 (5)	20 ± 1.60	25	75
Ofloxacin 5µg	15 (5)	20.79 ± 2.78	25	75
Ceftazidime 30µg	2 (18)	19.5 ± 2.78	90	10
Erythromycin 30µg	8 (12)	24.2 ± 2.53	60	40
Cefuroxime 30µg	13 (7)	22.23 ±1.27	35	65

DISCUSSION

Knowledge of the epidemiology of carrier rate of such organisms as methicillin resistant *Staphylococcus aureus* in a given community is very important for appropriate decision-making in preparation to combat infections they may cause due to their spread to susceptible members of the community. The prevalence of MRSA in our study (6.5%) was lower than 16.0% [25] recorded in one Nigerian University and 18.7% [26] recorded in a College of Education in Nigeria. The prevalence was higher than 0.65% [27] and 5.8% [28] reported in Universities outside of Nigeria. However, the prevalence of MRSA recorded in this study was lower than 7.4% [29] and 13.1% [30] recorded in two Universities outside Nigeria. Prevalence of MRSA among students of tertiary institutions of learning varies from

University to University. Prevalence of *Staphylococcus aureus* and invariably MRSA appear to vary in different localities.

In the present study, prevalence of MRSA was higher in males than in females. This position is supported by Ugwu and associate workers [25] who recorded higher nasal carriage of *S. aureus* and MRSA among males.

Among hospitalized patients, prevalence of MRSA varies widely ranging from 1.4% to 50% [31, 32], Solayide other researchers [21] recorded MRSA nasal carriage of 10% among elderly people in Lagos, Nigeria.

Staphylococcus aureus has the ability to change over time; hence MRSA will continue to be a problem in the future. In most tertiary institution, accommodation is a big challenge to many students. This often results in overcrowding of students in the hostels. Nasal or throat carriers of MRSA constitute reservoirs of the organism. The more the numbers of carriers of MRSA in a given community the easier the spread of the organism to their neighbors. Susceptible students in such setting are at great risk of infection by this organism posting a big challenge to effective treatment. The infection may spread readily also to immunocompromised individuals in the school.

The imprudent use of antibiotics in hospitals, underdose use of antibiotics, the easy accessibility of antibiotics without prescription; all result to increase in the chances of emergence of resistant strains. Spontaneous mutation of bacteria can also result in resistance. Antibiotics remove drug-sensitive competitors, leaving resistant bacteria behind to multiply as a result of natural selection [33].

Gentamicin, vancomycin, ofloxacin are the most effective agents against the isolated strains of *Staphylococcus aureus* in this study. The use of vancomycin as a substitute for methicillin [30] is supported by this work as the organism was 75% sensitive against vancomycin. Sensitivity against gentamicin (100%) was, however, better as there was none of the isolates that resisted the antibiotic.

Since complete eradication of MRSA may not be feasible, control of transmission appears to be the most appropriate remedy. This may be actualized through avoidance of transmission through hand contamination by practice of proper hand washing and avoidance of overcrowding because of transmission by nasal carriers. Administration of broad-spectrum

antibiotics for treating infections also increases the rate of MRSA and other resistant bacteria. Therefore, chemotherapy should rather be guided by sensitivity results of the incriminated organism against a set of antibiotics.

Detection of MRSA in the medical laboratories is of great importance. Likewise is the awareness of the route of its transmission through nasal and throat carriers in the community, institutions, army barracks and in prisons and other such living conditions that make close contact possible. Data on the prevalence study of MRSA in Nigerian Universities is inadequate. Many more studies are required to ascertain the risk level of MRSA and associated risk factors in tertiary institutions of learning in Nigeria.

CONCLUSION

MRSA will continue to be unless drastic efforts are adopted to create awareness especially in tertiary institution of learning. We report a total MRSA prevalence of 6.5% consisting of 5.8% males and 0.7% females in a Nigerian tertiary institution of learning in this study. This study has been done only in a few universities in Nigeria. More reports may give a clearer picture of the carriage rate of this organism in Nigerian higher institutions of learning.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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