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Methicillin Resistant *Staphylococcus aureus* in Wound Swabs of Patients Attending a Public Hospital in Warri Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author OEA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Author ORE managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to isolate, identify and determining the prevalence of Methicillin Resistant *Staphylococcus aureus* from wound swabs of patients attending a public hospital in Warri, Delta state.
Study Design: An experimental design.
Place and Duration of Study: The initial aspect of the work was carried out in public hospital Warri while the second phase was carried out in the medical microbiology laboratory of the University of Port Harcourt.
Methodology: A total of one hundred (100) wound swab samples were collected from patients in four (4) wards in a public hospital in Warri Delta state Nigeria. Samples were collected from male medical ward (MMW), male surgical ward (MSW), female medical ward (FMW) and female surgical ward (FSW). Sixty-eight (68) samples were from males and thirty-two (32) from females. The samples were evaluated using Mannitol salt agar and Oxoid BrillianceTM MRSA agar. The isolates

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were identified using morphology, colour indication, Gram reaction and biochemical tests. Antibiotic sensitivity was carried out using Kirby Bauer disc diffusion method.
Results: The study showed that 52 samples had significant growth of *Staphylococcus aureus* while 48 had no significant growth of the same bacteria. Nineteen (19) out of the fifty-two were confirmed to be MRSA. Erythromycin was found to be the most sensitive drug after the antibiotics sensitivity test while cloxacillin and cefuroxime showed the highest resistance using Kirby Bauer' disc diffusion technique. This study showed that the prevalence of MRSA in the public hospital studied is 19%.

Keywords: Antibiotics susceptibility; public hospital; MRSA; s wound.

1. INTRODUCTION

Resistance of microorganisms to commonly used antibiotics has become a worldwide problem [1]. The prevalence of resistant *S. aureus* has received considerable attention because of the role of this microorganism as a nosocomial pathogen in immune-compromised host. In particular, Methicillin Resistant *Staphylococcus aureus* (MRSA) has become a notorious etiologic agent for a wide variety of infections and it is one of the most important nosocomial pathogens worldwide. MRSA accounts for approximately 30-50% of hospital acquired *S. aureus* infections. MRSA is a strain of *S. aureus* that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class [2]. Its resistance to methicillin is due to the integration of *Staphylococcus* cassette chromosome *mec* (SCC*mec*) element into *S. aureus.* MRSA are most common in hospitals and other institutional health-care settings, such as nursing homes, where they tend to affect older people, those who are very ill, and people with a weakened immune system [3]. Methicillin and other semi synthetic penicillin represented important advances in the treatment of *S. aureus* infections, but within 2 years of their introduction the first clinical isolates of MRSA appeared [4]. During the past decade, healthy people within community settings have developed infections with new MRSA strains (community-associated MRSA), representing a new burden of disease [5]. CA-MRSA infections are usually manifested as skin infections, such as pimples and boils, and occur in otherwise healthy people. They are often misdiagnosed as “spider bites” and can cause serious infections if not treated early. *S. aureus* is most often spread to others by contaminated hands. The skin and mucous membranes are usually effective barriers against infection, however, if these barriers are breached (e.g. skin damage due to trauma, or mucosal damage due to viral infection), the organism may gain access to underlying tissues or the bloodstream and cause infection. Persons who are immune-compromised or who have invasive medical devices are particularly vulnerable to infection [6].

In the developing world, mortality associated with severe *S. aureus* infections far exceeds that in developed countries. Also recent studies have identified *S. aureus* as the main etiological agent of many infections in sub-Saharan Africa and a number of investigations have reported that *S. aureus* is among the most frequently encountered bacterial species in microbiology laboratories in Nigeria [7]. Recent reports have indicated that the prevalence of hospital-associated MRSA varies in health care institutions [6]. In the past 50 years, *S. aureus* has evolved to possess an impressive array of resistance tools that provide defenses against most antibiotics. However, acquisition of the MecA gene, which is a part of the mobile genetic element of the Staphylococcal cassette chromosome Mec (SCC*mec*) that is inserted into the staphylococcal chromosome. The production of the penicillin binding proteins has been altered and now results in the production of penicillin binding protein 2a(PBP2a). PBP2a has a reduced affinity for binding to beta-lactam antibiotics. As such PBP2a catalyzes the transpeptidization reaction required for peptidoglycan cross-linking, enabling cell wall synthesis in the presence of antibiotics. Resistance is therefore a consequence of the inability of PBP2a to interact with beta-lactam moieties.

This study is aimed at isolating, identifying and determining the prevalence of MRSA from wounds of patients attending a public hospital in Warri, Delta state.

2. MATERIALS AND METHODS

2.1 Source of Specimens

The study was carried out from April to December 2014. The specimens were obtained

from male and female patients both young and old attending a public hospital in Warri, Delta state Nigeria. A total of 100 specimens of wound swab from various etiologies were collected from various wards in the hospital.

2.2 Collection of Specimens

All specimens were collected by a medical scientist or an experienced nursing officer. Sterile swab sticks were used to aseptically collect wound exudates and pus from parts of the wound showing signs of infection. The specimen were collected from the following wards; male medical, male surgical, female medical and female surgical wards in the hospital. Swabs were stored in the refrigerator after collection and analyzed within 24 hours of collection.

2.3 Media Preparation

The media used were commercially available, they were; Mannitol salt agar (Lab M, UK), Nutrient agar (Lab M, UK), Mueller-Hinton broth, Mueller-Hinton agar (L: X biotech, USA). Tryptone soya broth (Oxiod, London). The above media were prepared and sterilized according to manufacturer's instruction where necessary. Brilliance oxiod MRSA agar (Oxiod, London) was ordered as prepared plates.

2.4 Preparation of Selective Supplements

2.4.1 Cefoxitn

The supplement was prepared by weighing 0.7 mg of powdered cefoxitin and suspended in 200 ml of distilled water; the solution was swirled severally to dissolve completely.

2.4.2 Aztreonam

The supplement was prepared by weighing 15 mg of powdered Aztreonam which was suspended in a solution containing 100 ml of dimethylformamide and 100 ml of methanol. The solution was swirled and agitated vigorously to dissolve completely.

2.5 Sodium Chloride

Sodium chloride was prepared by weighing 6.5 g of sodium chloride which was suspended in 100 ml of distilled water. It was left to stand for a few minutes and then swirled to dissolve completely.

2.5.1 Bacterial isolation

The swab specimens were inoculated on the surface of already prepared Mannitol salt agar using the streak plate method and were incubated aerobically for 24-48 hours at a temperature of 37℃ for the isolation of *S. aureus*. After incubation, the plates were observed for distinct colony growths which were further sub cultured on nutrient agar plates to obtain pure cultures.

2.5.2 Purification and preservation of isolates

From the primary culture, well-spaced discrete colonies were picked with a sterile wire loop and streaked on the Mannitol salt agar plates and incubated at 37℃ for 24 hours. Discrete colonies were picked using a sterile wire loop and transferred aseptically to nutrient agar slants in bijou bottles to avoid contamination. The agar slants were properly closed and incubated for 24 hours at 37℃ to allow growth before preservation in the refrigerator at 5-20℃. This served as stock cultures for storage and further analysis at 4℃.

2.5.3 Specimen analysis

From the stock cultures, the organisms were revived by sub culturing in an already prepared Mannitol salt agar plates from which distinct colonies were picked and inoculated into 10 ml of Mueller-Hinton broth supplemented with 6.5% sodium chloride and was incubated at 37℃ for 16-20 hours. One (1) ml of the enriched Mueller-Hinton broth was inoculated into 9 ml of Tryptone soya broth containing 10 µl of 3.5 mg/l cefoxitin and 75 mg/l aztreonam and was incubated further for a 16-20 hours at 37℃.

Using a sterile wire loop, one loopful of Tryptone soya broth was inoculated onto Brilliance oxiod MRSA agar using the streak plate method. This was done aseptically and was incubated for 24-48 hours at 37℃.

Based on morphology and color, presumptive MRSA colonies which appear denim blue on the Brilliance chromogenic agar plates were subjected to Gram reaction, catalase and coagulase test for proper identification.

The isolates were identified using colonial morphology characteristics. Gram staining was also carried out using the method described by Cheesbrough [8,9]. Biochemical test such as

catalase, coagulase, urease, citrate, indole, sugar fermentation and oxidase test were used to identify the isolates.

2.6 Antimicrobial Susceptibility Test

Antimicrobial susceptibility was carried out using Kirby Bauer method. An inoculating loop was used to pick a pure colony and was subculture into 5 ml of sterile nutrient broth (Oxoid) and incubated for a few hours at 37℃ until it became slightly turbid and the turbidity of each suspension was then matched to standard turbidity (0.5 Mac Farland Standard). A sterile cotton swab was dipped into standardized bacterial test suspension of each isolate and used to evenly inoculate the entire surface of the dried Petri dish containing Mueller-Hinton agar, a hockey stick was used to spread culture over the entire petri dish and allowed to dry for 5 minutes. Using a sterile forceps, a multiple disc containing a total of eight (8) antibiotics, Ofloxacin (5 µg), Cloxacillin (10 µg), Erythromycin (15 µg), Gentamycin (10 µg), Ceftazidime (30 µg), cefuroxime (30 µg), Ceftriaxone (30 µg), Augmentin (15 µg) was placed and lightly pressed on the agar plate containing the test organism. The inoculated petri dishes carrying antibiotics were then incubated at 37℃ for 18 - 24 hours after which the diameter of the zone of inhibition around each antibiotic disc was measured and isolates were classified as resistant or sensitive [10].

3. RESULTS AND DISCUSSION

A total of one hundred wound swab samples were collected from different wards in a public hospital in Warri, Nigeria. Sixty-eight were from males and thirty-two were from females. Fifty-two isolates of the 100 samples after being identified by their colonial morphological characteristics, Gram reaction and biochemical tests were found to be *S. aureus*. The isolates were negative for indole, urease, methyl-red and oxidase test. They were non- motile and positive for catalase, coagulase and citrate test. Thirty-eight of these were from males and fourteen were from females. From the100 samples, twenty-six samples were collected from male medical ward, forty-two from male surgical ward, thirteen from female medical ward and nineteen female surgical wards. The male surgical ward had the highest percentage distribution of *S. aureus* with 27 (52%), this was followed by the male medical ward with a frequency of 11 (21.1%) and then the female surgical ward with 9 (17.3%) and the least was female medical with a frequency of 5 (9.6%). From the fifty-two (52) *S. aureus* isolates, methicillin-sensitive *S. aureus* was found more with a percentage of 63.5% and MRSA at 36.5%. The 19 MRSA isolated were from wound sites such as boils, burns, surgical site, diabetic foot, bed sore and gunshot sites as illustrated below in Fig. 1. The least MRSA isolates were from bed sore and the gun shot. The MRSA at these two sites were not significant. MRSA were found more in boils, burns, surgical site and diabetic foot.

Fifty-two *S. aureus* isolates on agar plates were circular shapes, 1-3 mm in size, yellow in Mannitol salt agar, convex, opaque and smooth, isolates were also Gram positive cocci, catalase positive and coagulase positive. Other biochemical tests were also used to identify the organism. The colonies which were denim blue confirmed the presence of MRSA on the Brilliance oxoid agar. The nineteen (19) MRSA isolates obtained were analyzed for their antibiotic resistance profile. Maximum resistant was observed against Cloxacillin 19 (100%) and Cefuromine 19 (100%). Results showed that 18(94.7%) isolates were resistant to Ceftazidime and Ceftriazone while 17 (89.5%) isolates were resistant to Augumentin. Least resistance was observed against Erythromycin 5 (26.3%), Ofloxacin 5 (26.3%) and Gentamicin 7 (36.8%) as shown in Fig. 2. Erythromycin showed the highest susceptibility at 14 (73.7%). It was active against 14 out of the 19 MRSA isolates and was closely followed by Gentamicin and Ofloxacin which were active against a total of 12 (63.2%) isolates each as represented in Fig. 2. The chart shows 5% significant percentage value of each antibiotics.

Percentage resistance was highest to Cefuroxime 100% and Cloxacillin 100%, and Erythromycin and Ofloxacin showed the lowest resistance at 26.3% as represented in Fig. 3.

Two MRSA isolates showed an intermediate pattern towards Ofloxacin at 10.5%. Wounds provide favorable site(s) for microorganisms to grow and multiply leading to infection [11]. Methicillin resistance detection in staphylococci can be problematic in the clinical microbiology laboratory because of the heterogeneity of the bacterium under test. The detection of resistance in these isolates has been troubled due to variability in standard techniques used in determining methicillin resistance [12]. Although this study did not include polymerase chain

reaction (PCR) to detect *Mec*A gene which is regarded as the gold standard for determining methicillin resistance, Brilliance MRSA agar and sensitivity to or not with 10 µg cloxacillin discs were used. In this study, out of the 52 *S. aureus* isolates, 19 (36.5%) MRSA was recorded. This is lower than the study in Zaria by Onanuga and Oshogbo [13]. Results obtained from a study carried out in Kano showed higher prevalence rates of 69.0%, 47.8%, and 28.6% respectively. The differences in the design of these studies such as sample size and method of MRSA identification may account for the disparity in prevalence rate [14].

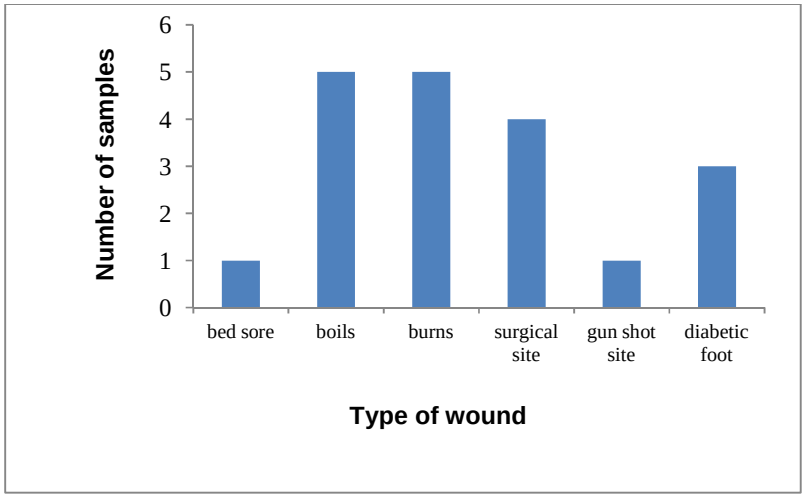

Fig. 1. Distribution of MRSA from different wound sites

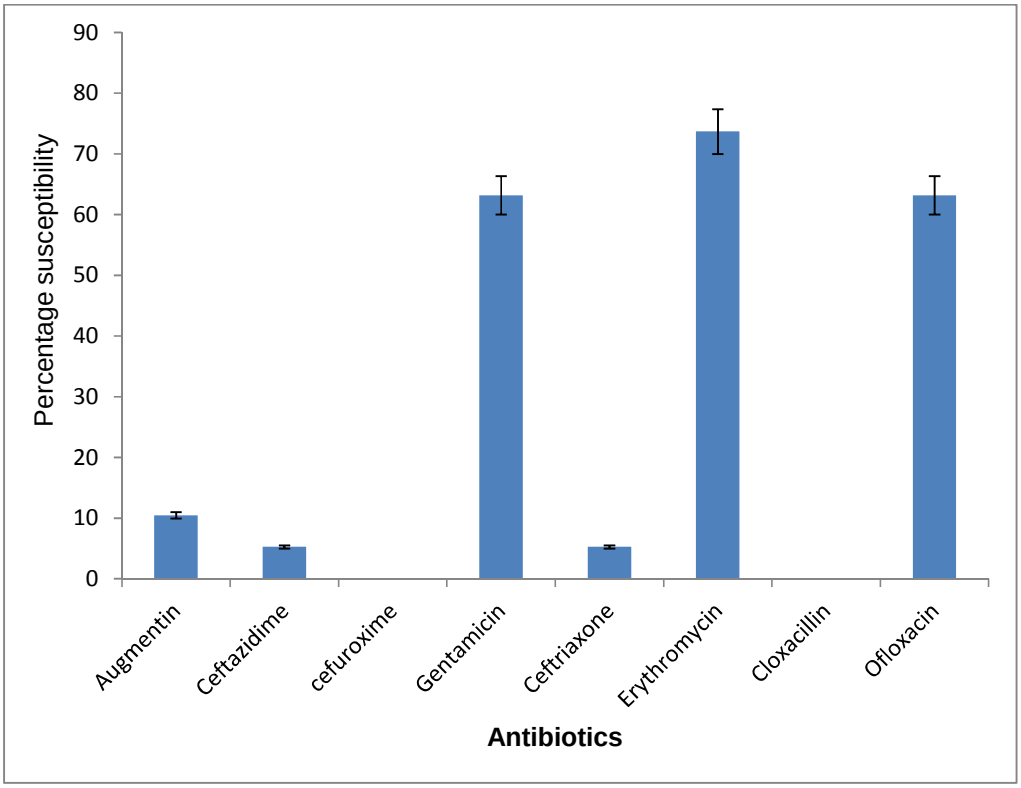

Fig. 2. Percentage of antibiotics susceptibility to MRSA isolates

Note: Error bars of antibiotics susceptibility to MRSA are indicated for each antibiotics showing 5% value

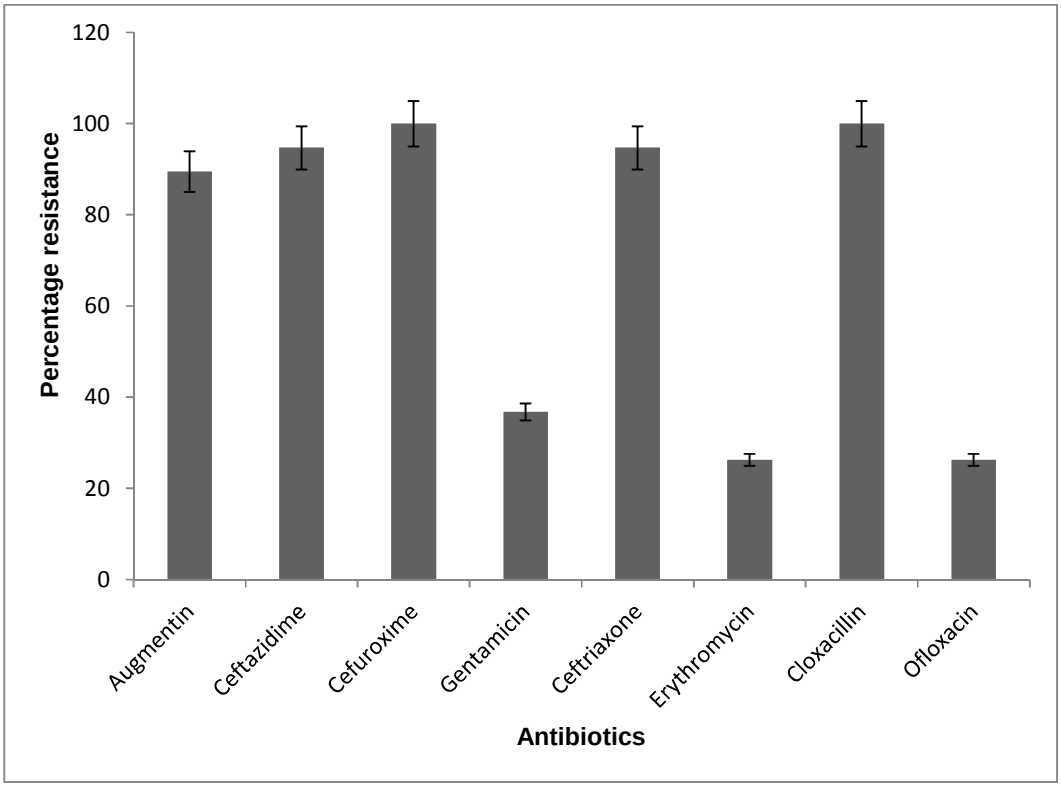

Fig. 3. Percentage of antibiotics resistance indicating 5% value

The MRSA isolated in this study showed 100% resistance to Cloxacilin, Cefuroxime and Ceftazidime. Identified MRSA isolates showed 94.7% resistance to Ceftriaxone which support the findings that MRSA strains are equally resistant to all beta-lactam antibiotics but had low resistance to Erythromycin (26.3%). The low resistance observed by the MRSA isolated in this study towards Erythromycin is lower than the reports of Hantash who observed a resistivity of 38 % to Erythromycin [15]. In this study, a high susceptibility to Ofloxacin, a Fluoroquinolone and Gentamycin in the MRSA isolated were recorded. The high sensitivity to Ofloxacin (63.2%) and Gentamycin (63.2%) supports some previous reports done in Bauchi [16] and in Jos [17]. The susceptibility of gentamycin, an aminoglycoside though very cheap, may be due to the complexity of the aminoglycoside and the route of administration while the fluroquinolones are newer drugs with mode of action centered on inhibition of the DNA replication which stops the multiplication of bacteria cells and are relatively expensive therefore are more likely not available for abuse [13]. Susceptibility of the MRSA isolated to Ofloxacin and Gentamycin supports the findings of Onanuga [13] which shows that these non-beta-lactam antibiotics may provide an opportunity for the recommendation of these drugs for the treatment of MRSA infections.

The prevalence of MRSA was found to be higher in Surgical Wards than in the General Wards. The high prevalence in the study could be due to a number of factors which include poor adherence to universal precautions by the health workers especially the use of gloves and proper hand washing in between patients. The other reason for the higher prevalence is indiscriminate and misuse of antimicrobials. This leads to selective pressure and antimicrobial resistance by microorganisms including MRSA.

Prevalence and spread of MRSA can be reduced by having a guide or an enlighten program on antibiotic stewardship especially in developing countries. This will promote the appropriate use of antimicrobials thereby reducing resistance and spread of infections caused by drug resistant organisms. Healthcare workers who carry MRSA in their nostrils should be advised to receive proper medical treatment to avoid the spread of the organism. In the Netherlands and Scandinavian countries, where extensive control

programs are in effect, human-adapted hospital strains of MRSA are rare among the people [18]. Less than 1% of *S. aureus* isolates from clinical specimens are Methicillin Resistant, and nasal carriage occurs in 0.03% of people admitted to the hospital in Netherland [19]. Danish control programs decreased the percentage of MRSA among *S. aureus* from 15% in 1971 to 0.2% in 1984 [19]. In the United States, approximately 1.5% of the population carried MRSA in 2003-2004 [20]. One recent U.S. study reported that, overall, 5.6% of its study population was colonized [21]. Based on recent studies it is clear that MRSA has become a global nosocomial pathogen with attendant therapeutic problems. Since complete eradication of MRSA may not be possible, control of transmission seems to be the appropriate goal. The first and the most effective way among these are to avoid transmission through hand contamination from personnel to patients. The use of broad-spectrum antibiotics for treating infections also increases the rate of MRSA and other resistant bacteria. Therefore chemotherapy should be guided by sensitivity of the probable causative organism. Accurate detection of MRSA by clinical laboratories is of great importance; also awareness should be created about the route of its transmission and the risk factors for infection such as indiscriminate antimicrobial use.

4. CONCLUSION

Methicillin Resistant *Staphylococcus aureus* is highly prevalent (19%) among patients with wound in a public hospital in Warri, Delta State. This study shows that there is need for early detection of MRSA as it will help in reducing the risk of hospital-acquired infections. Control of MRSA infections is essential and can be achieved by proper implementation of hospital control measures and regular surveillance activity for proper documentation and control measures aimed at combating spread.

The use of antibiotics inevitably requires the need for *in vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories. There should be an effective infection control committee to coordinate implementation of its policies especially regular hand washing and strict ward antisepsis to reduce nosocomial infections. Therefore, there should also be strict antibiotic prescription policies enforced by the appropriate authorities to contain the abuse of antibiotics and reduce acquisition of resistance by pathogens. Educational awareness should be encouraged to update health care workers with new intervention strategy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Khorvash F, Abdi F, Ataei B, Neisiani HF, Kashani HH, Narimani T. Nasal carriage of *Staphylococcus aureus*: Frequency and antibiotic resistance in healthy adults. J Res Med. 2012;2:139-232.
2. Que YA, Moreillon P. *Staphylococcus aureus* (Including Toxic Shock); In: Bennett JE, Dolin R. Eds. Principles and Practice of Infectious Diseases, 7th Ed. Philadelphia, Pa: Elsevier Churchill Livingstone. 2009; 195:2543-2578.
3. Davis KA, Stewart JJ, Crouch HK. Methicillin-Resistant *Staphylococcus aureus* (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clin Infect Dis. 2007;39:776-782.
4. Filice GA, Nyman JA, Lexau C, Lees CH, Bockstedt LA, Como-Sabetti K, Lesher JL, Lynfield R. Excess costs and utilization associated with methicillin resistance for patients with *Staphylococcus aureus* infection. Infect Control Hosp Epi. 2010;31: 4.
5. Popovich KJ, Weinstein RA, Hota B. Are Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? Clin Infect Dis. 2008;46:787–794.
6. Akujobi NC, Ilo IA, Egwuatu CC, Ezeanya CC. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among healthcare workers in a tertiary institution in Nigeria. Orient J Med. 2013;25:82-87.
7. Olowe OA, Kukoyi OO, Taiwo SS, Ojurongbe O, Opaleye OO, Bolaji OS, Adegoke AA, Makanjuola OB, Ogbolu DO, Terry Alli OA. Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from Ekiti State, Nigeria. Infect Drug Resist. 2013; 6:82-87.
8. Neetu PJ, Sevanan M. Biofilm resistant *Staphylococus aureus* and their antibiotic susceptibility pattern: An *in vitro* study. Current Research in Bacteriology. 2014; 7(1):1- 11.

9. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Ed. Cambridge University Press. 2006;7:64-66.
10. CLSI. Clinical and Laboratory Standards Institute, Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolates; Approved standard-Third edition, CLSI document M31-A3, Clinical and Laboratory Standards Institute, Wayne Pennsylvania, USA. 2008;28:1-99.
11. Kumar S, Easow UM, Singh R, Umdevi S, et al. Prevalence and current antibiogram of Staphylococci isolated from various clinical specimens in a tertiary care hospital in Pondicherry. Internet J. Microbial. 2012;10:1.
12. Terry-Alli OA, Ogbolu DO, Akorede E, Onemu OM, Okanlawon BM. Distribution of *mecA* gene amongst *Staphylococcus aureus* isolates from South-western Nigeria. Afri J Biomed Res. 2011;14:9–16.
13. Onanuga A, Temedie TC. Nasal carriage of multi-drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassoma in Niger Delta Region of Nigeria. Afr Health Sci. 2011;11:176-181.
14. Nwankwo EOK, Abdulhadi S, Magagi A, Ihesiulor G. Methicillin Resistant *Staphylococcus aureus* (MRSA) and their antibiotic sensitivity pattern in Kano, Nigeria. Afr J Clin Exper Microbiol. 2010; 11:129-136.
15. Nickerson EK, West TE, Day SJ. *Staphylococcus aureus* disease and drug resistance in resource-limited Countries in South and East Asia. Lancet Infec Dis. 2009;9:130-135.
16. Ghamba PE, Mangoro ZM, Waza DE. Reoccurrence and distribution of Methicillin resistant *Staphylococcus aureus* (MRSA) in clinical specimens in Bauchi, North eastern Nigeria. J Med Sci. 2012;3:506-511.
17. Ajoke OI, Okeke IO, Odeyemi OA, Okwori AEJ. Prevalence of methicillin-resistant *Staphylococcus aureus* from healthy community individuals volunteers in Jos south, Nigeria. J Micr Biotech Food Sci. 2012;1:1389-1405.
18. Kluytmans JA. Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency? Clin Microbiol Infect. 2010;16:11-5.
19. Leonard FC, Markey BK. Methicillin-resistant *Staphylococcus aureus* in animals: A review. Vet J. 2008;175:27-36.
20. Otter JA, French GL. Molecular epidemiology of community-associated methicillin- resistant *Staphylococcus aureus* in Europe. Lancet Infect Dis. 2010; 10:137-239.
21. Kottler S, Middleton JR, Perry J, Weese JS, Cohn LA. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* carriage in three populations. J Vet Intern Med. 2010; 24:132-139.

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