ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JANUARY 2019 VOL20 No.1
AJCEM/1903 http://www.ajol.info/journals/ajcem
COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v20i1.3
AFR. J. CLN. EXPER. MICROBIOL. 20 (1): 17-24

CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM APPARENTLY HEALTHY INDIVIDUALS IN MALETE, KWARA STATE, NIGERIA

Bale, M. I[1]; *Babatunde, S. K[2], Adedayo, M. R..[1] Ajiboye, A. E.[1] and Ajao, A. T.[1]

[1]Department of Biosciences and Biotechnology, College of Pure and Applied Sciences, Kwara State of University, Malete, Kwara State, Nigeria; [2]Department of Biological Sciences, Faculty of Science, Kings University, Odeomu, Osun State, Nigeria

***Correspondence: Dr. Shola Kola Babatunde, Department of Biological Sciences, Faculty of Science, Kings University, Odeomu, Osun State, Nigeria. E-mail: sk.babatunde@kingsuniversity.edu.ng or sholakemibab@yahoo.com Phone: 0803503550: 08074390025**

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common and continuously growing cause of nosocomial and community-acquired staphylococcal infections around the world. Screening for colonization with MRSA is a major aspect of control and limiting the spread of infections cause by this organism. We investigated the carriage of MRSA among apparently healthy individuals in four rural villages: Eleburu, Tapa, Atere and Apo all around semi-urban town-Malete, in Moro Local Government of Kwara State, Nigeria.

Methods: Nasal swabs were collected from volunteered individuals and were cultured on mannitol salt agar and blood agar for isolation and identification of *Staph aureus* using standard microbiological techniques. Susceptibility to cefoxitin disc (30 µg) was used to determine MRSA status of the isolates. Molecular method was used to detect the gene responsible for resistance among MRSA isolates. Antimicrobial susceptibility test to commonly prescribed antibiotics was carried out using discs diffusion method.

Results: Total number of individuals carrying *Staph aureus* in their nostrils was 42 (37.2 %). Antibiotics susceptibility profile of *Staph aureus* isolates showed 100 % resistance to cefuroxime, cefotaxime, cloxacillin and augmentin, and were 87 %, 81 %, 69 % and 23.8 % and 19 % resistant to tetracycline, ceftriaxone, erythromycin, ofloxacin and gentamicin respectively. A total of 6 (14%) Community –Acquired MRSA (CA-MRSA) isolates were recovered from individuals living in these villages. Molecular method detected *muc* and *mecA* genes in all the 6 (100%) CA-MRSA isolates and *lukS-lukF* was detected in 3 (50%) of the isolates.

Conclusion: Detection of CA-MRSA strains among these rural dweller indicates that they are harbouring enhance virulence organism that may manifest a more severe disease condition. The danger associated with high prevalence of multidrug resistant *Staph aureus* and CA-MRSA; and its consequential effects of poor drug administration in Nigeria was discussed. There is need to establish a more strict legislation and enforcement on drug control; and a body that would monitor production and appropriate use of antibiotics in the Nigeria.

KEYWORDS: *CA-MRSA, Staph aureus, Antibiotics, Rural Villages and Molecular Characterization*

CARACTÉRISATION DES ISOLATS DE STAPHYLOCOCCUS AUREUS RÉSISTANTS À LA MÉTHICILLINE PROVENANT DE PERSONNES APPAREMMENT SAINS À MALETE, ÉTAT DE KWARA (NIGÉRIA)

Bale, M. I[1]; *Babatunde, S. K[2], Adedayo, M. R..[1] Ajiboye, A. E.[1] and Ajao, A. T.[1]

1 Département des biosciences et de la biotechnologie, Collège des sciences pures et appliquées, Université de l'État de Kwara, Malete, État de Kwara, Nigéria; 2Département des sciences biologiques, faculté des sciences, université Kings, Odeomu, État d'Osun, Nigéria
*** Correspondance: Dr. Shola Kola Babatunde, Département des sciences biologiques, Faculté des sciences, Université Kings, Odeomu, État d'Osun, Nigéria. Adresse électronique: sk.babatunde@kingsuniversity.edu.ng ou sholakemibab@yahoo.com Téléphone: 0803503550: 08074390025**

ABSTRAIT
Contexte: Le Staphylococcus aureus résistant à la méthicilline (SARM) est une cause commune et en augmentation constante des infections staphylococciques nosocomiales et d'origine communautaire dans le monde. Le dépistage de la colonisation par le SARM est un aspect majeur du contrôle et de la limitation de la propagation des infections causées par cet organisme. Nous avons enquêté sur le transport de MRSA chez des individus apparemment en bonne santé dans quatre villages ruraux: Eleburu, Tapa, Atere et Apo tout autour d'une ville semi-urbaine-Malete, dans le gouvernement local de Moro dans l'État de Kwara, au Nigéria.
Méthodes: Des écouvillons nasaux ont été prélevés sur des individus volontaires et ont été cultivés sur gélose au sel de mannitol et sur gélose au sang en vue de l'isolement et de l'identification de Staph aureus à l'aide de techniques microbiologiques standard. La sensibilité à la céfoxitine (30 µg) a été utilisée pour déterminer l'état de SARM des isolats. La méthode moléculaire a été utilisée pour détecter le gène responsable de la résistance parmi les isolats de SARM. Un test de sensibilité aux antimicrobiens aux antibiotiques couramment prescrits a été réalisé à l'aide de la méthode de diffusion sur disques. Résultats: Le nombre total d'individus porteurs de Staph aureus dans leurs narines était de 42 (37,2%). Le profil de sensibilité aux antibiotiques des isolats de Staph aureus présentait une résistance à 100% au céfuroxime, au céfotaxime, à la cloxacilline et à l'augmentation, et à 87%, 81%, 69%, 23,8% et 19% respectivement.

Au total, 6 isolats (14%) de SARM acquis dans la communauté (CA-SARM) ont été retrouvés chez des individus vivant dans ces villages. La méthode moléculaire a détecté les gènes muc et mecA dans les 6 isolats (100%) de CA-MRSA et a détecté le lukS-lukF dans 3 (50%) des isolats. Conclusion: La détection de souches de SARM-CA chez ces habitants des zones rurales indique qu'elles hébergent un organisme de virulence qui pourrait se manifester par une maladie plus grave.
Le danger associé à la prévalence élevée de Staph aureus multirésistante et de CA-MRSA; et ses effets consécutifs à une mauvaise administration du médicament au Nigéria ont été discutés. Il est nécessaire de mettre en place une législation et une application plus strictes en matière de contrôle des drogues; et un organisme chargé de surveiller la production et l'utilisation appropriée des antibiotiques au Nigéria

MOTS-CLÉS: SARMAC, Staph aureus, antibiotiques, villages ruraux et caractérisation moléculaire

INTRODUCTION

Methicillin-resistance *Staph aureus* (MRSA) refers to isolates of *Staph aureus* that are resistant to antibiotics with B-lactam ring such as penicillins, cephalosporins and their derivatives from healthy individuals or patients. *Staph aureus* is an opportunistic pathogen that asymptomatically colonizing the nostrils, skin, glands and mucous membrane of both human and animal. It possesses a wide range of virulent factors and are responsible for a wide range of infections such as rashes, endovascular infections, inflammations of bones and the meninges, pneumonia, skin soft tissue infection (SSTIs), septic arthritis, endocarditis, sepsis, bloodstream infection and osteomyelitis [1], [2].

The first recorded emergence of methicillin resistance was in England in 1961 two years after introduction of methicillin [3]. MRSA organisms are also found to be resistant to other methicillin related antibiotics such as oxacillin, amoxicillin and penicillin. These organisms also acquire resistance to other groups of antibiotics that include clindamycin, cotrimoxazole, erythromycin and gentamicin [4]. The resistance is as a result of acquisition of resistant genes known as *mec A* gene which is a membrane-bound enzymes that catalyzes the transpeptidation reaction and encodes the production of PBP-2a a penicillin-binding protein-2a which is essential for cross-linkage of peptidoglycan layer [5]. Lately, a new methicillin resistance gene known as *mec*C gene has been reported and MRSA carrying this gene has been isolated from both human and animal [6], [7], [8].

Hospital acquired MRSA (HA-MRSA) has always been associated with prior exposure to hospitals and healthcare centres, however, recently MRSA infection in healthy individuals in community is on increase and it has been confirmed to be responsible for morbidity and mortality in various parts of the world [9], [10], and is referred to as community acquired methicillin-resistant *Staph aureus* (CA-MRSA) [11], [12].

HA-MRSA strains are genetically distinct from CA-MRSA, CA-MRSA strains are always resistant to the B-lactam and sometimes to erythromycin but largely remain susceptible to several other antibiotics. Many outbreaks of CA-MRSA have been reported in healthy populations and institutions such as Daycare centers, Military quarters and prisons [13].

Malete, a semi-urban town is surrounded by four rural villages; all are in Moro Local Government and Kwara North Senatorial District of Kwara State of Nigeria. These villages: Apo, Eleburu, Tapa and Atere have population of less than 500 each [14]. The residents are mainly farmers and herders. There are no schools or healthcare facilities in the villages. The pupils attend primary and secondary schools at Malete and the sick ones are cared for at Cottage Hospital, Malete.

In Nigeria, there is paucity of information on MRSA, particularly CA-MRSA in rural and semi-urban populations with its antecedent danger of ignorance. In other to meet with this need, we investigated the carriage of CA-MRSA stains in the nostril of some apparently healthy individuals in rural communities of Malete, Kwara State. We also screened to detect genes responsible for MRSA in individuals that were positive to MRSA.

MATERIALS AND METHODS

Sample Collection

Visits were made to each village heads (Baale) and his elders in council and obtained consent to collect nasal swab from only volunteered members of the villages. Further approval to carry out this research was obtained from Kwara State University research and ethical committee under the Community Development Centre (CDC). All subjects that have been admitted into the Hospital or Healthcare centre and those that were on any antimicrobial treatment in the past three months prior to sample collection were excluded from study. Samples were collected with sterile cotton swab pre-wetted with sterile saline; the swab was inserted into the two anterior nares and gently rotated. Samples were collected randomly from 113 apparently healthy individuals in the four rural villages of Malete that included Apo, Eleburu, Atere and Tapa between first and third weeks of August, September and October, 2017.

Culturing method

Samples collected were transported in ice pack box within one hour of collection to the Microbiology laboratory, Department of Biosciences and Biotechnology, Kwara State University, Malete. The samples were cultured on mannitol salt agar and blood agar plates. They were incubated aerobically at 35-37 ^{0}C for 18-24 hours. On blood agar, colonies that were light yellow to whitish cream and were 1-2 mm in diameter with some producing β-haemolysis or colonies on mannitol salt agar that were pinkish-yellow and were 0.5-1 mm in diameter were picked for further

tests. Further tests carried out to confirm that the isolates were *Staph aureus* are Gram staining, catalase, coagulase and DNAse tests as previously described [15], [16].

Antimicrobial Susceptibility Test

Antimicrobial susceptibility profile of each isolated *Staph aureus* was determined using standard disc diffusion method on Mueller-Hinton agar (Mast Diagnostics, Mast Group Ltd, Merseyside, UK) incubated aerobically at 37 ^{0}C for 18-24 hours using the following antibiotics discs; Cefuroxime (CXM_{30}), Cefotaxime (CF_{30}), Cloxacillin (CX_5), Augmentin (AU_{30}), Ceftriaxone (CRO_{30}), Tetracycline (TE_{30}), Erythromycin (E_{15}), Gentamicin (GEN_{10}) and Ofloxacin (OF_5). Zones of inhibition were measured in millimeters and interpreted following criteria of the Clinical and Laboratory Standard Institute [17].

Detection of Methicillin Resistance

Resistance to methicillin was determined using disc diffusion susceptibility method as described by guideline of the [18]. This was performed with $Cefoxitin_{30\mu g}$ antibiotics disc (Oxoid, UK) on Mueller-Hinton agar plate (Mast Diagnostics, Mast Group Ltd, Merseyside, UK); where there are no zone of inhibition or zone radius measures 2 mm or less, isolates were regarded as resistant [18]

Molecular Screening and Characterization

The genomes of the isolates were extracted using QIAamp DNA Mini Kit according to manufacturer's instructions. Methicillin resistant *Staph aureus* isolated that were phenotypically identified were subjected to molecular screening for the presence of *muc* gene [19] *mecA* gene, Panton valentine leucocidin (PVL) gene and *lukS-lukF* genes [19] using the appropriate primers as listed in Table 1. The PCR condition were initiated for denaturation at 95 ^{o}C for 2 minutes followed by 30 cycles of amplification with 94 ^{o}C for 30 minutes, annealing at 50 ^{o}C for 30 seconds, extension at 72 ^{o}C for 30 seconds and final extension at 72 ^{o}C for 4 minutes. The PCR products (10 µg each for *muc, mec* and *PVL* genes) were analyzed in 1.5% agarose gel. Electrophoresis was performed in TBE buffer at 180 volts for 1 hour, and gel was subsequently stained with ethidium bromide (10 mg/ml) (Sigma, UK). DNA bands were visualized using UV-illuminator and photographed.

RESULTS

Distribution of *Staph aureus* among villages

Staph aureus was recovered from the volunteered individuals from four rural villages studied. The total numbers of apparently healthy individuals carrying *Staph aureus* in their nostrils were 42 (37.2%) n=113; however the numbers recovered varied from one village (Table 2) to the other.

TABLE 1: SEQUENCE OF PCR PRIMER SET FOR IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS*, METHICILLIN RESISTANT GENES AND PVL GENE

Target genes	primer set	Primer sequence (5′→3′)	Amplicon Size (bp)
Staphylococcus aureus	*Nuc*	F-TCAGCAAATGCATCACAAACAG R-CGTAAATGCACTTGCTTCAAG	200bp
Methicillin resistance	*Mec A*	F-AAAATCGATGGTAAAGGTTGGC R-AGTTCTGCAGTACCGGATTTGC	500
Panton valentine leucocidin	(*lukS-lukF*)	F-ATCATTAGGTAAAATGTCTGGACATGATCCA R-GCATCAAGTGTATTGGATAGCAAAAGC	433

TABLE 2: *STAPH AUREUS* DISTRIBUTION IN VILLAGES

Villages	Total Sample Collected		Staph aureus isolated	
	Number	Percentage (%)	Number	Percentage (%)
Apo	38	34.0	13	34.2
Eleburu	27	24.0	9	33.3
Tapa	23	20.0	12	52.2
Atere	25	22.0	8	32.0
Total	**113**	**100**	**42**	**37.2**

Resistance Profile of CA-MRSA isolates to antibiotics

The percentage distribution of CA-MRSA showed that 42 (100 %) were resistance to cefuroxime, cefotaxime, cloxacillin and augmentin, while 34 (80.9 %) were resistant to ceftriaxone, 35 (87.5 %) are resistant to tetracycline, 29 (69.1 %) were resistant to erythromycin, 8 (19.1 %) were resistant to gentamicin and 10 (23.8 %) were resistant to ofloxacin.

Molecular analysis of the isolated MRSA

Molecular analysis on the 6 isolates that were methicillin resistant *Staph aureus* showed that *muc and mec A* genes were detected in all the 6 isolates (Plates 1; 2). While only 3 of the six Methicillin resistant isolates had *lukS-lukF* (Plate 3) and only 3 of the six methicillin resistant *Staph aureus* isolates had PVL gene detected (Plates 3).

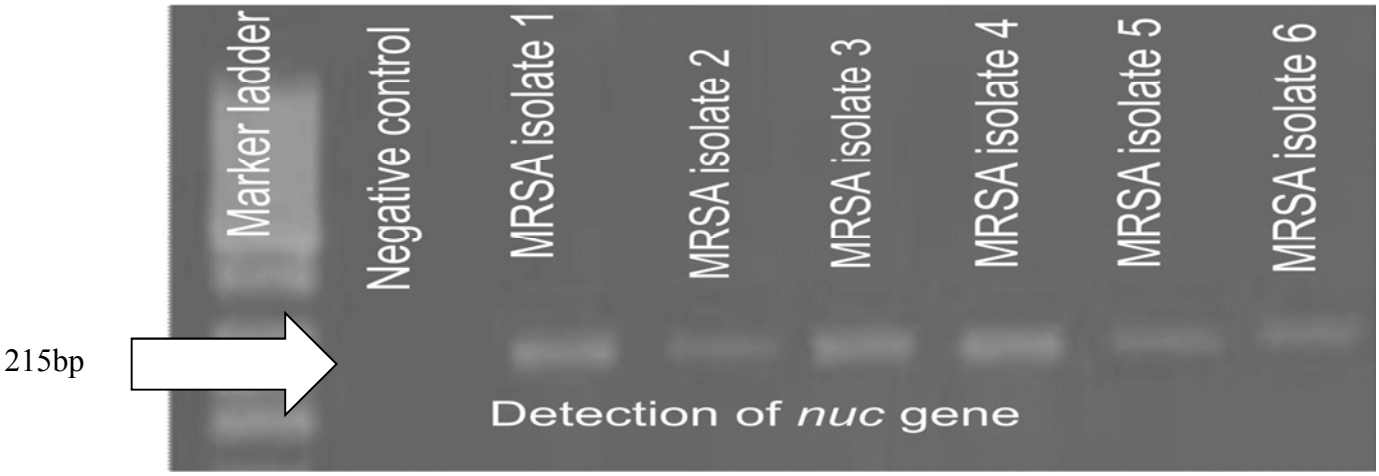

PLATE 1: PCR PRODUCTS AFTER GEL ELECTROPHORESIS SHOWING AMPLICONS DETECTION OF THE *MUC* GENES OF *S. AUREUS* ISOLATES.

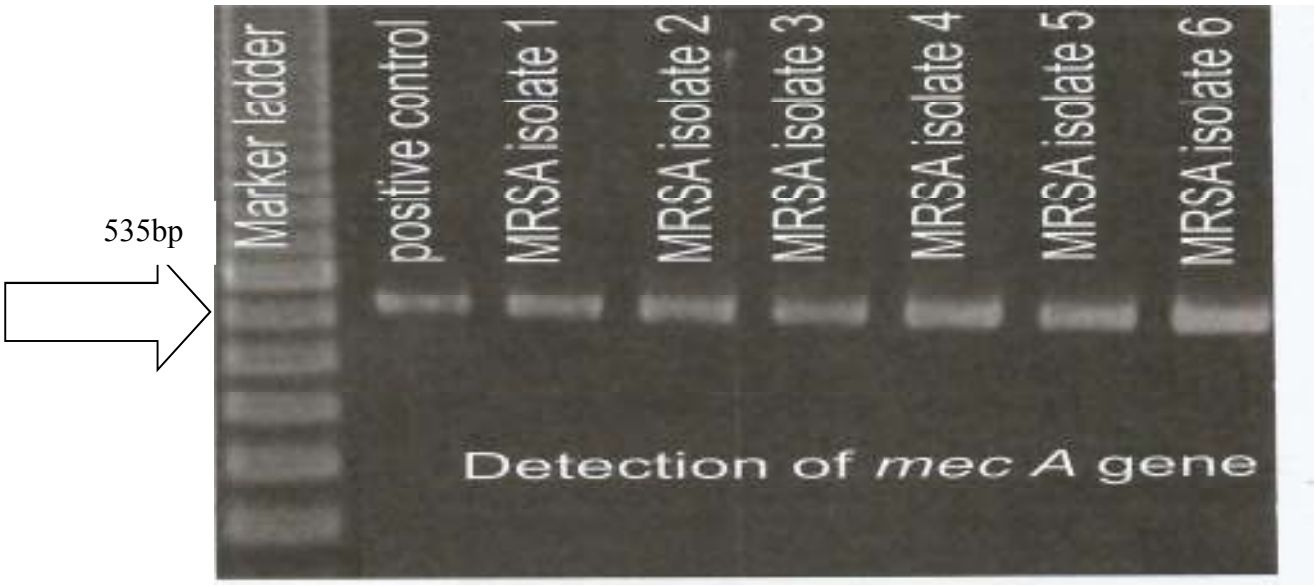

PLATE 2: PCR PRODUCTS AFTER GEL ELECTROPHORESIS SHOWING AMPLICONS FOR *MEC A* (535BP). DNA MOLECULAR WEIGHT MARKER (M) (535BP)

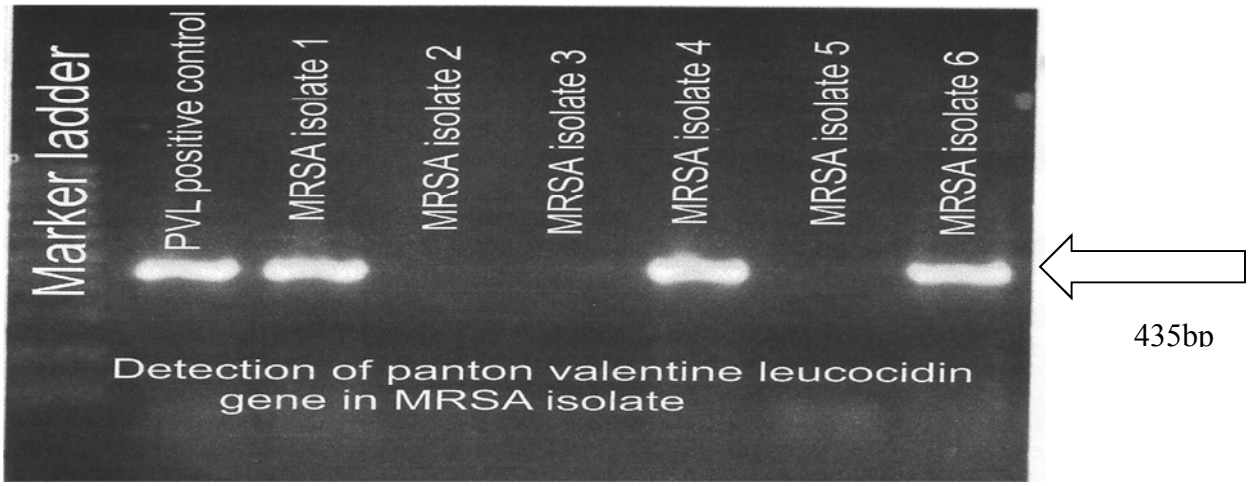

PLATE 3: PCR PRODUCTS AFTER GEL ELECTROPHORESIS SHOWING AMPLICON FOR *PVL* GENE DETECTED IN ONLY THREE OUT OF SIX CA-MRSA ISOLATED FROM MALETE COMMUNITIES. DNA MOLECULAR WEIGHT MARKER (M) (435BP)

DISCUSSION

Community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged as an important human pathogen in various parts of the world. This pathogen has varied distribution in both urban and rural community settings. In this study, we reported a prevalence of 37.2 % of *Staph aureus* in nostrils of apparently healthy individuals in rural communities of Malete. This prevalence is lower than a prevalence of 56.3 % reported by Chibuike and colleagues [20] in rural communities of Ururu among healthy individuals. Also a lower prevalence of 36.5 and 13.9% were reported by Egwuata *et al.* [21] in health workers in Lagos and Okwu *et al.* [22] among healthy individuals in Okada, Edo State Nigeria respectively. Various authors have recommended eradication of nasal carriage of *Staph aureus* to reduce transmission to others with the use of systemic antimicrobials, normal bacterial flora augmentation, antiseptic washes and topical antimicrobials [23], [24].

Antimicrobial resistance is one of the major threat posed by microorganisms to infection management in this twenty first century and *Staph aureus* has always been one of the major pathogen that possess ability to develop resistance to newly developed antimicrobial agents [25]. In this study, *Staph aureus* isolated were resistant to commonly available and recommended antibiotics such as cefuroxime, cefotaxime, cloxacillin and augmentin, but susceptible to ceftriaxone, tetracycline, erythromycin, gentamicin and ofloxacin in varying degrees. Susceptibility of erythromycin ranges from 5 to 64% in different geographic areas [26], while the susceptibility in this study is 30.9 %. There are many factors that may be adduced to the high resistance of this organism to antibiotics in these rural communities. Such factors included self-medication, availability and use of antibiotics without Doctor's prescription, irrational consumption rate of antibiotics, noncompliance to prescription and sales of fake or substandard drugs and transmission of resistant strains between individuals within the community.

Methicillin resistance is mediated among *Staph aureus* by the penicillin-binding protein (PBP)- 2a encoded by mecA gene. It was reported that all β-lactam antibiotics have poor affinity when PBP is altered, and such microbe would not be killed when exposed to therapeutic concentration. The mecA gene is found to be located on a mobile genetic element of staphylococcal chromosome cassette (SCCmec) [27]. In this study, the prevalence of methicillin resistance *Staph aureus* was 14.3 %. This is prevalence is lower when compared with what other researchers reported among apparently healthy individuals. Prevalence of 22.0, 47.6 and 60.6 % were reported by Akerele *et al.* [28] in Ekosundun, Benin City; Adelowo *et al.* [29] in Maiduguri and Chibuike et al. [21] in Uturu rural community respectively.

The major objective of screening for *mecA* gene is to compare results of antibiotic susceptibility by disc diffusion method with gene analysis results in *Staph aureus* isolates. The phenotypic expression of antibiotic resistant genes has been reported to be affected by different conditions such as the incubation temperature and time, medium inoculated, inoculum size, test agents, pH, and ionic strength of NaCl [30]. The *mecA* gene may be heterogeneously expressed and, therefore, all MRSA strains may not be detectable with phenotypical methods and the test require at least 24 h for evaluation of the results. However, the detection of *mecA* gene by PCR techniques is considered the gold standard method. All the six isolates that were cefoxitin resistant were also positive for this gene detection method;

confirming that they are all methicillin-resistant *Staph aureus*

The present study showed strong correlation between genotypic and phenotypic analysis, the results were consistent with previous studies which showed a perfect and strong correlation between the results obtained by the phenotypic antibiotic resistance determination and PCR-based assays [31], [32]. The critical parameters for success of a PCR-based assay for the detection of multidrug resistant bacteria like MRSA are the reliability, accuracy, fast and sensitivity and results were obtained within hours [33]. However the cefoxitin disc has been shown to have specificity 97-100 % to detected *mec A* gene [34].

Previous reports on MRSA in North-central, Nigeria were based phenotypic method, to our best knowledge this is the first report based on the detection of *mecA* gene in CA-MRSA among healthy individuals in rural communities of North-central, Nigeria. However, *mecA* gene has been discovered from other different part of Nigeria such reports included, a study in Benin city, Nigeria, 4 isolates representing 11 % were confirmed to carry *mecA* gene using molecular technique [35], Esan *et al.* [36] confirmed only one MRSA isolate from health care institutions from Ekiti and Ondo states and Shittu *et al.* [37], detected two MRSA isolates with *mecA* gene were detected in Ile-Ife, one from Lagos and two from Ibadan (all in South-western Nigeria)

In the North-eastern Nigeria, five MRSA isolates with *mec A* gene were detected in Maiduguri by Okon *et al*. [38] reported the detection of 12.5 % MRSA from clinical specimens from six tertiary hospitals.

PCR result for the detection PVL (*lukS-lukF*) gene in this research showed that out of 6 MRSA isolates screened only 3 of them were PVL positive, representing a percentage of 50 % (n=6). This finding was higher compared to the report of Sani *et al*. [39] who detected PVL gene prevalence of 3.25 and 8.13 % amongst MRSA from hospitalized and non-hospitalized patients in Benin. However, this difference may be due to the size of our samples. Another report by Breurec *et al.* [40] detected a 57 % *lukS-lukF* genes encoding Panton–Valentine Leukocidin (PVL) prevalent in MRSA isolates from Cameroon, Niger, and Senegal (West and Central Africa).

It appears that misuse and overuse of these antibiotics could have contributed to increase in antibiotics resistance in these rural communities as it has been observed in other part of the world. Therefore, to prevent resistance and treatment failures, we advocate proper monitoring of antibiotics usage and collection of data on antibiotic susceptibility testing pattern, public enlightenment on the effectiveness of various antibiotics and the enactment of drug policies in Nigeria; are urgently needed to move in line with the global trends.

Multidrug resistant organisms are defined as organisms that have acquired non-susceptibility to at least one agent in three or more classes of antimicrobial [41], [42], [43], [44]. By this definition, all the six CA-MRSA isolated in this community are multidrug resistant *Staph aureus*.

Conclusion: The detection of methicillin-resistant *Staph aureus* carriage among individuals in these remote rural communities of Malete indicated the extensive distribution of this organism and also indicated that the abuse of drug is on high side in Nigeria. Unlicensed patent medicine dealer and drug hawkers may be the main source of drugs distribution to these villages.

Acknowledgment: We acknowledged the cooperation of members of Apo, Eleburu, Atere and Tapa communities, Director of Community Development Centre (CDC), Kwara State University, Malete and technical staff of Microbiology laboratory for their support.

REFERENCES

1. **Aklilu E, Zunita Z, Hassan L, Chen HC. Phenotypic and genotypic characterization of methicillin-resistant Staphylococcus aureus (MRSA) isolated from dogs and cats at University Veterinary Hospital, Universiti Putra Malaysia. Tropical Biomedical 2010; 27(3): 483-492.**

2. **Kolawole, DO, Adeniran, A, Frieder Schaumburg, A L. Akinyoola, OO, Lawal, YB. Amusa, Robin K and Karsten B (2013) Characterization of Colonizing Staphylococcus aureus Isolated from Surgical Wards' Patients in a Nigerian University Hospital Public Library of Science 2013; (PLOS ONE) 8(7): e68721. doi:10.1371/journal.pone.0068721.**

3. **Sakoulas G, Moellering RC, Jr., Increasing antibiotic resistance among methicillin-resistant Staphylococcus aureus strains. J. Clin and Infect Dis 2008; 46(5):360-367.**

4. **Chambers HF. The changing epidemiology of Staphylococcus aureus. Emerg Infect Dis 2001; 7:178-182.**

5. **Cuny C, Lothar H, Wieler W and Wolfgang W. Livestock Associated MRSA: The Impact on Humans. Antibiotics 2015; 4, 521-543; doi:10.3390/ antibiotics 4040521**

6. **Harrison EM, Paterson GK, Holden MTG, Morgan FJE, Larsen AR, Petersen A, Leroy S, De Vliegher S, Perreten V, Fox LK, Lam TJGM, Sampimon OC, Zadoks RS, Peacock SJ, Parkhill J, Holmes MA. A Staphylococcus xylosus Isolate with a New mecC Allotype. Antimicrobial Agents Chemotherapy 2013; 57(3): 1524-1528. http://dx.doi. org/10.1128/AAC.01882-12.**

7. **Perrero MC, Valverde A, Fernández-Llario P, Díez-Guerrier A, Mateos A, Lavín S, Canton R, Fernandez Garayzabel J, Domenguez L. Staphylococcus aureus carrying mecC gene in animals and urban wastewater,**

Spain. Emerging Infectious Disease 2014; 20(5): 899-901. http://dx.doi.org/10. 3201 / eid2005.130426

8. Paterson GK, Morgan FJE, Harrison EM, Peacock SJ, Parkhill J, Zadoks RN, Holmes MA. Prevalence and properties of mecC methicillinresistant Staphylococcus aureus (MRSA) in bovine bulk tank milk in Great Britain. Journal of Antimicrob. Chemotherapy 2014; 69(3): 598-602. http://dx.doi. org/10. 1093/jac/dkt417 PMid:24155057 PMCid:PMC3922150

9. Davis SL, Perri MB, Donabedian SM, Manierski C, Singh A, et al. Epidemiology and outcomes of community-associated methicillin-resistant Staphylococcus aureus infection. Journal of Clinical Microbiology 2007; 45: 1705–1711.

10. Persoons D, Van Hoorebeke S, Hermans K, Butaye P, De Kruif A, Haesebrouck F, et al. Methicillin-resistant Staphylococcus aureus in poultry. Emerg Infect Dis. 2013; 15(3):452-3.

11. CDC. Community-associated MRSA (CA-MRSA) information for clinicians. February, 2005 http://www.cdc.gov/ncidod/hip/ARESIST/ca_mrsa_clinician.htm.

12. Tenover, FC. and Goering, RV. Methicillin-resistant staphylococcus aureus strain USA 300: origin and epidemiology. Journal of Antimicrobial Chemotherapy 2009; 64(3):441-446.

13. Centre for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant Staphylococcus aureus - Minnesota and North Dakota 1997-1999. MMWWR Morb Mortal Wkly 1999; Rep 48(32): 707-710.

14. National Population Commission: (NPC, 2006) Nigerian Population Census, 2006

15. Barrow G.I. and Feltham RKA. In: Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Ed., Cambridge University Press, Cambridge, UK. 1993, Pages: 331.

16. Greenwood D., Slack R., Peutherer J., Barer M. Medical Microbiology. Churchhill Livingstone publisher 2007; 17th Edition. Pp 172-177

17. CLSI. Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard—Eleventh Edition. CLSI document M02-A11 2012, Wayne, PA,USA.

18. Milheirico C, Oliveira DC. and de Lencastre, H. Multiplex PCR Strategy for Subtyping the Staphylococcal Cassette Chromosome mec Type IV in Methicillin-Resistant Staphylococcus aureus: SCCmec IV Multiplex. Journal of Antimicrobial Chemotherapy 2007; 60:42–48.

19. Lina G., Piemont, Y, Godial-Garmot F., Bes M., Peter MO., Gauduchon V., Vandenesch F. and Ettiene J. Involvement of Panton-Valentine Leukocidin Producing Staphylococcus aureus in Primary Skin Infections and Pneumonia. Clini infect Dis, 1999; 29(5):1128-1132

20. Chibuike I, Reginald AO, Solomon U. C., Ifeanyi AO, Conrad Jacobs, C. J. Nduka, N J 1 and Kelechi UO. Prevalence and Antibiotic Susceptibility Patterns of Methicillin-Resistant Staphylococcus aureus (MRSA) Isolated from Healthy Inhabitants of Uturu Rural Communities, Abia State, Nigeria Journal of Natural Sciences Research 2013; Vol.3, No.10,

21. Egwuala CC, Egwuatu TO, Iwuafor AA, Akujobi CN. Nnachi AU, Aghanya IN, Ogunsola FT and Oduyebo OO. Time-Kill Effect of Crude Extracts of Garcinia kola Seeds on Methicillin-Resistant Staphylococcus aureus from the Anterior Nares of Healthcare Workers at a Tertiary Hospital in Nigeria Journal of Advances in Medical and Pharmaceutical Sciences 2016; 8(3): 1-11, Article no.JAMPS.27005 ISSN: 2394-1111

22. Okwu M. Uchechukwu OM and Onyinye PO. Prevalence and antimicrobial susceptibility profiles of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) isolates among healthy individuals in Okada, South-South, Nigeria.US Open Pharmaceutical, Biological & Chemical Sciences Journal 2014; Vol. 1, No. 1, March, pp. 1 - 9 Available online at http://arepub.com/Journals.php

23. Klutymans J, van Belkum A, Verbrugh H. Carriage of Staph aureus: Epidemiology, Underlying Mechanisms, and Associated Risk. Clinical Microbiol. Rev. 1997;10: 505-520

24. Laupland KB, Conly JM. Treatment of S aureus Colonization and Prophylaxis for Infection with Topical Intranasal Mupirocin: An Evidence-based Review. Clini Infect Dis 2003; 37(7): 933-938

25. Joo EJ, Chung DR, Kim SH, Back JY, Lee NY, Cho SY, Ha YE, Kang CI, Peak KR, Song JH. Emergence of Community-Genotype-Methicillin-Resistance Staph aureus on Korean Hospital: Clinical Characteristics of Nosocomial Infections by Community-Genotype Stain. Infect. Chemotherapeutic 2017; 49: 109-116.

26. Kuehnert MJ, Hill H, Mc Quillan G, et al. Prevalence of Staphylococcus aureus colonization in the United States—2001-2002. Proceedings of the IDSA 2004 Annual Meeting, abstract 2004; 487.

27. Ito T, Kuwahara K, Hiramatsu K. Staphylococcal Cassette Chromosome mec (SCCmec) Analysis of MRSA. Methods Mol. Biol. 2007; 391: 87-102

28. Akerele JO, Obasuyi O and Omede D. Prevalence of Methicillin-Resistant Staphylococcus aureus among Healthy Residents of Ekosodin Community in Benin-City, Nigeria Tropical Journal of Pharmaceutical Research 2015; 14 (8): 1495-1499 ISSN: 1596-5996 1596-9827

29. Adelowo KA, Okon K O, Denue BA, Ladan J, Tahir F, Uba A. Methicillin-Resistant Staphylococcus Aureus (MRSA) Colonisation level among Patients seen at a

Tertiary Hospital in Maiduguri, Nigeria Journal of Medicine and Medical Sciences 2014; Vol. 5(10) pp. 238-244, DOI: http;/dx.doi.org/10.14303/jmms.2014.220

30. Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F. Comparison of polymerase chain reaction and conventional methods in detecting methicillin resistant Staphylococcus aureus. J. Infect Dev Ctries. 2008; 2: 46-5013

31. Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, Ouellette, M. and Bergeron MG. Correlation between the Resistance Genotype Determined by Multiplex PCR Assays and the Antibiotic Susceptibility Patterns of Staphylococcus aureus and Staphylococcus epidermidis. Antimicrobial Agents and Chemotherapy 2000; 44(2): 231–238. 182

32. Strommenger B, Kettlitz C, Weniger T, Harmsen D, Friedrich AW, and Witte W. Assignment of Staphylococcus isolates to groups by spa typing, smaI macrorestriction analysis, and multilocus sequence typing. Journal of Clinical Microbiology 2006; 44: 2533-2540.

33. Melendez DP, Greenwood-Quaintance KE, Berbari EF, Osmon DR, Jayawant N, Mandrekar JN, Hanssen AD and Patel R. Evaluation of a Genus- and Group-Specific Rapid PCR Assay Panel on Synovial Fluid for Diagnosis of Prosthetic Knee Infection. Journal of Clinical Microbiology 2016; 54(1): 120-126

34. Swenson JM, Tenover FC, Cefoxitin Disk Study Group. Results of disk diffusion testing with cefoxitin correlate with presence of mecA in Staphylococcus spp. J Clin Microbiol 2005; 43: 3818-3823.

35. Obasuyi O. (2013). Molecular identification of methicillin resistant Staphylococcus aureus in Benin City, Nigeria. African Journal of Clinical and Experimental Microbiology 2013; 14 (1): 1-4

36. Esan CO, Famurewa O, Johnson LJ. and Shittu A0. Characterization of Staphylococcus aureus isolates obtained from health care institutions in Ekiti and Ondo States, South-Western Nigeria. African Journal of Microbiology Research 2009; 3(12):962-968

37. Shittu AO, Okon K, Adesida S, Oyedara O, Witte W, Strommenger B, Layer F and Nübe U. Antibiotic resistance and molecular epidemiology of Staphylococcus aureus in Nigeria. BioMed Central Microbiology; 2011;11: 92.

38. 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 Journal of Medicine and Medical Sciences 2013; 4 (5): 214-220.

39. Sani H, Baba-Moussa F, Ahoyo TA., Mousse W, Anagonou S, Gbenou JD, Prevost G, Kotochoni SO and Baba-Moussa L. Antibiotic Susceptibility and Toxins Production of Staphylococcus aureus isolated from Clinical Samples from Benin. African Journal of Microbiology Research 2011; 5(18):2797-2803

40. Breurec S, Zriouil SB, Fall C, Boisier P, Brisse S, Djibo S, Etienne J, Fonkoua MC, Perrier-Gros-Claude JD, Pouillot R, Ramarokoto CE, Randrianirina F, Tall A, Thiberge JM; Working Group on Staphylococcus aureus infections, Laurent F, Garin B. Epidemiology of methicillin-resistant Staphylococcus aureus lineages in five major African towns: emergence and spread of atypical clones. Clin Microbial Infect. 2011; 17(2): 160-165

41. Nikaido H. Multidrug Resistance in Bacteria. Annual Review of Biochemistry. 2009; 78:119-146. http://dx.doi.org/10.1146/annual review of biochemistry 78.082907.145923

42. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G. Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infecion 2012; 18:268-281. http://dx.doi.org/ 10.1111/ j.1469-0691.2011.03570.x

43. CDC-Centers for Disease Control and Prevention (2013). 1600 Clifton Rd. Atlanta, GA 30333, USA 800-CDC-INFO (800-232-4636) TTY 2013; (888) 232-6348.

44. World Health Organization. Antimicrobial Resistance Fact sheet N°194, Updated April Yamaoka T. (2007). The bactericidal effects of anti-MRSA agents with rifampicin and sulfamethoxazole-trimethoprim against intracellular phagocytized MRSA. Journal of Infection Chemotherapy 2014; 13: 141–146