ORIGINAL ARTICLE

pISSN: 1907-3062 / eISSN: 2407-2230

Molecular characterization of methicillin-resistant staphylococci among apparently healthy students

Helen Oroboghae Ogefere* and Lawrence Ayodele Ogunleye*,**

ABSTRACT

BACKGROUND

Staphylococcus aureus are widely considered a major factor of nosocomial and community-acquired infections. This work was aimed at determining the prevalence of methicillin-resistant *S. aureus* (MRSA) among apparently healthy students.

METHODS

A cross-sectional study was conducted involving 400 nasal swab samples randomly collected from students using sterile swab sticks and processed to recover *S. aureus* using standard microbiological techniques. Conventional methods were used to identify the isolates and antibiotic susceptibility tests were performed using Kirby-Bauer disc diffusion method according to performance standards of Clinical and Laboratory Standard Institute guidelines. Methicillin-resistance was detected phenotypically using cefoxitin 30µg discs. Bacterial deoxyribonucleic acid (DNA) extraction was done on cefoxitin-resistant staphylococci isolates only using ZymoResearch (ZR) fungal/bacterial DNA MiniPrep™ kit. A polymerase chain reaction assay targeting the 16S rRNA, nuc, and *mecA* genes on 1.0% agarose gel electrophoresis stained with ethidium bromide was used to identify *S.aureus* and detect methicillin resistance.

RESULTS

The overall prevalence of MRSA was 5.8% using phenotypic methods. PCR amplification of the 23 phenotypically confirmed MRSA using 16S rRNA and *nuc* genes identified staphylococci 23/23(100%) and *S. aureus* 23/23(100%) at band size 886bp and 225bp respectively. However, 16(69.6%) were positive for *mecA* gene at band size 532bp by PCR method. Poor level of susceptibility was recorded among the MRSA namely to erythromycin (26.6%), cloxacillin (0%), augmentin (0%), cefuroxime (0%), ceftriaxone (0%) and ceftazidime (0%). Ofloxacin was the most effective antibiotic (60.9%).

CONCLUSION

Active antimicrobial surveillance of pathogenic staphylococci is important to analyze the infections and transmission rate for possible control measures.

Keywords: Methicillin-resistance, staphylococci, students, antibiotics, *mecA, nuc.*

*Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria
**Medical Laboratory Unit, Cottage Hospital, Auchi Polytechnic, Auchi, Edo State, Nigeria.

Correspondence:
Helen Oroboghae Ogefere
Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria
e-mail: helenogefere@yahoo.com
phone number: +2348023509447

Date of first submission, September 17, 2018
Date of final revised submission, March 19, 2019
Date of acceptance, March 20, 2019

Cite this article as: Ogefere HO, Ogunleye LA. LMolecular characterization of methicillin-resistant staphylococci among apparently healthy students. Univ Med 2019;38:25-32. doi: 10.18051/UnivMed.2019.v38.25-32

DOI: http://dx.doi.org/10.18051/UnivMed.2019.v38.25-32

INTRODUCTION

The widespread resistance across various species of staphylococci has been reported in recent years.[1] The most notable example is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the launch of methicillin in the early 1960s and has now become a major public health concern with its prevalence increasing globally.[2] MRSA can cause the same types of infections as other *S. aureus* isolates such as: skin and soft tissue infections, including impetigo, folliculitis, furunculosis, abscesses, cellulitis, and wound infections.[2] The MRSA has also been reported to cause invasive infections such as: pneumonia, endocarditis, osteomyelitis, septic arthritis, meningitis, septicemia, toxic shock and staphylococcal scalded skin syndromes in both infants and adults.[2]

The MRSA strains are resistant to nearly all beta-lactam antibiotics by producing an alternative penicillin-binding protein (PBP) known as PBP-2a.[3] This protein is encoded by the *mecA* gene and has a low affinity to many beta-lactam antibiotics. MRSA strains are not only resistant to beta-lactams and cephalosporins, but also often show resistance to a wide range of antibiotics.[3] The *mecA* is part of a mobile genetic element called staphylococcal cassette chromosome (SCCmec). The SCCmec is flanked by cassette chromosome recombinase genes (*ccrA* or *ccrB* or *ccrC*) that permit intra- and interspecies horizontal transmission of SCC*mec*.[3, 4] *S. aureus* has four PBPs, (PBP1, PBP2, PBP3, and PBP4), with PBP2a/2' and PBP2 being responsible for peptidoglycan synthesis with transpeptidase and transglycosylase activities.[3] In Nigeria, the prevalence of hospital associated-MRSA in clinical samples also varies from one region to another; 19.2% in Ekiti, 28.6% in Kano, 12.5% in Maiduguri and 38.0% in Benin.[5-9] Several reports of studies on prevalence of community-associated MRSA (CA-MRSA) are also emerging in Nigeria; 10.8% in apparently healthy school children in Okada, Edo State, 41% and 56.7% in apparently healthy university students in Edo and Ogun states respectively, and 60.7% in otherwise healthy inhabitants of Uturu communities in Abia State.[10-12]

Characterization of the 16S rRNA gene is now well-established as a standard method for identifying and classifying species, genera and families of bacteria including staphylococci.[13] The nuc gene, which encodes thermonuclease, is widely used as a specific target for the identification of *S. aureus* by polymerase chain reaction (PCR).[14] Numerous in-house PCR based assays targeting the nuc gene alone or in combination with the *mecA* gene have been designed for fast screening or identification of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA).[14] In recent years, detection of *mecA* by PCR is also considered as the gold standard for identification of MRSA.[9, 14]

Cefoxitin, being a cephamycin, is a stronger inducer of the *mecA* regulatory system than oxacillin. The cefoxitin disc test is easier to read than the oxacillin disc diffusion test and more accurate for detection of *mecA* mediated resistance.[15] Many PCR based molecular methods were developed as alternative ways for accurate identification of MRSA.[16] Most reports in our locality concentrate on phenotypic methods of identifying methicillin-resistant staphylococci especially among apparently healthy subjects and neglecting molecular characterization to identify the specific bacteria and genes responsible for antimicrobial resistance. This study therefore aimed to use the PCR assay to target three genes: a genus-specific 16S rRNA to detect staphylococcal DNA; *nuc*, which encodes the *S. aureus*—specific region of the thermonuclease gene and *mecA*, a determinant of methicillin resistance among apparently healthy students of a tertiary institution in Auchi, Edo State, Nigeria.

METHODS

Research design

This study was of cross-sectional design and was conducted using 400 apparently healthy students (consisting of 167 males and 233 females) of Auchi Polytechnic, Auchi, Edo State, Nigeria. Informed consent was obtained from all participants prior to specimen collection. Institutional ethical approval was obtained before study commencement. The duration of the study was from July 2016 to November 2017.

Specimen collection and processing

A total of 400 anterior nasal swab specimens were collected from the participants. All samples were collected from the left anterior nares using sterile swab stick soaked in sterile saline, labelled, packaged and transported immediately to the Microbiology laboratory for analysis. *Staphylococcus aureus* isolates were identified using API-Staph system (API System; bioMerieux, Paris, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out following the recommendation of the British Society for Antimicrobial Chemotherapy (BSAC) method.[17] The test colonies were emulsified in sterile distilled water and the turbidity matched with 0.5 McFarland. Once matched, a sterile cotton wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on side of the test tube. The entire surface of Mueller–Hinton agar plate was seeded by swabbing in three directions with the swab. The antibiotic discs were placed on the plate with the use of a sterile forceps. The antimicrobial agents tested were ofloxacin (5µg), amoxicillin-clavulanate (30µg), ceftazidime (30g), erythromycin (5µg), cloxacillin (5µg), ceftriaxone (30µg), cefuroxime (30µg) and gentamicin (10µg). The plates were incubated at 37^0C for 18 – 24 hours.

Detection of cefoxitin resistance

All *Staphylococcus aureus* isolated were screened for methicillin-resistance by following CLSI guidelines using 30 µg cefoxitin discs (Abtek U.K).[15] Plates were read after incubation at 35°C for 18 h. Zone diameter d" 21mm was deemed to indicate cefoxitin resistance.

Bacterial DNA extraction

Bacterial DNA extraction was done on the 23 cefoxitin-resistant staphylococci isolates only. The DNA of the representative bacterial isolates was extracted using ZR fungal/bacterial DNA Mini Prep ™ kit (Zymo Research Corporation, USA) following the manufacturer's instruction.

PCR amplification of 16S rRNA, *nuc* and *mecA* genes

The 23 cefoxitin-resistant staphylococci eluted DNA extracts were amplified for 16S rRNA, nuc and *mecA* genes by PCR using their respective primers as shown in Table 1. Briefly, a volume of 25µl PCR reaction mixture consisting of 12.5µl of PCR master mix, primers (1.25µl each), double distilled water (5µl), and DNA template (5µl) was used for PCR.

Table 1: List of primers amplified by PCR

Primer Code	Oligonucleotide Sequence (5'- 3')	Annealing Temp (^{O}C)	Expected Amplicon Size (bp)
16S rRNA	16S-1 (F) 5'GTGCCAGCAGCCGCGGTAA 3' 16S-2 (R) 5'AGACCCGGGAACGTATTCAC 3'	57	886
nuc	(F) 5'TCAGCAAATGCATCACAAACAG3' (R) 5'CGTAAATGCACTTGCTTCAGG 3'	57	225
mecA	*mecA* F 5'AAAATCGATGGTAAAGGTTGGC 3' *mecA* R 5'AGTTCTGCAGTACCGGATTTGC 3'	53	532

Table 2. Prevalence rate of MRSA isolated from the nares of apparently healthy students using cefoxitin disc

Gender	*S. aureus* (n,%)			Cefoxitin resistance (n,%)		
	positive	negative	p value	positive	Negative	p value
Gender						
Male	99 (59.3)	87 (40.7)	0.553	8 (8.1)	159 (91.9)	0.5219
Female	130 (55.8)	103 (44.2)		15 (11.5)	218 (87.5)	

Amplifications were performed using GeneAmp* PCR system 9700 thermocycler (Applied Biosystems, USA) beginning with an initial denaturation at 94º C for 3 min followed by 30 cycles of 30 s denaturation step at 94ºC, annealing at 57º C for 30 s, extension at 72º C for 30 s, and a final 7 min extension at 72ºC followed by a hold at 4ºC. The 10µl of PCR products was electrophoresed on 1% Tris-acetate-EDTA agarose gel stained with ethidium bromide for 60 min with 90V current and viewed under UV trans illuminator (Dolphin-DOC plus, Wealtec Corporation).[18]

Statistical analysis

Statistical analysis was by the Chi (X^2) square test and Fischer's exact test where appropriate using INSTAT® software. A p value of <0.05 was deemed statistically significant.

Ethical approval

Ethical approval for this study was sought and obtained from Auchi Polytechnic Health Ethical Committee, Auchi with registration number: AP/HC.14/VOL.1/69.

RESULTS

Out of 400 apparently healthy students screened, 229 (57.3%) were culture positive for *S. aureus*. Twenty-three (7.7%) of these isolates were methicillin resistant using cefoxitin disc. This comprised 8 (8.1%) males and 15 (11.5%) females. Gender did not however significantly affect the distribution of MRSA (p=0.5219) (Table 2).

The prevalence of specific genes 16S rRNA, *nuc* and *mecA* among cefoxitin-resistant *S. aureus* was 100%, 100%, and 69.6%

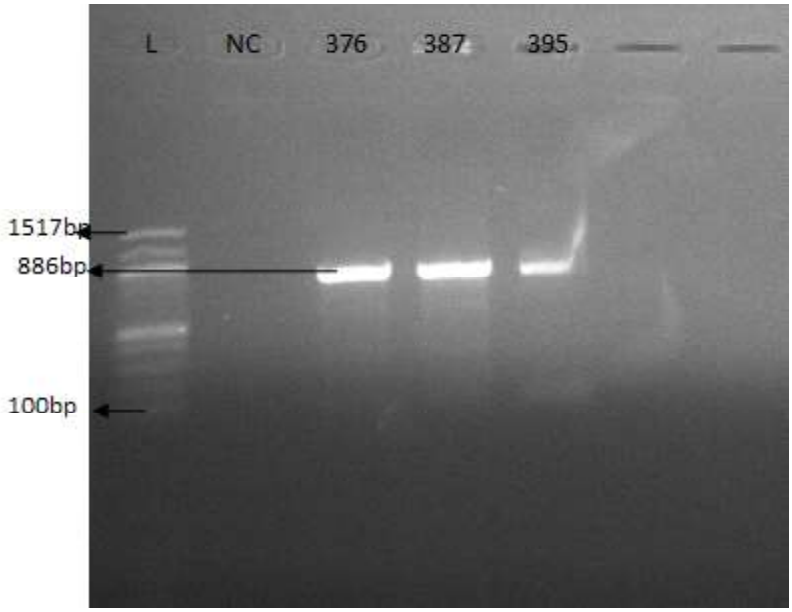

Figure 1. Polymerase chain reaction of 16S rRNA gene in cefoxitin-resistant staphylococci isolates using 1.0% agarose gel electrophoresis stained with ethidium bromide to identify staphylococci. Left-hand lane is 100bp-1517bp DNA ladder (molecular marker) while NC is a no DNA template control

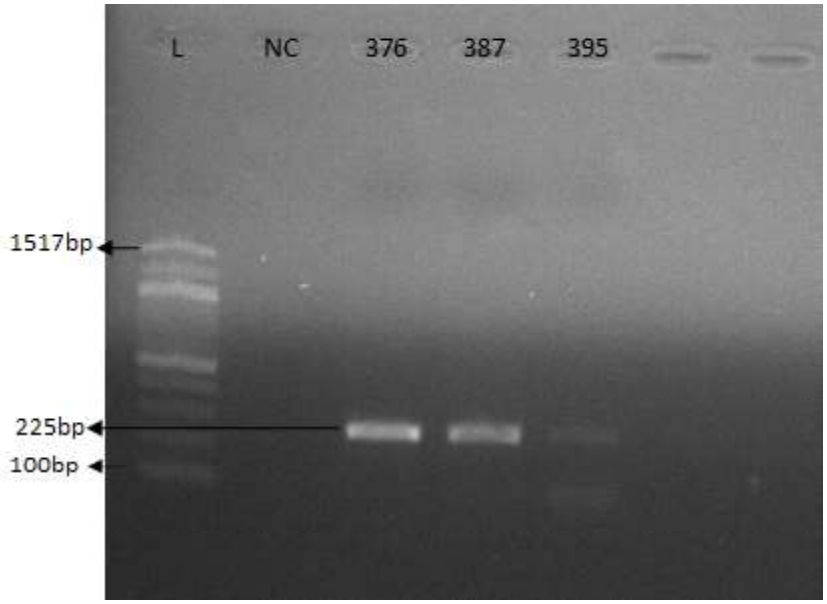

Figure 2. Polymerase chain reaction of nuc gene in cefoxitin-resistant staphylococci isolates using 1.0% agarose gel electrophoresis stained with ethidium bromide to identify S*taphylococcus aureus*. L is 100bp-1517bp DNA ladder (molecular marker) while NC is a no DNA template control

respectively. Polymerase chain reaction results for detection of these genes can be seen in Figure 1, 2, and 3 respectively. Gender also did not significantly affect the distribution of *mecA* gene among cefoxitin-resistant *S. aureus* as 16 (69.6%) isolates harbored the gene, in a male-to-female ratio of 6:10 (p=0.975).

Methicillin resistant *S. aureus* showed poor susceptibility to commonly available antibiotics as 0% susceptibility was observed for ceftazidime, cefuroxime, ceftriaxone, cloxacillin and amoxicillin-clavulanate (augmentin). A poor level of susceptibility was recorded among the MRSA namely to erythromycin (26.6%), The most active antibiotic was ofloxacin (60.9%).

DISCUSSION

The prevalence rate of MRSA in this study was 5.8% (using cefoxitin disc). This finding is

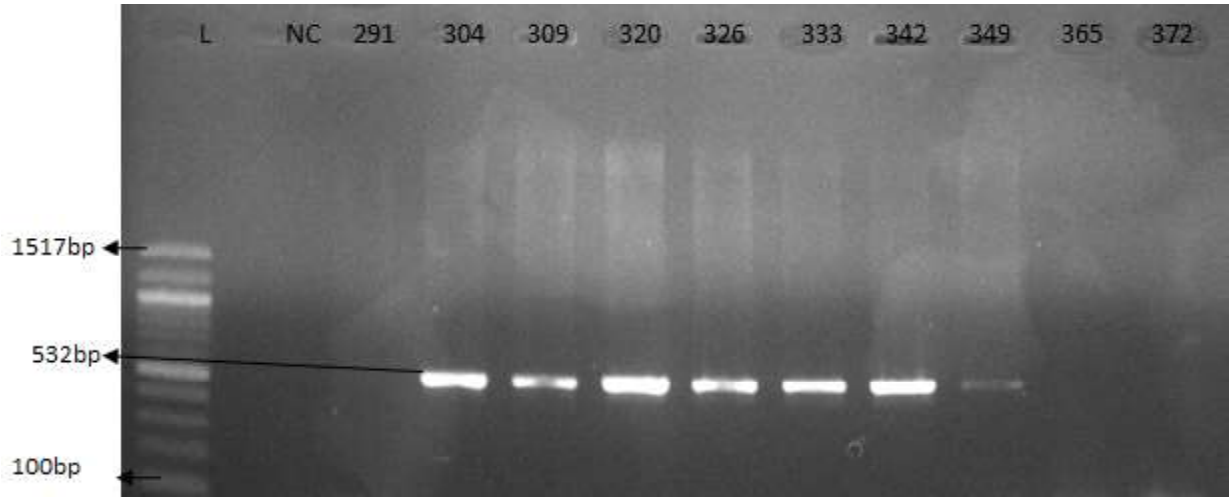

Figure 3.Polymerase chain reaction results for detection of *mec*A gene from confirmed cefoxitin-resistant *Staphylococcus aureus* isolates on 1.0% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1517bp DNA ladder (molecular marker) while NC is a no DNA template control

in agreement with the work of Mahde et al.[19] which reported community-associated MRSA among healthy university students (4.2%). Also, Okwu et al.[10] reported a prevalence of 10.8% of community-associated MRSA in apparently healthy school children in Okada, Edo State, Nigeria. A study carried out in Colombia by Bettin et al. showed that the MRSA carriage status by medical students in their clinical rotations was 1.6%.[20] A study in Thailand reported 1% prevalence among university students while Shadi recorded 6.7% MRSA colonization of nares among medical students in Jeddah, Saudi Arabia.[21, 22] On the contrary, Eke et al.[11] reported 41% MRSA in apparently healthy university students in Ekpoma, Edo State, Nigeria. The difference in prevalence rates recorded could be attributed to method of identification of *S. aureus* isolates used to detect MRSA. Most other studies in Nigeria reported above used phenotypic identification as against the genotypic method used in this study. Ayeni and Odumosu had reported 85% misidentification rate of other microorganisms as *Staphylococus aureus* in Southern Nigeria when comparing phenotypic methods with genotypic (16S rRNA).[13]

Polymerase chain reaction (PCR) amplification of 16S rRNA genes to identify staphylococci among the 23 MRSA of apparently healthy subjects was positive (100%). This is in line with work of Degaim et al.[23] where all isolates were positive for both 16S rRNA genes and *mecA* genes (100%). Ayeni and Odumosu similarly showed the reliability of this genotypic test when compared with phenotypic tests in identification of *S. aureus*.[13] This current study results agreed with Makgotlho,[24] who showed that all isolates 97/97 (100%) have 16S rRNA gene while *mecA* gene was detected in 96/97 (99%) of the MRSA isolates, the 1/97(1%) which did not show the presence of *mecA* gene was, however, phenotypically identified as MRSA.

In this study, the PCR amplification of *nuc* gene to detect *Staphylococcus aureus* among MRSA showed 100% positive results. According to Vremera et al.[25] the *nuc* gene in *S. aureus* encodes the thermonuclease enzyme and amplification of *nuc* gene is a potential method for rapid diagnosis of *S. aureus* infection. Therefore, the primer used in this study confirmed the ability of PCR as a fast and reliable method for detection of the *nuc* gene to identify *S. aureus* strains. Also, of the 23 MRSA (using phenotypic method), 16 (69.6%) were confirmed to be *mecA* positive by the PCR method. This is also similar to the work of Ibadin et al. [9] where sensitivity and specificity of *mecA* gene in detection of MRSA was 89.5% and 90.3% respectively. Phenotypically methicillin-resistant strains without *mecA* gene and methicillin sensitive strains harboring *mecA* gene have been shown in previous studies.[13] In this study, phenotypic and genotypic methods for detection of MRSA were used. The results of this study showed that 5.8% and 4.0% of *S. aureus* isolates were recognized as MRSA by cefoxitin disc diffusion test and PCR method respectively. Considering that detection of the *mecA* gene by PCR method is a gold standard method for identifying methicillin-resistance in *S. aureus* isolates, therefore the prevalence of MRSA in this study was 4.0%.

In this study, a poor level of susceptibility was recorded among the MRSA namely to erythromycin (26.6%), cloxacillin (0%), augmentin (0%), cefuroxime (0%), ceftriaxone (0%) and ceftazidime (0%). This implies that MRSA isolates are generally resistant to beta-lactam antibiotics as previously reported.[9,10] However, some MRSA isolates were susceptible to gentamicin (47%) and ofloxacin (60.9%). The sensitivity to the non-beta-lactam antibiotics was also similar to previous findings and it is recommended that non-beta-lactam antibiotics should be the preferred drugs for the treatment of community-acquired (CA) MRSA infections.[8, 9] The moderately high resistance (52.2%) recorded for gentamicin was similar to the report on *S. aureus* from clinical samples (44.4%).[9] Ofloxacin (with sensitivity of 60.9%)

appeared to be the best antibiotic of choice for the treatment of MRSA infections in Auchi, Edo State and its surrounding communities.

A limitation of this study is hinged on the fact that only phenotypically identified MRSA strains were subjected to molecular analysis. More far reaching recommendations for routine microbiological practice may have been reached if all phenotypically identified *S. aureus* were screened for the *nuc* gene. The study however holds important clinical implications as the role of carriers of MRSA is continually being explored in opportunistic infections and nosocomial outbreaks. Further studies that explore nasal carriage of hospital-acquired and community-acquired MRSA strains among apparently healthy subjects may deepen understanding of the superbug-MRSA in our region.

CONCLUSIONS

This present study established the phenotypic and genotypic prevalence rates of MRSA among apparently healthy subjects in the Auchi Polytechnic to be 5.8% and 4.0%, respectively. It showed that the PCR technique is a very useful tool in detecting and identifying MRSA using 16S rRNA, *nuc* and *mecA* genes. In comparison with conventional methods, genotypic analysis of these three genes is still the gold standard for detecting MRSA. Ofloxacin was found to be most effective antibiotic against MRSA.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

ACKNOWLEDGEMENT

The authors are grateful to all study participants for their cooperation and to the management of Auchi Polytechnic, Auchi, for the approval to carry out this research.

CONTRIBUTORS

HOO took part in the conception and design of the study. LAO took part in data generation. HOO and LAO contributed to data analysis and interpretation. Both authors participated in writing and approving the final draft of the manuscript.

REFERENCES

1. Peng Q, Hou B, Zhou S, et al. Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in a teaching hospital, Shontou, China. Afr J Microbiol 2010;4:844-8.
2. Rongpharpi SR, Hazarika NK, Kalita H. The prevalence of nasal carriage of *Staphylococcus aureus* among healthcare workers at a tertiary care hospital in Assam with special reference to MRSA. J Clin Diagn Res 2013;7:257–60. doi: 10.7860/JCDR/2013/4320.2741.
3. Garza-González E, Morfin-Otero R, Llaca-Diaz JM, et al. Staphylococcal cassette chromosome (SCCmec) in methicillin-resistant coagulase negative staphylococci. A review and the experience in a tertiary-care setting. Epidemiol Infect 2010;138:645–54. DOI: https://doi.org/10.1017/S0950268809991361
4. Treesirichod A, Hantagoo S, Prommalikit O. Nasal carriage and antimicrobial susceptibility of *Staphylococcus aureus* among medical students at the HRH Princess Maha Chakri Sirindhorn Medical Center, Thailand: a follow-up study. J Infect Public Health 2014;7:205-9. doi: 10.1016/j.jiph.2012.12.004.
5. Olowe OA, Kukoyi OO, Taiwo SS, et al. Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from Ekiti state, Nigeria. Infect Dr Resist 2013;6:87–92. DOI: http://dx.doi.org/10.2147/IDR.S48809
6. Nwankwo BOK, Sale A, Magagi A, et al. Methicillin-resistant *Staphylococcus aureus* and their antibiotic susceptibility pattern in Kano, Nigeria. Afr J Clini Exper Microbiol 2010;11:1595–689. DOI: http://dx.doi.org/10.4314/ajcem.v11i1.44088.
7. Onemu OS, Ophori EA. Prevalence of multi-drug resistant *Staphylococcus aureus* in clinical specimens obtained from patients attending the

university of Benin teaching hospital, Benin City, Nigeria. J Nat Sci Res 2013;5:154-9.
8. Shittu AO, Usman H, Adamu N, et al. Epidemiology and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus r*ecovered from tertiary hospitals in North-eastern, Nigeria. J Med Medic Sci 2013;4:214-20.
9. Ibadin EE, Enabulele IO, Muinah F. Prevalence of *mecA* gene among staphylococci from clinical samples of a tertiary hospital in Benin City, Nigeria. Afr Health Sci 2017;17:100-10. DOI: https://dx.doi.org/10.4314/ahs.v17i4.7.
10. Okwu M, Bamgbala S, Aborisade W. Prevalence of nasal carriage of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) among healthy primary school children in Okada, Nigeria. J Natur Sci Res 2012;2:61-5.
11. Eke S, Abdulkadiri S, Okoro CJ, et al. The prevalence and resistivity pattern of *Staphylococcus aureus* isolates from apparently healthy university students in Ekpoma, Edo State, Nigeria. Inter J Bas Appl Innov Res 2012;1:183-7.
12. Ayepola, OO, Taiwo OS, Anifowose A, et al. Nasal carriage of *Staphylococcus aureus* and associated risk factors among students in a Nigerian University. Acta Sci Microbiol 2018;1:6-8. doi: 10.31080/ASMI.2018.01.0010.
13. Ayeni FA, Odumosu BT. False identification of other microorganisms as *Staphylococcus aureus* in Southern Nigeria. Trop J Pharm Res 2016;15: 1941-5. DOI: http://dx.doi.org/10.4314/tjpr.v15i9.19
14. Liu Y, Zhang J, Ji Y. PCR-based PCR-based approaches for the detection of clinical methicillin-resistant *Staphylococcus aureus*. Open Microbiol J 2016;10:45-56. doi:10.2174/1874285801610010045.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing approved standard M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA;2013.
16. Biswajit B, Shibendu B, Bappa M. Isolation of imipenem resistant *Staphylococcus aureus* from post-operation pus sample in oral and maxillofacial infections. Res J Pharm Bio Chem Sci 2012;3:896-900.
17. Andrews JM. BSAC standardized disc susceptibility testing method (version 8). J Antimicrob Chemother 2009;64:454-89.
18. Kaya EG, Karakoc E, Yagci S, et al. Evaluation of phenotypic and genotypic methods for detection of methicillin resistance in *Staphylococcus aureus*. Afr J Microbiol Res 2009;3:925-9.
19. Mahde SA, Reem QM, Nawfal RH. Nasal carriage rates of *Staphylococcus aureus* and CA-methicillin resistant *Staphylococcus aureus* among university students. J Microbiol Res 2015;5:123-7. doi:10.5923/j.microbiology.20150504.01
20. Bettin A, Causil C, Reyes N. Molecular identification and antimicrobial susceptibility of *Staphylococcus aureus* nasal isolates from medical students in Cartagena, Colombia. Brazil J Infect Dis 2012;16:329–34. doi: 10.1016/j.bjid.2012.06.017.
21. Kitti T, Boonyonying K, Sitthisak S. Prevalence of methicillin-resistance *Staphylococcus aureus* among university students in Thailand. Southeast Asian J Trop Med Pub Hea 2011;42: 1498-504.
22. Zakai SA. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization among medical students in Jeddah, Saudi Arabia. Saud Med J 2015;36:807-12. https://doi.org/10.15537/smj.2015.7.11609.
23. Degaim ZD, Shani WS, Hamim SS. In Virulence factors of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from burn patients. Inter J Curr Microbiol Appl Sci 2015;4:898-906.
24. Makgotlho PE, Kock MM, Hoosen A, et al. Molecular identification and genotyping of MRSA isolates. FEMS Immun Med Microbiol 2009;57:104–15.
25. Vremera T, Iancu LS, Logigan C, et al. Optimization of triplex real time PCR for detecting *Staphylococcus aureus mecA, pvl* and *nuc* genes. Roman Arch Microbiol Immun 2011;70:69–73.