Sokoto Journal of Medical Laboratory Science 2021; 6(2): 22 - 26

SJMLS - 6 (2) - 004

Detection of mecA Gene in the Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*

Raji, M.I.O. [*1], **Haido, A.U.** [1], **Garba, I.** [2] **and Shuaibu, A.B.** [3]

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria [1], *Department of Medical Microbiology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria* [2], *Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria* [3]

[*]Corresponding author: rajimuda@gmail.com

Abstract

Resistance coding in *Staphylococcus aureus* could be phenotypic or genotypic. In genotypic coding, mecA gene codes for the production of the altered penicillin-binding protein responsible for methicillin resistance. This study aimed at determining the nature of methicillin resistance observed in staphylococcal isolates. Ninety-six (48 each) clinical isolates of *Staphylococcus aureus* were collected from two health facilities in Sokoto namely Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Specialist Hospital, and screened for Methicillin-resistant *Staphylococcus aureus* (MRSA) using Oxacillin-resistant screening agar base (ORSAB). Ten (5 each) of the 38 MRSA isolates obtained from the two health facilities were subjected to DNA extraction using DNA extraction mini-kit (Qiagen K.K., Tokyo, Japan). Polymerase Chain Reaction (PCR) was then carried out to amplify the extracted DNAs and the PCR products were subjected to agarose gel electrophoresis to detect the presence or absence of mecA gene. The result of ORSAB showed that 38 out of 96 clinical isolates were MRSA with 18 (37.5%) MRSA isolates from UDUTH and 20 (41.7%) MRSA isolates from Specialist Hospital. Agarose gel electrophoresis analysis of the PCR products showed that all isolates were mecA negative since no band showed the targeted DNA primer. The phenotypic methicillin resistance in the studied MRSA isolates were not coded by mecA gene.

Keywords: *Staphylococcus aureus*, MRSA, mecA gene, penicillin-binding protein.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is a major pathogen responsible for severe nosocomial infections due to its multi-drug resistance that limits treatment options (Cikman *et al*., 2019). The strains are capable of developing resistance to β-lactam antibiotics due to the change created on penicillin-binding protein 2a (PBP 2a) in their genomes (Olayinka *et al.*, 2009). The altered PBP 2a is encoded by mecA gene which is located in a genetic element called the Staphylococcal Chromosome Cassette (SCCmec) in different form and genetic content (Reichmann and Pinho, 2017). While β-lactam resistance is mostly attributed to mutations in the mecA gene, other factors may also be considered responsible for resistance in MRSA (Mogahid *et al*., 2015). Previous studies have reported occurrences of MRSA in health facilities in other parts of Nigeria (Nwakwo *et al*., 2010; Akanbi and Mbel, 2013; Nwokah *et. al*., 2016) and in Sokoto metropolis (Olowo-Okere *et al*., 2017; Adeiza *et. al*., 2020a; Adeiza *et al*., 2020b). Thus, the need to carry out this research to determine the source of resistance of the clinical isolates of MRSA from Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital in Sokoto metropolis – whether it is through mecA gene or otherwise.

Material and Methods

Isolation and characterization of Methicillin-resistant Staphylococcus aureus

Ninety-six (48 each) clinical isolates of *Staphylococcus aureus* collected from two health facilities namely Usmanu Danfodiyo University Teaching Hospital (UDUTH) and

Specialist Hospital, Sokoto were screened for Methicillin-resistant *Staphylococcus aureus* (MRSA) using Oxacillin-resistant screening agar base (ORSAB). The agar was prepared in a 500ml conical flask according to the manufacturer's instructions and poured in plates and allowed to solidify. Fresh cultures of the clinical isolates were inoculated on the ORSAB and incubated at 37 °C for 24 hours after which cultures with characteristics blue colour were sought for and confirm to be Methicillin-resistant *Staphylococcus aureus*.

Detection of mecA gene using PCR analysis
DNA extraction
Ten (5 each) of the 38 MRSA isolates obtained from the two health facilities were subjected to DNA extraction. DNA extraction of the bacterial cells was carried out by using a DNA extraction kit (QIAamp DNA mini kit; Qiagen K.K., Tokyo, Japan). Heating block was first set at 60°C before starting the extraction. Into a 2ml tube was added 200µl of the bacterial cells in liquid medium. Four hundred microliter (400µl) of lyses buffer and 10µl proteinase K were added to the sample and the tube was placed on heat block at 60°C for minimum of 1 hour. Four hundred microliter (400 µl) of phenol chloroform (1:1) was added to the lysate and vortexed briefly. The mixture was centrifuged at 10000rpm for 10 minutes to separate the phases. The upper layer was carefully removed with a pipette without taking the white interphase which contained the DNA. For the second time, 400µl of chloroform was added to the remainder and vortexed briefly. The mixture was centrifuged again at 10000rpm for 10 minutes to separate the phases and the upper layer was carefully removed without taking the white interphase. To the remainder, was added 400µl of 100% ethanol and 20µl of 3M sodium acetate. This was mixed by inverting the tube several times and the tube was incubated at –20°C overnight. On the following day, the tube was centrifuged at maximum speed for 10 – 30 minutes in refrigerated centrifuge and the ethanol was removed. Four hundred microliter (400µl) of 70% cold ethanol was added and centrifuged at maximum speed for 5 minutes at 4°C to precipitate the DNA because DNA is not miscible in alcohol. This step was repeated again to get more DNA precipitated and the salt totally removed. All traces of ethanol were removed by spinning the tube for 30 seconds at high speed and the DNA was dry out by leaving the tube open for 3 – 10 minutes. The pellet was re-suspended in 50µl sterile water for further analysis.

PCR analysis
The standard PCR assay was performed using the DNA amplification instrument master cycler gradient (Eppendorf, Germany) to identify MRSA strains. Cellular DNA was obtained from staphylococci colonies grown overnight on blood agar plates using DNA extraction kit (Qiagen, China) in accordance with the manufacturer's instructions. The mecA specific primer pairs used for amplification of 533 base pair (bp) fragments are forward (MECA-F), 5AAAATCGATGGTAAAGGTTGGC-3 and reverse (MECA-R) 5-AGTTCTGCAGTACCGGATTTGC-3. A volume of (1µL) of prepared DNA (0.5µg) was added to a final volume of 25µL PCR mixture containing 11µL 2× master mix (Ampliqon Co., Denmark), including 1× PCR buffer, 1.5mmol\L mgcl2, 0.15mmol\L dNTP, and 1.25IU Taq DNA polymerase, (Ampliqon Co., Denmark), 0.5µL of 5.5 µmol\L of each primer and 9µL of nuclease free water. The PCR cycling protocol was applied as following: initial Denaturation at 94°C for 5 min, followed by 30 cycles of Denaturation at 95°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 2 min, followed by a final extension at 72°C for 7 min. The amplified products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide to determine presence or absence of mecA gene.

Results
The result of ORSAB showed that 38 out of 96 clinical isolates from the two health facilities were MRSA with 18 MRSA isolates from UDUTH and 20 MRSA isolates from Specialist Hospital (Table1). Table 2 shows the mecA specific primer pairs used in DNA amplification, and its sequence with length of 22nt and product size of 533bp for forward and reverse primers. The result of agarose gel electrophoresis showed absence of mecA gene in all of the 10 isolates subjected to PCR analysis, as represented in figure 1.

Table 1: Prevalence of MRSA in Clinical Isolates from two Health Facilities in Sokoto Metropolis

Health Facility	Clinical *S. aureus* Collected	MRSA	MRSA (%)
UDUTH	48	18	37.5
Specialist Hospital	48	20	41.7
Total	96	38	

Table 2: mecA Primer and its sequence

Primer	Sequence	Length(nt)	product size(bp)
Forward	5-AAAATCGATGGTAAAGGTTGGC -3	**22**	**533**
Reverse	5- AGTTCTGCAGTACCGGATTTGC -3	**22**	**533**

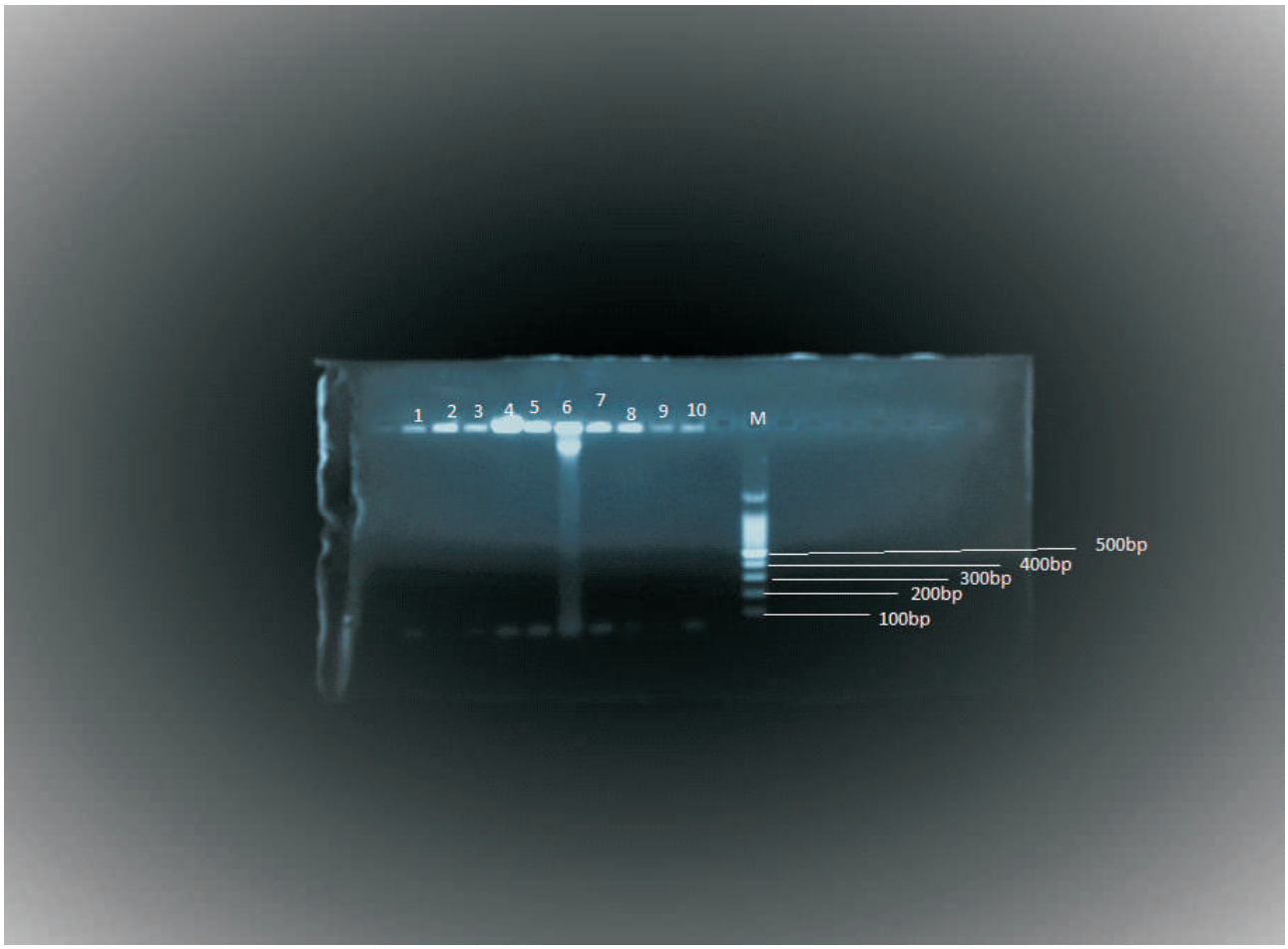

Fig 1: Agarose gel electrophoresis (2%) for detection of mecA gene using multiplex PCR products with all PCR products being mecA negative
Lane 1-10 = Isolate 1-10; M = Maker

Discussion

The percentage prevalence of MRSA in the two health facilities studied calls for concern. The study on detection of methicillin resistance *Staphylococcus aureus* among clinical isolates of *Staphylococcus aureus* from patients attending Usmanu Danfodiyo University Teaching Hospital and Specialist hospital, Sokoto revealed that there were no mecA genes in the resistant strains of the *Staphylococcus aureus* analyzed. This result is similar to those of Olayinka *et al*. (2009) and Mogahid *et al*. (2015) in Zaria, Nigeria and Shandi City, Sudan respectively. It was reported that this type of resistance might be as a result of hyper production of β- lactamase. Agarose gel electrophoresis analysis of the amplified PCR products of MRSA isolates did not reveal any mecA gene and this might be caused by several factors such as presence of mecC or hyper production of β- lactamase as earlier mentioned being responsible for coding of resistance in the studied strains of MRSA. In contrast to the result of this study is the result of Fernando *et al.* (2014) where a conclusion was made that although there are obviously differences in biochemistry between mecA and mecC-encoded PBP2a, mecC nonetheless confers methicillin resistance, and such strains need to be identified correctly as MRSA in diagnostic laboratories. Furthermore, their study reported for the first time the presence of mecC-carrying MRSA strains isolated from humans in Spain which gives the possibility of assuming that absence of mecA in the resistant strains studied might have been caused among other possibilities by mecC. A study carried out in Turkey revealed that isolates found to be MRSA with MecC gene in the absence of mecA gene which is a homolog of mecC, has been reported (Cikman *et al.* , 2019). Degain *et al.* , (2019) in their molecular study of mecA and mecC genes profile of clinical isolates of *Staphylococcus aureus* in a teaching hospital in South of Iraq established the significance of mec C gene in MRSA recognition than mecA gene. However, it was revealed by Dogan *et al*. (2016) that mecC gene harboring *Staphylococcus aureus* cannot be detected by mecA gene-specific PCR, multiple-task screening that combine mecA, mecC and other screening is therefore of paramount requirement for detection of resistant strains *Staphylococcus aureus*.

Conclusion

Electrophoresis gel analysis of mecA gene of the studied MRSA isolates showed that phenotypic resistance observed on the isolates were not coded by mecA gene. It was therefore concluded that the presence of mecA gene is not the only determining factor for resistance coding in Methicillin-resistant *Staphylococcus aureus.* Researchers should therefore go beyond mecA gene screening in the detection of Methicillin-resistant *Staphylococcus aureus.*

References

Adeiza, S., Onaolapo, J. and Olayinka, B (2020a). Prevalence, risk-factors and antimicrobial susceptibility profile of methicillin-resistant Staphylococcus aureus (MRSA) obtained from nares of patients and staff of Sokoto state-owned hospitals in Nigeria. GMS Hygiene and Infection Control, **15(2196-5226)**: 1-17.

Adeiza, S.S., Onaolapo, J.A. and Olayinka, B.O. (2020b). Molecular detection of chloramphenicol-florfenicol resistance (cfr) genes among linezolid resistant MRSA isolates in Sokoto State, Nigeria. Journal of Health and Biological Science; **8(1)**: 1-6.

Akanbi, B.O. and Mbe1, J.U. (2013). Occurrence of methicillin and vancomycin resistant staphylococcus aureus in University of Abuja Teaching Hospital, Abuja, Nigeria. African Journal of Clinical and Experimental Microbiology; **14(1)**: 10-13.

Cikman, A., Aydin, M., Gulhan, B., et.al. (2019). Absence of the mecC gene in methicillin-resistant Staphylococcus aureus isolated from various clinical samples: The first multi-centered study in Turkey. Journal of Infection and Public Health;**12**: 528–533.

Degaim, Z.D., Mater, A.D. and Al-Malky, H.K. (2019). mec A and mec C genes profile of Clinical Isolates of Staphylococcus aureus. Project, University of Thi-Qar, Iraq; 675-682.

Doğan, E., Kilic, A., Turutoglu, H., Ozturk, D. and Turkyilmaz, S. (2016). Screening of Staphylococcus aureus isolates for mecA and

mecC genes carriage. Ankara Üniversitesi Veteriner Fakültesi Dergisi; **63**: 389-391.

Fernando, G., Emiliac, C., Mercedes, M., Mercedes, B., Pilar, T., Juan, C., Carmen, B., Julia, P., Pilar, A. and Ana, V. (2014). Methicillin-resistant Staphylococcus aureus carrying the mecC gene: emergence in Spain and report of a fatal case of bacteraemia, Journal of Antimicrobial Chemotherapy; 45-50.

Mogahid, M.E., Hani, A.O., Hassan, A.H., Miskelyemen, A.E. and Leila, M.A. (2015). Absence of the mecA Gene in Methicillin Resistant Staphylococcus aureus isolated from different clinical specimens in Shendi City, Sudan. BioMed Research International; 1-5.

Nwakwo, B.O.K., Abdulhadi, S., Magaji, A., Ihesiulor, G. (2010). Methicillin-resistant Staphylococcus aureus and their antibiotic susceptibility pattern in Kano, Nigeria. African Journal of Clinical and Experimental Microbiology; **11(1)**: 1595-689.

Nwokah, E.G., Abey, S.D. and Wachukwu, C.K. (2016). mecA gene profile of methicillin-resistant Staphylococcus aureus isolates from clinical sources in Port Harcourt, Nigeria. American Journal of Biomedical and Life Sciences; **4(3)**: 41-48.

Olowo-Okere, A., Atata, R.F., Abass, A., Shuaibu, A.S., Ungokore, H.Y. and Tanko, N. (2017). Incedence and Antibiotic Susceptibility Profile of Staphylococcus aureus isolates from Wounds of Patients at Specialist Hospital, Sokoto Nigeria. Journal of Medical Bacteriology; **6(3,4)**: 44-50.

Olayinka, B.O., Olayinka, A.T., Obajuluwa, A.F., Onaolapo, J.A. and Olurinola, P.F. (2009). Absence of mecA Gene in Methicillin-resistant Staphylococcus aureus isolates. African Journal of Infectious Diseases; 3(2): 49 – 56.

Reichmann, N.T. and Pinho, M.G. (2017). Role of SCCmec type in resistance to the synergistic activity of oxacillin and cefoxitin in MRSA. Science Reports; 7(1):6154.

Citation: Raji, M.I.O., Haido, A.U., Garba, I. and Shuaibu, A.B. Detection of mecA Gene in the Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*. *Sokoto Journal of Medical Laboratory Science;6(2): 22 -26*