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Molecular Determination of Resistance Gene in Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Skin and Wound

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Abstract

The wide spread use of antibiotic resulted in the development of resistance to antibiotics through acquisition of the mobile cassette chromosome carrying the Methicillin-resistant gene *mecA***.** The study was aimed to characterize the resistance gene in Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from skin and wound samples in Kano Metropolis, Northwestern, Nigeria. A total of 235 *S. aureus* isolates were identified and subjected to MRSA screening. MRSA were phenotypically identified by antibiotic susceptibility testing using agar disc diffusion method. The suspected MRSA were subjected to polymerase chain reaction (PCR). The PCR products were subjected to gel electrophoresis and a DNA ladder were loaded into the gel wells. The gel was examined for the presence of specific amplicons of the expected size for mecA, which is 192bp. Out of a total of 235 isolates of *Staphylococcus aureus*, only 11 (4.7%) strains were found to be Methicillin resistant *Staphylococcus aureus* (MRSA). Five out of the 11 isolates show the presence of DNA bands of the expected size for *MecA* gene which indicated the presence of the resistance genes in the bacterial isolates. It is concluded that *MecA* gene is one of the gene responsible for methicillin resistance in MRSA.

Keywords: Methicillin resistant *staphylococcus aureus* (MRSA); Resistance; Wound; Skin.

1. Introduction

The problems concerning microbial resistance to the available antibiotics especially in the hospitals are fast growing. One of such problem is the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) which has become a global threat to antimicrobial chemotherapy. The MRSA bacteria belong to the *Staphylococcus aureus* bacteria family. *Staphylococcus aureus* is common bacterium which lives harmlessly on the skin and in the nose of around a third of healthy people. When it does cause infection 'ordinary' *Staphylococcus aureus* is sensitive to most commonly used antibiotics. MRSA is a particular type of *Staphylococcus aureus* that has developed resistance to several antibiotics. Only a few antibiotics will act against MRSA [1]. The wide spread use of antibiotic resulted in the development of resistance to antibiotics through acquisition of the mobile cassette chromosome carrying the Methicillin-resistant gene mecA [2, 3].

Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Resistant Coagulase-Negative Staphylococci (MR-CoNS) has been identified as multidrug-resistant zoonotic pathogens in humans and many animal species [4, 5]. Even through, coagulase negative Staphylococci may also be a normal flora for skin and mucous membranes of human and animal species [6]. The MRSA was first noted in 1961, about two years after the antibiotic methicillin was initially used to treat Staph aureus and other infectious bacteria. The resistance to methicillin was due to a penicillin-binding protein coded for by a mobile genetic element termed the methicillin-resistance gene –mecA [7]. In recent years, the gene has continued to evolve so that many MRSA strains are currently resistant to several different antibiotics such as penicillin, oxacillin and cefoxitin.

MRSA is still considered as an emerging pathogen and public health threats result from the spread of hospital-acquired as well as community-acquired MRSA [8]. The study was aimed to characterize resistance gene in Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolated from infected skin samples in Kano Metropolis, Northwestern, Nigeria.

2. Materials and Methods

2.1. Sampling Site

Suspected samples of *Staphylococcus aureus* were isolated from wound and skin of patients attending Murtala Muhammad Specialist Hospital, Sheikh Muhammad Jiddah General Hospital and Muhammad Abdullahi Wase

Specialist Hospital all in Kano, Nigeria were collected and used in the study. In addition three (3) motor parks namely Mallam Kato square, Kano line and Yan-Kaba were selected for collection of wound and skin of patients i.e. community base. Kano State is located in the Northwestern Nigeria at latitude $11^0$3'N and longitude $8^0$3'E. It share borders with Kaduna state to the south- west, Bauchi state to the South-East, Jigawa state to the East and Katsina state to the North. It has a total area of 20,131 Km^2 and estimated population of 13.4 million [9].

2.2. Identification and Characterization of *S. Aureus*

The isolates were subjected to confirmatory tests as *Staphylococcus aureus* by conventional microbiological methods: Gram staining and Biochemical tests (DNase test, Catalase test, Coagulase test and Mannitol fermentation test) as described by Cheesbrough [10].

2.3. Phenotypic Identification of MRSA

The confirmed *S. aureus* suspension adjusted to 0.5 McFarland was subjected to antibiotic susceptibility testing using agar disc diffusion method as described by Bauer, *et al.* [11]. Mueller Hinton agar (MHA) plates were inoculated with overnight culture of the isolate by streak plating. The 1 µg oxacillin and 30 µg cefoxitin sensitivity discs was then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were be incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to oxacillin and cefoxitin discs based on zones of produced. Zones of inhibition were interpreted according to CLSI [12] criteria: susceptible, >13 mm; intermediate, 11–12 mm; and resistant <10 mm [13].

2.4. Molecular Identification of *MRSA* Resistant Gene

The bacterial isolates were cultured in nutrient broth tubes and incubated at 37 °C for 24 hours. The bacterial cells were harvested through centrifugation at 10,000 g for 5 minutes. Genomic DNA was extracted from the harvested cells using a commercially available kit according to the manufacturer's instruction (Quick-DNA™ Microprep Kit manufactured by Zymo Research). Specific primers for the MecA gene (F: TCCAGATTACAACTTCACCAGG, R: CCACTTCATATCTTGTAACG) was designed using NCBI Primer-BLAST. A PCR reaction mixture of 12.5 µL was prepared as follows: 6.25 µL master mix (Zymo Taq premix (Zymo Research), 4.25 µL sterile DNase free water, 1 µL DNA template, 0.5 µL forward primer, 0.5 µL reverse primer. The PCR reactions were run with the following parameters on thermocycler (Techne Techgene, model FTGENE5D): Initial denaturation at 95°C for 5 minutes, followed by 35 cycles of

- Denaturation at 95°C for 30 seconds
- Annealing at a temperatures 50.3 for 30 seconds
- Extension at 72°C for 30 seconds
- Then final extension at 72°C for 5 minutes.

The PCR products were subjected to gel electrophoresis. A 1.5% agarose gel was prepared by dissolving 1.5 g of agarose powder into 100ml X1 TAE buffer, bring to boil in a microwave after which 1ul ethidium bromide was added. This was poured into a gel tank fitted with comb, and was allowed to solidified. The PCR products and a DNA ladder were loaded into the gel wells, and were run at a 100 voltage until the DNA bands migrated sufficiently, after which it was visualized under UV transluminator (Technegene). The gel was examined for the presence of specific amplicons of the expected size for mecA, which is 192bp.

2.5. Ethical Approval

Ethical clearance for the study was obtained from Health Service Management Board Kano State with following Approval number: NHREC/17/03//2018; and reference number: Ref: SHREC/2021/2970

3. Results

3.1. Identification of Methicillin Resistant *Staphylococcus Aureus* (MRSA)

The identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) is presented in Table 1. Isolates were tested using oxacillin and cefoxitin disc diffusion test for detection of MRSA. Out of a total of 235 isolates of *Staphylococcus aureus*, only 11 (4.7%) strains were found to be Methicillin resistant *Staphylococcus aureus* (MRSA).

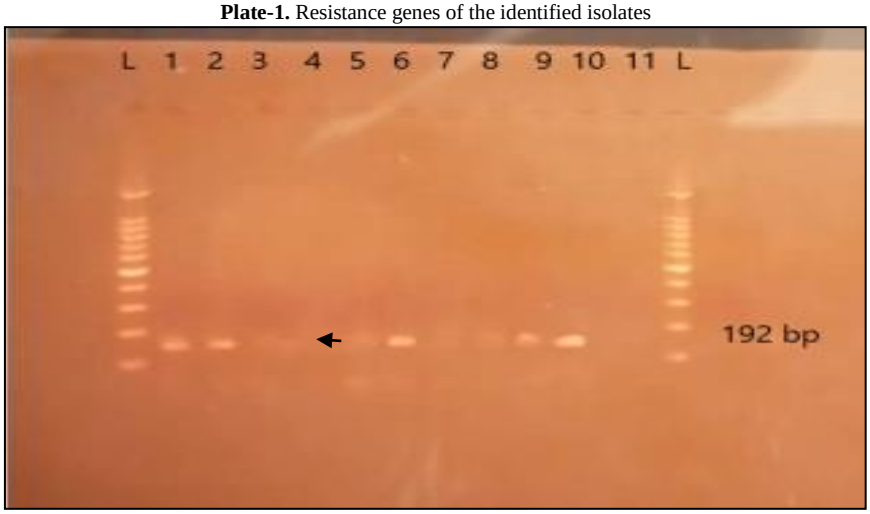
Table-1. Identification of Methicillin resistant *Staphylococcus aureus* (MRSA)

Isolate Code	Oxacillin disc (1µg)	Cefoxitin disc (10µg)	Status
IMH_8	10	06	MRSA
IMH_{12}	08	08	MRSA
IWH_{23}	10	08	MRSA
IWH_{45}	06	10	MRSA
IJH_{13}	10	10	MRSA
IJH_{21}	08	10	MRSA
IWH_3	10	06	MRSA
IWH_{13}	08	08	MRSA
IWH_{43}	10	06	MRSA
IKC_{19}	06	08	MRSA
IYC_{21}	08	10	MRSA

3.2. Molecular detection of Resistance Gene of the Isolates

The presence of DNA bands of the expected size for MecA indicated the presence of these resistance genes in the bacterial isolates.

Plate-1. Resistance genes of the identified isolates



4. Discussion

Infections caused by methicillin-resistant *S. aureus* (MRSA) represent a growing public health problem and a challenge for health care institutions [14]. The present study was aimed to characterize the resistance gene in Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from skin and wound samples in Kano Metropolis, Northwestern, Nigeria.

In this study, out of a total of 235 isolates of *Staphylococcus aureus*, only 11 (4.7%) of the *staphylococcus* species isolated were MRSA and most of them are from Hospital based wound infection. This finding was in conformity with that of Nas, *et al.* [15] who found higher number of MRSA in wound sample. Higher number of Methicillin Resistance *Staphylococcus aureus* (MRSA) in infected wound is due to the fact that the organisms colonize human skin tissue. This result is in accordance with the work of Falagas, *et al.* [16] in South Africa reported a slightly higher prevalence frequency of 24 % and 20 % for HA-MRSA and CA-MRSA respectively. On the other hand, this study is in contrary with the research conducted in Osogbo, South-western Nigeria by Olowe, *et al.* [17] who reported the isolation of 22.6% MRSA from S. aureus isolates.

Molecular characterization of the resistant gene in MRSA showed that 5 out of the 11 isolates indicated the presence of *MecA* gene (plate 1). A gene known as *mec*A gene is responsible for the resistance to methicillin which codes for penicillin-binding protein PBP 2A [3]. The wide spread use of antibiotic resulted in the development of resistance to antibiotics through acquisition of the mobile cassette chromosome carrying the methicillin-resistant gene *mecA* [3] and *mecC* [7]. The resistance to methicillin was due to a penicillin-binding protein coded for by a mobile genetic element termed the methicillin-resistance gene –*mecA* [7]. In recent years, the gene has continued to evolve so that many MRSA strains are currently resistant to several different antibiotics such as penicillin, oxacillin and amoxicillin [18].

5. Conclusion

In this study, out of a total of 235 isolates of *Staphylococcus aureus*, only 11 (4.7%) of the *Staphylococcus* species isolated were MRSA and most of them are from Hospital based wound infection. However, 5 out the 11 MRSA showed the presence of MecA gene which is responsible for resistance against some beta lactam drugs. It is recommended that individual should practice good personal hygiene to avoid the spread of infection with *Staphylococcus* species

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