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The Molecular Detection of mecA Genes of *Staphylococcus Aureus*

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Abstract

Antibiotic resistance is common among pathogenic bacteria associated with community acquired and nosocomial infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have become a global health problem particularly in hospital setup causing simple skin infections to life threatening infections. The present study aimed to investigate the presence of *mec*A genes in MRSA from pigs, using Polymerase Chain Reaction. One hundred *S. aureus* isolates of blood samples from Pigs in Bariga, Lagos State were collected from Molecular Biology and Biotechnology Unit, Nigeria Institute of Medical Research. Methicillin resistance was determined by Kirby-Bauer's disc diffusion method. The PCR was used for *mec*A gene detection from MRSA strains. Twenty-five pure *Staphylococcus aureus* isolates were identified based on cultural characteristics, biochemical reactions and positive slide coagulase test. Out of these, 11 (44%) strains were MRSA by phenotypic method. Amplification of *mec*A gene for all the 11 MRSA isolates was negative when visualized on 2% agarose gel electrophoresis. Eleven strains of MRSA were found among *Staphylococcus aureus* isolates of blood samples from Pigs. The MRSA phenotype observed in the isolates was not the classical *mec*A mediated resistance. Hence, it is highly recommended to consider alternative mechanisms for β-lactams resistance that may compete with *mec*A gene in the emergence of MRSA phenomenon in Nigeria.

Keywords: Antibiotics; *Staphylococcus Aureus*; MRSA; *mec*A Gene; PCR Amplification

Introduction

Staphylococcus aureus is one of the most frequent bacterial pathogens encountered in humans where it causes skin infections, soft tissue infections, osteomyelitis, bacteremia, septicemia and respiratory tract infections in the community, as well as postoperative and catheter-related infections in hospitals [1] Methicillin resistant *S. aureus* (MRSA) have become major public health problem worldwide [2]. The burden of MRSA continues to raise with a rate of 14% of all *S. aureus* strains from clinically significant samples in New South Wales, Australia [3]. The rising colonization rates lead to the increasing of infection rates in the community and in hospitals. The consequence to the health care system is longer hospital stays and greater costs, which approximately double the expenditure per patient [4]. The patient risks include significantly higher mortality and morbidity rates with invasive MRSA infection [5]. Within U.S. hospitals, nearly 60% of nosocomial *S. aureus* infections acquired in intensive care units are methicillin resistant [5]. Health care workers may carry MRSA on their hands or clothes following their contact either with asymptomatic carriers or patients who have clinical infection, which may then, unknowingly transmit the organism to other patients. The contaminated environmental surfaces also contribute to MRSA transmission. Thus, symptomatic patients constitute a small portion of the actual reservoir of MRSA within hospitals resulting in an iceberg phenomenon [6]. The worldwide emergence of community acquired methicillin resistant *S. aureus* (CA-MRSA) can have severe public health

implications [7]. The differentiation between community-acquired MRSA and hospital acquired MRSA (HA-MRSA) is becoming difficult to understand, since CA-MRSA could spread into hospitals [8]. The risk of acquiring MRSA in the hospitals increased by severity of illness, length of stay, use of intravascular devices and the intensity of exposure to infected patients [9-12]. Infection control measures include screening, and segregation of positive patients, eradication of carriage and good standards of general hygiene [13-17].

Molecular study of antibiotic resistance gene from *Staphylococcus aureus* its amplification and sequencing of *mec*A gene which is responsible for most of the β-lactams antibiotics resistance including methicillin will give insight on how to design new synthetic drugs to control community acquired infections of *S. aureus.*

Methods

Collection and Identification of Isolates

A total of one hundred isolates of *S. aureus* from blood samples of pigs in Bariga, Lagos State were collected from the Molecular Biology and Biotechnology division of the Nigeria Institute of Medical Research Yaba, Lagos State. The isolates were sub cultured on Brucella medium after which Gram staining was carried out to confirm that the isolates are pure.

Mannitol salt agar was then used as selective medium for secondary isolation of the *staphylococci*. Isolates were inoculated unto Mannitol Salt agar plates and incubated at 37 °C for 24 to 48 hrs. Plates were examined for growth of colonies with the characteristic golden coloration.

Biochemical test such as the coagulase test was performed to confirm *S. aureus* strains for twenty-five isolates. The isolates were characterized by their Gram stain characteristics, growth on Mannitol Salt Agar and slide coagulase tests to confirm the *S. aureus* isolates [18].

Antimicrobial Susceptibility Test

Susceptibility test was done for all the isolates obtained against the following antibiotics: Methicillin, Oxacillin and Vancomycin (Oxoid, UK) by modified Kirby-Bauer technique. Nutrient agar medium containing 5% of sodium chloride was prepared, distributed in 20ml aliquots into bottles and sterilized at 121°C for I5 mins [19]. Overnight cultures of the confirmed *S. aureus* isolates were emulsified in 3ml of Phosphate buffered solution (PBS) and turbidity adjusted to 0.5 McFarland were inoculated unto the NA plates by swabbing. The paper disk (methicillin (5 µg), oxacillin (1µg) and vancomycin (30µg), were placed on the dried agar plates and incubated for 18 hours at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic discs were measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug were interpreted using the criteria published by the Clinical Laboratory Standards Institute (CLSI) [20,21]

Detection of *mec* A Genes by PCR and Bacterial Genomic DNA Extraction

Eleven MRSA isolates identified by phenotypic method were subjected to detection of *mec*A gene using PCR. An overnight culture in Brain Heart Infusion (BHI) broth was collected by centrifugation and processed according to the procedure of Arakere, et al. [22]. The isolated DNA was stored at -20 °C till further use.

PCR Protocol

A three step PCR method reported by Oliveira, et al. [23] was carried out in a thermal cycler (Gradient thermocycler, Biologix, China) The primers used for amplification were; forward primer sequence mecA1 (AAAATCGATGGTAAAGGTTGGC) and reverse primer sequence mecA2 (5'AGTTCTGCAGTACCGGATTTTGC 3'). The conditions of PCR were as described by Murakami, et al. [24] which includes an initial denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 60seconds, 62 °C for 30seconds, and 72 °C for 35seconds, annealing at 53 °C for 30seconds with a final extension at 72 °C for 10 min. The PCR product was finally visualized under UV transilluminator on 2% agarose and the image captured with 16MP Nikon Camera.

Results

Out of a total of 100 isolates, 25 were pure Gram-positive cocci. of the total 25 *S. aureus* isolates studied, 11(44%) isolates were MRSA, 15 (60%) were resistant to Oxacillin and none was resistant to Vancomycin (inhibition zone of 12 mm or less by Kirby-Bauer's disc diffusion method). The susceptibility pattern of the organism against the various antibiotics is shown in figure 1. 44% of the organisms were resistant with Methicillin while 56% were sensitive the antibiotic, 60% and 40% of the organisms were sensitive and resistant to Oxacillin respectively, while no strain was seen resistant to Vancomycin as they were all (100%) sensitive (Figure 1). PCR results indicated that 11 (44%) MRSA isolates were negative for *mec*A genes (absence of the corresponding band) (Figure 2).

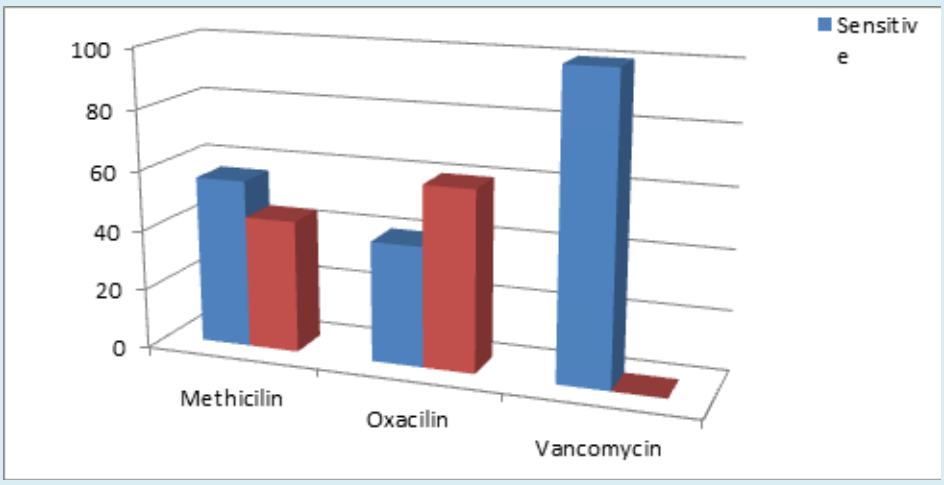

Figure 1: Proportion of isolates that were sensitive and resistant to the various antibiotics.

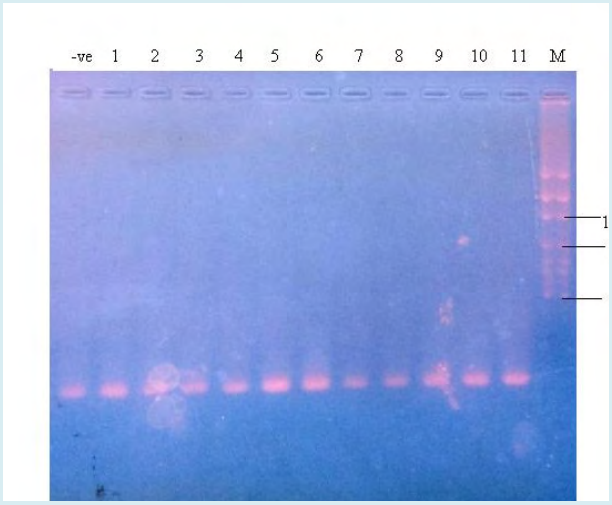

Figure 2: Amplicon of ***mec***A gene: Last lane (right) molecular weight marker; Lane 1-11 *mec*A negative; First lane (left) internal negative control.

Discussion

There was a high proportion of isolates that were resistant to methicillin and this is very worrisome particularly as the isolates were obtained from pigs. A previous study in Netherlands reported 39% MRSA out of 540 pig isolates. Perhaps the low number of isolates used in the study could have accounted for the high proportion of MRSA [25]. Findings of *mec*A gene were the major evidence for the detection of MRSA isolates [26]. However, findings in this project suggests low burden of the *mec*A gene, this may open the door to search for other intrinsic factors that may compete with *mec*A gene in producing resistance phenomenon in regions with high prevalence of MRSA in pigs. Also, a previous study in Nigeria reported the complete absence of *mec*A genes as well as the gene product of PBP2a in isolates which were phenotypically MRSA suggesting a probability of hyper production of β-lactamase as a cause of the phenomenon [27]. Moreover, recently Ba and colleagues mentioned specific alterations in different amino acids present in protein binding proteins cascade (PBPs 1, 2, and 3) which may be the basis of resistance [28]. These alterations were found to include three amino acid substitutions which were identical and were present in PBPs 1, 2, and 3. The same amino acid was found to have two other different substitutions in PBP1, both the identical and different amino acid substitutions were observed in isolates from different multilocus types. These findings provided clear evidence that there were mechanisms other than the presence of *mec*A gene responsible for beta-lactam resistance of MRSA and that molecular methods alone were not enough for confirmed characterization of MRSA isolates, a point that should be under consideration by regional and reference laboratories.

Conclusion

The phenotypic methicillin resistance observed in the isolates may not be due to the production of the altered PBP2a encoded by the *mec*A gene, but other factors such as hyper production of β-lactamase. MRSA without *mec*A gene were also being implicated in the cause of some severe infections. This study indicates that MRSA could be much more frequent among persons having contact with pigs than among other persons outside hospitals [29].

In conclusion, these findings indicate increasing prevalence of MRSA in pigs. However, the absence of *mec*A gene in a considerable percentage of MRSA isolates requires investigating the alternative genetic possibilities related to the resistance phenomena. A concerted research in similar

area of study using methods that simplifies extraction and amplification of target DNA is required in other parts of Nigeria. This is to provide a clearer and broader picture on the existence of MRSA isolates in Nigeria.

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