



Short Communication

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Non detection of *mecA* gene in methicillin resistant *Staphylococcus aureus* isolates from pigs

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Abstract:

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) have become a global health problem causing infections in both humans and livestock, ranging from skin and soft tissue to life threatening blood stream infections. The *mecA* gene is known to confer resistance to MRSA isolates. This study investigated the carriage of *mecA* gene by MRSA isolates from pigs.

Methods: One hundred non duplicate staphylococcal isolates recovered from blood samples of pigs in Bariga district of Lagos State at the Molecular Biology and Biotechnology unit of the Nigerian Institute of Medical Research were used in the study. *S. aureus* was identified by cultural characteristics, and positive catalase, coagulase and deoxyribonuclease tests. Phenotypic methicillin resistance was determined by the modified Kirby Bauer disk diffusion method and *mecA* gene was detected by conventional polymerase chain reaction (PCR) assay.

Results: Twenty-five *S. aureus* were identified, of which 11 (44%) were MRSA by phenotypic method. All the isolates were *mecA* negative on PCR.

Conclusion: The MRSA phenotype observed in the pig isolates in this study appears not to be the classical *mecA* mediated resistance. There may be alternative mechanisms of resistance in MRSA isolates in pigs.

Key words: MRSA, phenotypic, *mecA* gene, PCR, pigs

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Non détection du gène *mecA* dans les isolats de porc provenant de *Staphylococcus aureus* résistant à la méthicilline

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Abstrait

Contexte: Le *Staphylococcus aureus* résistant à la méthicilline (SARM) est devenu un problème de santé mondial provoquant des infections chez l'homme et le bétail, allant de la peau et des tissus mous aux infections du flux sanguin constituant un danger de mort. On sait que le gène *mecA* confère une résistance aux isolats de SARM. Cette étude portait sur le portage du gène *mecA* par des isolats de SARM chez le porc.

Méthodes: Une certaine d'isolats de staphylocoques non dupliqués, récupérés à partir d'échantillons de sang de porcs dans le district de Bariga, dans l'État de Lagos, de l'unité de biologie moléculaire et de biotechnologie de l'Institut nigérian de recherche médicale ont été utilisés dans l'étude. *S. aureus* a été identifié par ses caractéristiques culturelles et par des tests positifs à la catalase, à la coagulase et à la désoxyribonucléase. La résistance phénotypique à la méthicilline a été déterminée par la méthode de diffusion sur disque de Kirby Bauer modifiée et le gène *mecA* a été détecté par un test classique de réaction en chaîne de la polymérase (PCR).

Résultats: 25 *S. aureus* ont été identifiés, dont 11 (44%) étaient des SARM par méthode phénotypique. Tous les isolats étaient *mecA* négatifs en PCR.

Conclusion: le phénotype de MRSA observé dans les isolats de porc dans cette étude ne semble pas être la résistance à médiation classique de *mecA*. Il peut exister d'autres mécanismes de résistance des isolats de SARM chez les porcs

Mots-clés: MRSA, phénotypique, gène *mecA*, PCR, porcs

Introduction:

Staphylococcus aureus is one of the most frequent bacterial pathogens encountered in humans where it causes variety of hospital and community associated infections ranging from mild skin and soft tissue infections, catheter-associated urinary tract infections, surgical site infections, osteomyelitis, to life threatening bacteraemia, septicemia and endocarditis (1). Infections by methicillin resistant *S. aureus* (MRSA) strains have become major public health problem worldwide (2). Since 2000, there have also been reports of zoonotic transmission of livestock-associated MRSA clonal complex 398 (LA-MRSA CC398) leading to increasing number of human infections in Denmark and other European countries with industrial pig production (3, 4, 5).

In Africa, there are only few reports of MRSA in pigs (6, 7, 8) and particularly in Nigeria, only two studies have reported MRSA in pigs (9, 10) with one of them reporting genetically confirmed MRSA strains in pigs that appeared to have originated from a typical community associated MRSA (CA-MRSA) clone in the human population (10).

The *mecA* gene is known to

mediate resistance to methicillin and other β -lactamase resistant penicillins but some reports have shown absence of *mecA* gene in MRSA strains (11, 12). The aim of this study therefore is to investigate the genetic basis of MRSA isolates from pigs in Nigeria

Methods:

Study setting and culture isolation

A total of 100 staphylococci isolated at the Molecular Biology and Biotechnology division of the Nigerian Institute of Medical Research Lagos from non duplicate blood samples of pigs in Bariga district of Lagos, were employed in the study. The staphylococcal isolates were first subcultured on Brucella medium to confirm purity and secondarily isolated on Mannitol salt agar after incubating cultures at 37°C for 24 hours. *S. aureus* isolates were identified by characteristic golden yellow colouration, and positive catalase, tube coagulase and deoxyribonuclease (13, 14).

Detection of methicillin resistance by phenotypic test

Resistance to methicillin was performed on all confirmed *S. aureus*

isolates by the modified Kirby-Bauer technique using methicillin (5 µg) and oxacillin (1 µg) disks (Oxoid, UK) performed on Mueller Hinton (MH) agar plates containing 5% sodium chloride, which were incubated at 35°C for full 24 hours (15). The diameter of zone of growth inhibition around each disk was measured and interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines (16).

Detection of *mecA* gene by PCR

PCR for amplification of *mecA* gene was performed on the 11 MRSA isolates identified by phenotypic method. DNA was extracted by subjecting overnight cultures of each isolate grown in Brain Heart Infusion (BHI) broth to centrifugation and processing according to the procedure of Arakere et al., (17). The isolated DNA was stored at -20°C until further use. PCR was performed as previously described (18) in a thermal cycler (Gradient Thermocycler, Biologix, China) with *mecA1* forward primer 5'-AAAATCGATGGTAAAGGTTGGC-3' and *mecA2* reverse primer sequence 5'-AGTTCTGCAGTACCGGATTTTGC-3' (18).

The PCR conditions were as described by Oliveira et al., (19) and included an initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 60 seconds, 53°C for 30 seconds, and 72°C for 35 seconds with a final extension at 72°C for 10 minutes. The PCR products were electrophoresed on 2% agarose gel, visualized under UV transilluminator and the image captured with 16 MP Nikon Camera. Amplification of *mecA* gene was expected to produce a band pattern at 533 bp (18).

Results:

Twenty five of the 100 (25%) staphylococci were confirmed as *S. aureus* out of which 11 were resistant to both methicillin and oxacillin. The prevalence of MRSA in the pig population studied is 11% (11/100) but the rate among the *S. aureus* population from the pigs is 44% (11/25). PCR results indicated that all 11 MRSA isolates did not amplify for *mecA*

gene with absence of the expected band at 533 bp (Fig 1).

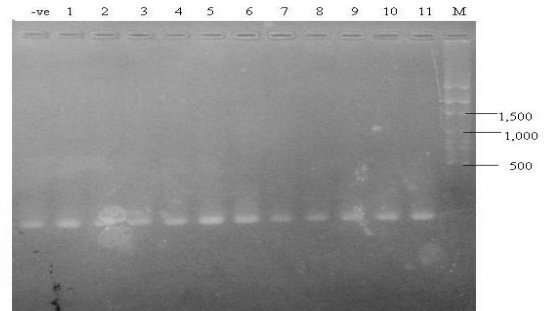


Fig 1: Gel electrophoresis of *mecA* gene

lane M = molecular weight marker; lane 1-11 = *mecA* negative; lane 1 - internal negative control

Discussion:

Although the prevalence of 11% for MRSA isolates among the 100 pigs in this study is lower than the 39% reported in the Netherlands among 540 pigs (20), the MRSA rate of 44% among the *S. aureus* population in the pigs is high. This is very worrisome as many studies including some from Nigeria have shown possible transmission of MRSA infections from pigs to human and vice versa (3-10). A high rate of MRSA in pigs therefore makes them a reservoir and potential source of MRSA transmission and outbreaks, which may constitute a public health challenge in the nearest future.

All the MRSA isolates in our study did not contain the *mecA* gene that is the classical gene responsible for methicillin resistance carried by a unique class of mobile genetic elements, staphylococcal cassette chromosome *mec* (*SCCmec*) (21) originally found in MRSA (22). Other *mec* genes such as *mecC* in *S. aureus* (23, 24) and *mecB* and *mecD* in *Micrococcus caseolyticus* (25, 26) have however been described to mediate methicillin resistance, with capacity for transfer of these genes from one species to another. We did not investigate other *mec* gene types in our study, which is one of the limitations.

Some researchers in Nigeria and elsewhere have also reported absence of *mecA* gene as well as the gene product, PBP2a or PBP2', in phenotypic MRSA

strains and have suggested the possibility of hyper-production of β -lactamase as a cause of this phenomenon (11, 12). We also did not test for this in our study.

Although these were obvious limitations in this study, the possibility of *S. aureus* exhibiting resistance to methicillin and other beta-lactamase resistant penicillins other than through *mecA*-mediated resistance should be considered, especially among livestock MRSA strains.

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