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Identification of the Methicillin-Resistant Gene *mecA* in *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant public health concern due to its resistance to β -lactam antibiotics and its ability to cause a wide range of infections. This study aimed to detect the presence of the *mecA* gene responsible for methicillin resistance in *Staphylococcus aureus* isolates obtained from patients with skin infections. A total of ten (10) swab samples isolates originally collected from patients with urinary skin infections and had been previously processed and stored by our research team was used. Identification of isolates was carried out using colonial morphology, Gram staining, microscopy, and biochemical tests including catalase, coagulase, citrate, and methyl red tests. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method with ampicillin and amoxicillin. Molecular detection of the *mecA* gene was carried out using Polymerase Chain Reaction (PCR) followed by agarose gel electrophoresis. The antimicrobial susceptibility results revealed that 80% of the isolates were resistant to amoxicillin, while 20% showed resistance to both ampicillin and amoxicillin. PCR amplification targeting the *mecA* gene (336 bp) showed that none of the tested isolates carried the *mecA* gene despite some isolates showing phenotypic resistance to β -lactam antibiotics. The absence of the *mecA* gene in resistant isolates suggests the possible involvement of other resistance mechanisms. Despite existing studies on MRSA, limited molecular data exist on *mecA* gene prevalence in this region, and discrepancies between phenotypic and genotypic resistance remain poorly understood. This study highlights the importance of combining phenotypic antimicrobial susceptibility testing with molecular techniques for accurate detection of MRSA. The findings also indicate the need for further investigation into alternative genetic mechanisms responsible for methicillin resistance in *Staphylococcus aureus*.

Keywords: *Staphylococcus aureus*; Methicillin-resistant *Staphylococcus aureus* (MRSA); *mecA* gene; Antimicrobial resistance; β -lactam antibiotics.

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INTRODUCTION

Staphylococcus aureus is a non-motile, aerobic or anaerobic Gram-positive bacterium that is usually found in the nasal membranes and skin of warm-blooded animals and man, in whom it could cause a range of infections from such as skin infections, urinary tract infection and food poisoning (Projan & Novick, 1997). Although the *Staphylococcus* genus includes 52 species and 28 subspecies, *S. aureus* is by far the most clinically relevant and is perhaps the pathogen of very much concern because of its intrinsic virulence, its ability to cause various life-threatening infections, and its capacity to adapt to different environmental conditions (Wertheim et al., 2005). Usually, the infections caused by this bacterium are treated with a variety of antibiotics especially β -lactams and β -lactamase inhibitors. However, in recent times, these bacteria have increasingly become resistant to these antibiotics. The evolution of increasingly resistant bacterial strains is caused by factors such as constant usage and abuse of antimicrobials, the extensive use of these agents as growth enhancers in animal feed, and with the increase in travels both regionally and internationally, these antimicrobial-resistance bacterial strains have been able to cross borders with ease (Lowy, 2003). Among the most important of these antimicrobial-resistance bacterial resistant strains, is the methicillin-resistant *Staphylococcus aureus* (MRSA). Methicillin was the first antibiotic that was modified to neutralize bacterial resistance mechanism. It was tailored to be withstand the attack of the staphylococcal enzyme penicillinase-resistant antistaphylococcal penicillin. However, very soon after its use, it was reported in 1961 that some strains of *S. aureus* had developed resistance to methicillin (Pantosti et al., 2007). The production of an additional penicillin-binding protein (PBP2a) designated is responsible for the methicillin resistance. The *mecA* gene (the gene responsible for methicillin resistance) is part of a mobile genetic element found in all MRSA strains (Katayama et al., 2005). The PBP2a is encoded by the *mecA* gene with reduced affinity for beta lactams and penicillin generally and thus enables staphylococci to survive these agents at high concentrations. Thus, resistance to methicillin confers resistance to all β -lactam agents including cephalosporins. PBPs are membrane-bound enzymes that catalyze the transpeptidation reaction that is necessary for cross-linkage of peptidoglycan chains. PBP2a differs from other PBPs in that its active site blocks binding of all β -lactams but allows the transpeptidation reaction to proceed (Lim & Strynadka, 2002). The *mecA* gene is carried on a structurally complex mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) that can also transport determinants of resistance to other antimicrobials, virulence determinants and other genes important for bacterial survival in stress conditions (Pantosti et al., 2007). Despite numerous reports on methicillin-resistant *Staphylococcus aureus* (MRSA), there is still limited molecular data on the prevalence of the *mecA* gene among clinical isolates in many healthcare settings. Additionally, discrepancies between phenotypic resistance and the absence of the *mecA* gene suggest the

existence of alternative resistance mechanisms that are not well understood. Therefore, further studies combining phenotypic and molecular methods are necessary to accurately detect MRSA and better understand the genetic basis of antimicrobial resistance.

Materials and Methods

Sample collection

A total of ten (10) bacterial isolates were obtained from the microbiology laboratory of Federal University Gusau, Nigeria. These isolates were originally collected from patients with urinary tract infections and had been previously processed and stored by our research team. No direct patient sampling was conducted during this study.

Culture

The swab samples were inoculated on Mannitol Salt Agar (MSA). Inoculation was carried out by gently rubbing/rolling the swab bearing the specimen on the surface of the media to a size of 2 cm to produce a pool of inoculum and then streaked out with wire loop flamed at intervals. After the inoculation, the plates were incubated aerobically at 37°C for 24 hours.

Identification of colonies

Using Morphology and Gram staining, colonies that developed were identified. Colonies from MSA were examined using colonial morphology and gram staining and re-inoculated on another set of fresh MSA plates. This was done by subculturing the suspected colonies of staphylococci from the previous plates on the freshly prepared MSA plates to produce a pool. From the pool, further streaking was made to four quadrants, sterilizing the wire loop between every quadrant. Care was taken to separate growths that showed mannitol fermentation from non-mannitol fermenters. Plates were incubated at 37°C for 24 hours.

Morphology

A presumptive identification of staphylococci was made by observing the growths of pure colonies. Some of these presumptive characteristics include the size, shape, surface, edge, odour and consistency of the colonies.

Gram staining

The slide was washed and cleaned (to make it dust and grease free) and allowed to dry. With the help of inoculating loop, a loop full of cultured bacteria were taken and spread uniformly on the slides, this is called a smear preparation. The smear was allowed to dry in air and then heat fixed. Heat fixing kills bacteria and adheres them to the slide. The crystal violet (Primary stain) flooded on the smear for 1 minute, then it was run off by using tap water. Gently gram's iodine was flooded over the smear for 1 minute, at this moment the smear appeared purple in color. Then the smear was decolorized by flooding the slides with 95% alcohol for 30 seconds. The safranin (counter stain) also flooded over the slides and kept it for 45 seconds, then it was run off with distilled water. The

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slides were allowed to blot dry with bibulous paper and observed under microscope, as described by (Coico, 2006).

Microscopy

Immersion oil was dropped on the slides and viewed at x100 objectives lens, Gram-positive bacteria seen as purple color while gram negative bacteria seen as pink color with round shape (Cocci) or rod shape (Bacilli), as described by (Davies et al., 1983).

Biochemical tests

Catalase test

Catalase test was carried out as described by Oyeleke & Manga (2008). A little portion of the bacterial growth was transferred with a sterilized wire loop to a drop of 3% hydrogen peroxide on a clean glass slide. The presence of bubbling indicated a positive catalase.

Coagulase test

The test was carried out to identify staphylococcus aureus which produces the enzyme coagulase. A drop of distilled water was placed on each end of a slide, a colony of the test organism was emulsified in each of the drops to make two thin suspensions, a loop full of plasma was added to one of the suspensions and mixed gently. Clumping of the organisms within 10 seconds indicated a positive result, as described by (Cheesbrough et al., 2000).

Citrate

A little portion of each isolate was inoculated into koser's citrate media and incubated at 37°C for 24hours, a positive citrate test was confirmed by formation of bright blue color while the initial green color indicated a negative test, as described by (Oyeleke & Manga, 2008).

Methyl red test

Methyl red broth was prepared in a test tubes, then inoculated the broth aseptically with 2 loop full of respective bacterial culture, then the test tube was incubated at 37°C for 24hours, the methyl red indicator few drops was added in the incubated test tubes (Cheesbrough, 2000).

Antimicrobial susceptibility test

About 7.6 g of Muller Hinton agar was suspend in 200ml of distilled water, the solution was heated to boil until it dissolved completely, it was also autoclaved at 121°C for 15minutes, the media was poured into the Petri dish and allowed to solidify (Andrew et al., 1999). To identify MRSA, samples previously identified as *S. aureus* were tested for resistance to Ampicillin and Amoxicillin by the Kirby Bauer discs diffusion method. This was done by suspending the growths from MSA in saline solution and was adjusted to match the turbidity standard of 0.5 McFarland standards. The standardized suspension was used to inoculate the surface of the Mueller Hinton agar plates using sterile cotton swab. Ampicillin and Amoxicillin discs were carefully placed on the center of the plates, the plates were then incubated at 37°C for 24hrs after which zone diameters of inhibition around the disc

were measured using transparent meter rule. Zones equal to or wider than 22mm were recorded as sensitive, while zones smaller than this were recorded as resistant to Methicillin.

DNA extraction from cultured cell

The cultured cells were treated by 200µl of 1xPBS (phosphate buffer saline), and then transferred into 1.5 microcentrifuge tube and centrifuged for 5minutes at 12000rpm. 10µl of RNase A was added and mixed thoroughly, then 20µl of proteinase K was added to the pellet to degrade the proteins inside, then the mixture was incubated for 2minutes at room temperature. Then, 300µl of lysis buffer was added to the sample and mixed immediately by the vortex mixer and incubated for 10minutes at 60°C. 25µl of absolute ethanol was pipetted and homogenized, the lysates were carefully transferred into the upper reservoir of the binding column tube (fitted in a collection tube) without wetting the rim, the tube were closed and centrifuged at 12,000rpm for 1minute. 500µl of WA1 buffer was also added without wetting the rim, the tubes were closed and centrifuged at 8000rpm for 1minute, the solution from the collection tube was discarded and the collection tube was reused. Then 500µl of W2 buffer was added without wetting the rim, then the tubes were closed and centrifuged at 8000rpm for 1minute. The resulting solution from the collection tubes were discarded and the tubes were reused. The pellet was centrifuged once more at 13000rpm for 1minute to completely removed ethanol to ensure that no droplet clinging to the bottom of binding column tube. The binding column tubes were transferred to a new 1.5ml tube for elution, and 70µl of EA buffer was added onto binding column tubes, then it was allowed to stand for at least 1minute at room temperature, followed by centrifuged at 8000rpm for 1minute to elute. The pure DNA were obtained in the 1.5ml container (using Genomic DNA Extraction Kit (K-3032)).

Polymerase chain reaction (PCR)

PCR was carried out in 20µl PCR volumes containing 2.0µl of template DNA, 6µl of molecular grade water, 2.0µl of primer for *MecA* gene detection and 10µl multiplex PCR premix (containing Taq polymerase, loading dye, dNTP and PCR buffer). Amplification of DNA was performed in thermal cycler (Accpower*, Hotstart PCR premix, South Korea) with cycling parameter. The PCR tubes was placed in the thermal cycler and the cycling program was started. The PCR products were analyzed in 1.5% agarose gel containing 12µl of ethidium bromide in tris-acetate EDTA buffer and the gel was photographed under ultraviolet illuminator using gel documentation system (Bio-Rad, USA). Further, 100-2000bp DNA ladder was included in the run. The forward and backward primer were used, forward primer attaches to the antisense strand of the DNA that runs 3' to 5' directions. The primers anneal to the DNA strand and bring about amplification. Reverse primer binds to the sense strand of the DNA that runs in the 5' to 3' directions.

Table 1: Forward and backward MecA gene primer, with the gene base pair

Gene	Sequence	Band size (bp)
MecA	F- GTTGTAGTTGTCGGGTTTGG R - CTTCCACATACCTCTTCTTAAAC	336

Gel electrophoresis

Agarose gel electrophoresis: Agarose gel which is commonly used for separating larger DNA fragments were used. 1.5g of Agarose powder was measured into a conical flask, 1x TAE buffer was used and made it up to 100ml, the solution was heated to dissolve completely; after heating the solution was allowed to cool to 40-45°C, ethidium bromide was added to the solution. The prepared agarose gel was poured on the casting tray and comb was inserted, after it solidified, it was taken to electrophoresis tank. The DNA ladder was added in the first well made by the comb, followed by PCR products, and it was run for 30minutes. The agarose gel was removed and taken to gel documentation.

Results

Table 2: Antimicrobial susceptibility test of *S.aureus* against ampicillin and amoxicillin

S/No	Ampicillin (30 µg)	Amoxicillin (20 µg)
1	0	0
2	0	0
3	0	22
4	32	0
5	36	15
6	14	0
7	25	0
8	30	0
9	24	0
10	13	0

Key: CLSI (2022) was used to interpret the zone of inhibition (mm) of Ampicillin (30 µg) and Amoxil (20 µg) which is Susceptible ≥ 20, Intermediate 15-19, Resistant ≤ 14

PCR Amplification of mecA Gene

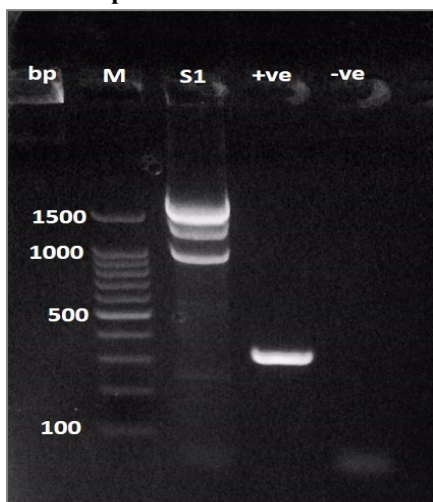


Figure. 1 Polymerase chain reaction result: the first well shows DNA marker, and the next well indicates negative mecA gene which was supposed to be identified at 336bps as shown by the positive control in the third well. The thick multiple bands found in the same well with the DNA are non-relevant genes.

Discussion

A total of ten samples were isolated and identified via gram staining, microscopy and biochemical tests as staphylococcus aureus. Antibiotic susceptibility shows that 80% of the isolates were resistant to Amoxicillin out of the ten (10) samples, only 20% (2) were both resistant to Ampicillin and Amoxicillin. PCR amplification of the mecA gene provided a comprehensive framework to understand the mecA gene. One of the resistant isolates was screened as staphylococcus aureus positive with PCR, however, the mecA gene was negative in the isolate. This is similar to research worldwide such as (Bignardi et al., 1996) where there was absence of mecA gene in resistant staphylococcus aureus isolates. Although several genes which had almost the same primer sequence with the mecA primer appeared on the gel as they had been amplified due to their correlation in PCR conditions with the mecA gene. However, these visualized genes were seen as irrelevant since they are not the gene of interest. This outcome of the research therefore indicates that there might be other intrinsic factors that confer resistance other than the mecA gene as discovered by Ba and his colleagues (Ba et al., 2014).

Conclusion

This study investigated the presence of the mecA gene in Staphylococcus aureus isolates using both phenotypic and molecular methods. Although some isolates exhibited resistance to β-lactam antibiotics based on antimicrobial susceptibility testing, none of the isolates were found to carry the mecA gene by PCR analysis. This finding highlights a discrepancy between phenotypic resistance and genotypic detection, suggesting that alternative resistance mechanisms may be involved. The results emphasize the limitation of relying solely on phenotypic methods for MRSA detection and underscore the importance of combining molecular techniques for accurate identification. These findings have important implications for clinical diagnosis and antibiotic stewardship, as misidentification of resistance can affect treatment decisions. Therefore, further studies with larger sample sizes and investigation of other resistance genes are recommended to better understand the mechanisms of methicillin resistance in S. aureus.

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