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## Original article

# Antibiogram and detection of *mecA* gene among MRSA at Specialist Hospital Sokoto

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### ABSTRACT

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has become more widespread all over the world and it is important to determine methicillin resistance genes in different regions. The major goals of this work were to identify the *mec-A* gene related with MRSA and to assess the antibiogram of clinical isolates of *S. aureus*. **Methods:** Using normal microbiological techniques, 30 clinical Staphylococcal isolates from various specimens were processed to isolate *S. aureus*. The antibiotic susceptibility test was completed using the Kirby-Bauer disc-diffusion method in accordance with EUCAST criteria. Cefoxitin (30 g) discs were used to screen for MRSA isolates, and the standard polymerase chain reaction (PCR) was used to amplify the *mec-A* gene. **Results:** *Staphylococcus aureus* predominance was 66.6 percent (n = 20) among the 30 bacterial growths. Methicillin-resistant *Staphylococcus aureus* prevalence was 100% (n = 20), and multidrug resistance was present in 85% (17/20) of the cases (MDR). The majority of the *S. aureus* isolates were resistant to penicillin (95.2%), cefoxitin (100%), tigecycline (60%) and the combination antibiotics quinipristin-dalfopristin (50%) as well as tobramycin (30) and trimethoprim-methotrexate (20). The results of the PCR show that four out of the twelve isolates analyzed were *mecA* gene. **Conclusion:** Without taking antibiotic resistance into account and avoiding antibiotic use, fighting these superbugs won't be achievable. This might quickly escalate into an unmanageable situation. According to this study, MRSA is more common than previously believed and about 80% of isolates are multidrug resistant.

### Introduction

*Staphylococcus aureus* (*S.aureus*) is one of the most well-known and widely spread bacterial pathogens, causing an unknown number of uncomplicated skin infections each year, and hundreds of thousands to millions of more serious, invasive infections worldwide [1,2]. Pneumonia, surgical site, prosthetic joint, cardiovascular infections and respiratory tract infections, as well as nosocomial bacteremia, are all caused by *S aureus* [3]. By nature, *S. aureus* is susceptible to every antibiotic that has been developed by mankind.

However, this pathogen also demonstrated the ability to develop antibiotic resistance mechanisms to aid its survival against antibiotics [4]. Shortly after the usage of penicillin commercially, *S. aureus* strains that are resistant to penicillin were widely spread worldwide [5]. In addition to molecular characterization, antibiotic resistance, notably regarding the emergence and evolution of multidrug-resistant (MDR) *S aureus*, has become a major focal point in research across the world. Later, superbug bacteria called methicillin-resistant *S*

*aureus* (MRSA) that are resistant to multiple antibiotics emerged from medical settings, causing difficulties in treating persistent *S aureus* infections. Methicillin-resistant *S aureus*, which begins with resistance to methicillin or most beta-lactam antibiotics gradually develops co-resistance to vancomycin [6,7], limits the use of alternative anti-infective drugs and threatens patient's health. Hence, drug resistance should be closely monitored to provide the basis for clinical antibacterial infection treatment, including exploring antibiotic resistance of *S aureus* isolated from clinical settings. Methicillin-Resistant *S aureus* has increased in prevalence over the past 40 years, from minor irritation to a serious public health threats [8]. One of the most hazardous organisms MRSA, causes infections ranging from mild skin infections to more severe illnesses to potentially fatal conditions such as septicemia, meningitis, endocarditis, and pneumonia [9]. More than half of all nosocomial infections are caused by MRSA [10]. Methicillin resistant *S aureus* infections are widespread in the health economy due to their association with increased morbidity, death, inferior outcomes, and greater costs [11]. In order to combat MRSA, infection control measures must be reinforced, as well as antimicrobials must be used wisely. In *S aureus*, methicillin resistance is mediated by a mutated protein called penicillin-binding protein with a low affinity (PBP2a). PBP2a is encoded by the *mecA* gene and is found in a chromosomal mobile genetic element called SCCmec. Because MRSA is linked to multiple drug resistance and a higher treatment cost, precise and timely MRSA identification is critical in the clinical context for MRSA infection management. Our present study aimed to study the antibiotic susceptibility and detection of *mecA* gene associated with MRSA from clinical isolates.

## Material and Methods

### Sample collection and identification

From July 2021 to December 2021, this descriptive study was carried out at the Specialist Hospital in Sokoto. This research was conducted in the Department of Microbiology at the Faculty of Life Sciences Kebbi State University of Science and Technology Aliero. Thirty (30) suspected staphylococcal isolates were collected using nutrient agar for additional examination from clinical samples including blood, sputum, wound swabs, and urine that were given to the hospital's medical

microbiology section throughout a Five-month period. The collected clinical isolates were delivered right away to the KSUSTA microbiology lab within a period of two to three hours. Using impregnated swab sticks, suspected *S aureus* isolates were inoculated onto nutrient agar and incubated for 18 hours at 37°C. Growths from the nutrient agar were streaked over sterile mannitol salt agar (MSA), then incubated for 18 hours at 37°C to get purified single distinct golden yellow colonies that were assumed to be *S aureus*. According to [12], the presumptively detected *S aureus* was subjected to culturing on mannitol salt agar and further identified by Gram stain, catalase and coagulase tests.

### Determination of antibiotic sensitivity of *S. aureus* isolates

The ability of *S. aureus* strains to withstand several antibiotics was assessed using the Kirby-Bauer disc diffusion method. Separate colonies of isolates from nutrient agar plates were emulsified in 5 ml of sterile physiological saline and the turbidity was adjusted to 0.5 McFarland standard (about  $1.5 \times 10^8$  cfu/ml) for analysis. The standardized suspension was injected on Muller Hinton agar using sterile swab to ensure equal dispersion and confluent development. The various antibiotics' sensitivity discs were positioned aseptically utilizing sterile forceps on the dry, inoculated surface of the agar. The plates were then incubated at 37°C for 18 hours after the discs had been applied for 30 minutes. The plates were analyzed for zones of inhibition and result interpretation using EUCAST after incubation. Tobramycin (10 mg), Cefoxitin (30 mg), trimethoprim-sulfamethoxazole (5 mg), tigecycline (30 mg), quinipristin and dalfopristin (10 mg), and penicillin (10 mg) are some of the antibiotics that will be utilized, as per (EUCAST) guidelines.

### Phenotypic detection of MRSA by cefoxitin disc-diffusion method

The modified Kirby-Bauer disc-diffusion method was used to evaluate the susceptibility of *S. aureus* isolates to cefoxitin (30 g), following EUCAST recommendations (Oxoid Limited UK). These *S. aureus* strains were identified as MRSA after showing that they were resistant to cefoxitin (isolates with a zone of inhibition 21 mm in diameter) [13]. Oxoid Limited UK donated antibiotic susceptibility discs for the study.

### Molecular detection of *mec A* gene from *Staphylococcus aureus* isolates

#### DNA extraction

Genomic DNA extraction was completed Using the Zymo Research Protocol technique [14]. The extracted cell pellets were shaken loose before being thoroughly mixed by vortexing with 200µL of deionized water. After 400µL of the lysis solution had been added, the mixture was mixed. The mixture was then incubated at 70°C for 15 minutes to completely lyse all cells and make them appear viscous to prevent clogging the zymo-spin column. A zymo-spin™ IV spin was transferred exactly 400µL of supernatant and centrifuged for one minute at 7000 rpm with a filter in a collection tube. Additionally, the filtrate in the collecting tube from the previous stage received approximately 1200µL of DNA binding buffers. Exactly 800µL of the mixture from the last step was transferred to a new collecting tube, centrifuged at 10000g for one minute, and then placed on a zymo spin IIC Column. The flow-through stage from earlier was repeated after discarding the collecting tube used. One minute was spent centrifuging a fresh collection tube with a zymo spin column and 200µL of DNA pre-wash buffer at a speed of 10,000. Before being centrifuged at 10,000 g for one minute, the zymo spin column received roughly 500µL of DNA wash buffer. The zymo spin was transferred to a clean 1.5 ml microcentrifuge tube after the column matrix had been directly filled with 100µL of DNA elution buffer. The DNA was then separated by centrifuging for one minute at 10,000 g. [14]

#### DNA amplification of *mecA* gene

After external reaction tuning to achieve a better amplification, the *mecA* gene was amplified using end point PCR. The procedure that was followed was; For each isolate, the following components were added to a single 25-µl reaction in the thin-walled PCR tubes that were labelled. Viz: The PCR

tube was filled with 12.5µL of Top Taq PCR master mix (Qiagen, USA), 1.0µL of forward primer (Table 1), 1.0µL of reverse primer (Table 1), 5.0µL of temple DNA (genomic DNA), and 4.5µL of nuclease-free water. Water free of nucleases served as the model for unfavorable management. The tubes were spun down, and the Applied Biosystem 9700 thermal cycler was used to carry out the PCR. The cycler was set up to perform 40 cycles of initial denaturation at 97°C for 5 minutes, followed by annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and final denaturation at 72°C for 40 cycles. The amplified products were dissolved in agarose gel at a concentration of 2%. In a 250 ml conical flask, 2g of agarose powder was dissolved in 100 ml tris acetate EDTA, microwaved for 2 minutes, and then cooled. Before stirring and gently pouring into a gel caster with a comb inserted, 5µL of ethidium bromide was added. This was given 30 minutes to set up. The gel slab was put into a BioRad micro tank that had been filled with 1X TAE after the comb and adhesives had been carefully removed. Amplicons, 100-bp plus ladder, an 8µL negative control solution from Biolabs in the UK, and amplified product were injected into the designated wells. 70 Volts were used for the electrophoresis for a whole hour. The gel slab was taken out, put on the BioRad XRS gel imager, and examined with a UV transilluminator. The expected amplicon size was considered when documenting and interpreting the gel.

#### Statistical analysis

The analysis was conducted by the principles of descriptive and inferential statistics using graphs, figures and diagrams to explain the results.

**Table 1.** Primer sequence and PCR conditions used in amplification of *mecA* gene

Target gene	Primer sequences	PCR condition	Size (bp)	Reference
<i>MecA</i>	5'-TCCAGATTACAACCTCACCAGG-3' 5'-CCACTTCATATCTTGTAACG3'	32 cycles of 94°C for 30s, 53°C for 30s, and 72°C for 50s	162	Oliveira and Lencastre, 2002

## Results

### Isolation and identification of *S. aureus* isolates

Initial identification by growth on mannitol salt agar showed that only 26/30(86.6%) isolates were

suspected to be *Staphylococcus aureus* based on the formation of golden yellow colonies that indicates fermentation of phenol red in mannitol salt agarA. Further identification showed that 20/30(27.6%) were coagulase positive while 6/30(20.8%) were

coagulase-negative, final identification confirmed 20/30(26.8%) of the isolates to be *Staphylococcus aureus* as shown in **figure (1)**. The majority of *S. aureus* isolates were from wound (40 %), blood (30%), and sputum (5%) while urine samples (25%). A breakdown of the prevalence of (26.7%) in wound samples was recorded as shown in **table (2)**. The percentage of *S aureus* isolates by specimen source was shown in **figure (2)**.

#### Antibiotic susceptibility of *S. aureus* isolates

The *S. aureus* isolates were generally resistant to penicillin (95.2%), cefoxitin (100%), tigecycline (60%), Quinipristin- dalfopristin (50%), tobramycin, (30) and trimethoprim and methotrexate (20) as shown in **table (3)**. **Figure 3** shows the percentage resistance of the antibiotics tested in this study.

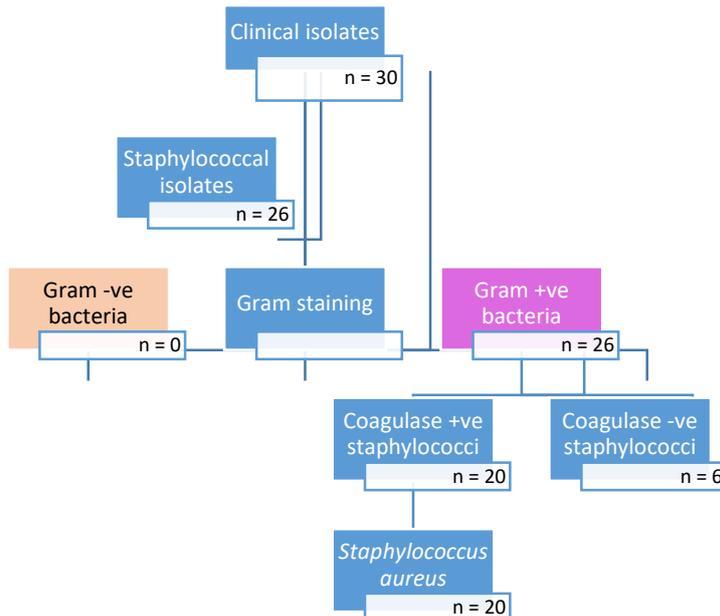
**Table 2.** Distribution of *Staphylococcus aureus* isolates by specimen.

Isolate source	<i>S. aureus</i> =20	Prevalence %
Blood	6(30)	20
Urine	5(25)	16.7
Wound swab	8(40)	26.7
Sputum	1(5)	3.33

**Table 3.** Distribution of methicillin-resistant *Staphylococcus aureus* in clinical isolates.

Sample type	MRSA	MSSA
Blood	6(30)	0(0)
Urine	5(25)	0(0)
Sputum	1(5)	0(0)
Wound swab	8(40)	0(0)
Total	20(100)	0(0)

**Figure 1.** Isolation and identification of *S. aureus* clinical isolate.



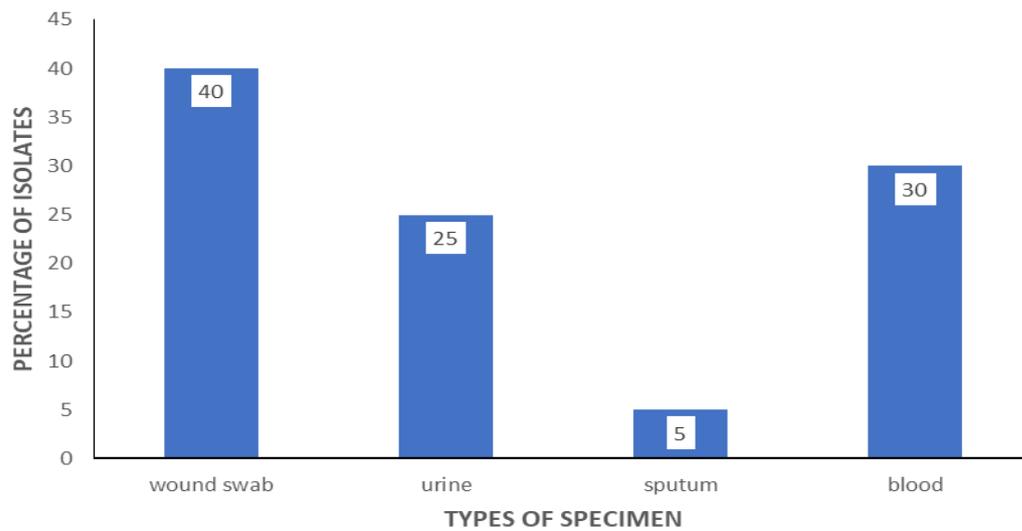
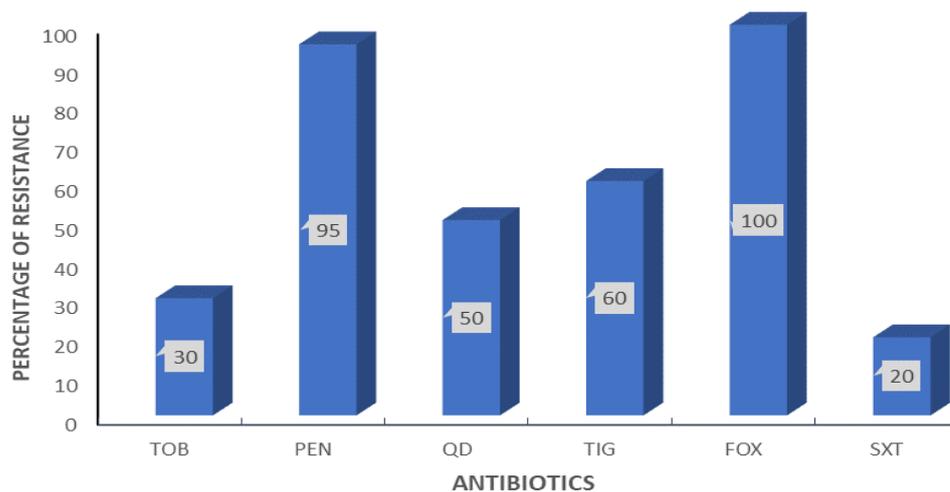
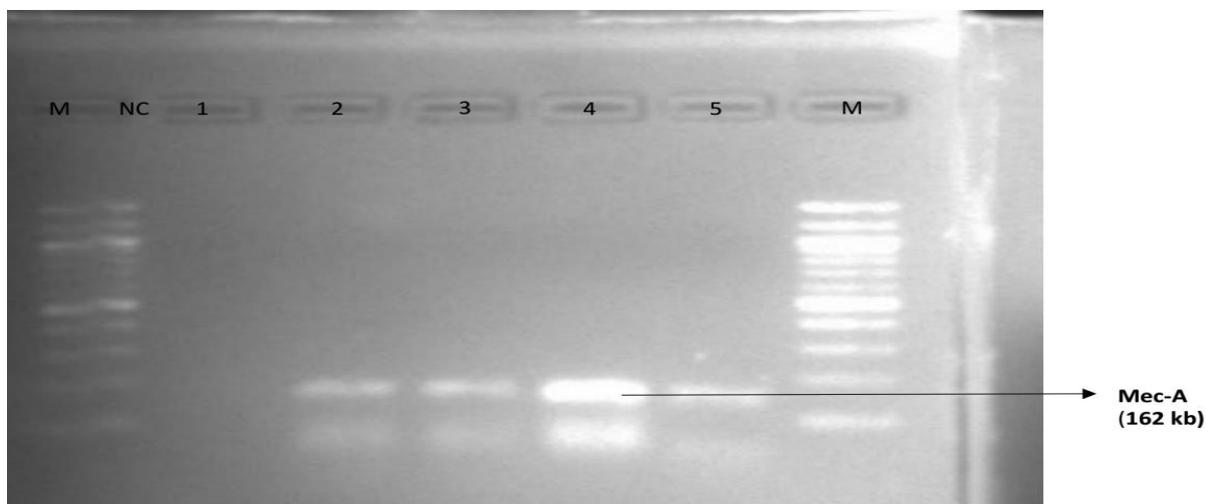
Resistance to cefoxitin indicates MRSA. A total of 16 (80%) of the *S. aureus* isolates were MDR.

#### Phenotypic detection of methicillin resistant *Staphylococcus aureus*

A total of 20 (100%) of *S. aureus* isolates tested phenotypically using cefoxitin disc were MRSA and 0(0%) were MSSA as shown in **table (4)**. The highest percentage distribution was 8(40%) from wound swabs isolates.

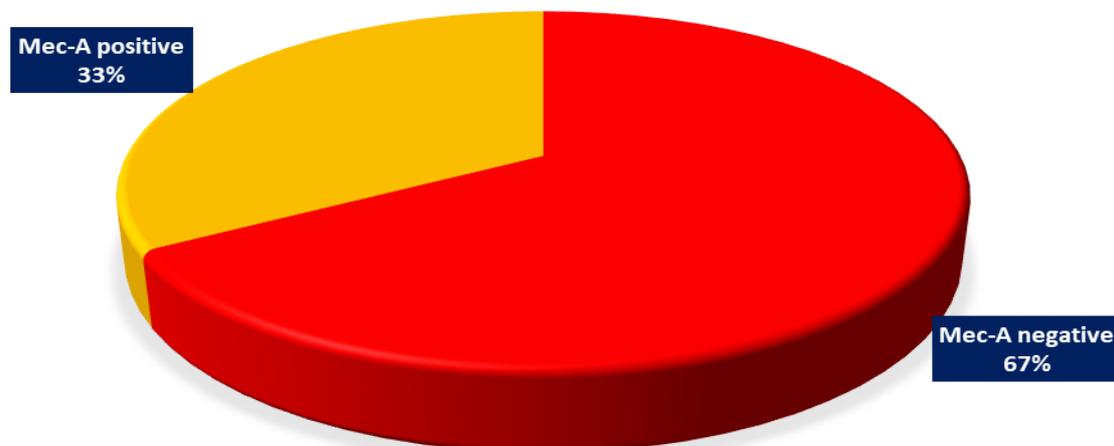
#### Detection of mec-A gene using PCR

As indicated in **figure (4)**, the mecA gene was discovered using polymerase chain reaction. **Figure 5** shows that out of 12 MRSA isolates examined by PCR, 4 (33%) were mecA positive and 8 (67%) were mecA negative.

**Figure 2.** Percentage of isolates by specimens' source.**Figure 3.** Resistance of *Staphylococcus aureus* isolates to tested antibiotics.**Figure 4:** Agarose gel electrophoresis image of *mec-A* gene at 162 bp. Lane M: 1000bp Molecular DNA ladder Lane NC was control Lane 2: w10, Lane 3: B5, Lane 4: U2 and Lane 5: W5 were the representatives of the *mecA* positive *S aureus* isolates.

Key: W = wound, U = urine, SP = sputum, W = blood, bp = base pair.

**Figure 5.** Percentage of *S aureus* isolates with *mecA* positive.



### Discussion

More reports on the prevalence and antibiotic resistance of *S. aureus* have surfaced in recent years. Because these bacteria are becoming increasingly resistant to routinely used antibacterial medicines, there is an immediate need for accurate drug-resistant strain detection to control infection-related risk factors. As a result, the current work attempted to investigate the molecular identification of the *mecA* gene in MRSA.

Antibiotic resistance is growing at a much faster than new medications are being introduced into clinical practice, putting the world's health at risk. Because of the extensive use of antibiotics as a result of the overuse and manufacture of antibiotics, many microorganisms have acquired resistance to them, leaving these treatments worthless. The prevalence rate of MRSA was found to be 66.6% in the current investigation. This conclusion is consistent with [15] and significantly higher than the previous report, which found a 52% prevalence rate of MRSA in 2017 in Karachi [16] and [17]. MRSA prevalence was reported to be 50% in Lahore by [18]. In Europe, however, a frequency of MRSA of 65% was seen in *S. aureus* isolated from ICUs [19]. This is in contrast to research conducted in Nigeria, Ido-Ekiti which discovered 31% MRSA [20], reported 32.2% MRSA in Peshawar, Pakistan, Nepal, [21] reported 70.6% MRSA in Nepal, and [22] reported 29.23 percent MRSA. MRSA detection is critical for patient care and efficient use of infection control resources. Methicillin resistant

*S. aureus* is the main pathogen that has arisen in the previous four decades, causing nosocomial and community-acquired infections. The administration of appropriate antibiotic therapy and the management of nosocomial transmission of MRSA strains need rapid and precise diagnosis of methicillin resistant *S. aureus*. The pattern of antibiotic resistance demonstrates that Tobramycin resistance was found in 30% of MRSA isolates, quinupritin/dalfopristin resistance was found in 50%, and tigecycline resistance was found in 60%, and penicillin resistance was found in 95%. and cefoxitin was found in 98%. The cefoxitin resistance found in our study is in conformity with the finding of [23] who reported 100% resistance. The overall trend of antibiotic resistance is developing, according to this study, and the time period of resistance development is concerning. Tobramycin and trimethoprim-sulfamethoxazole are the only antibiotics that can currently be used to treat MRSA infections, as they have shown the least resistance of any of the antibiotics evaluated in this study. Antibiotic resistance must be taken into account, and overuse of antibiotics must be avoided, or else the fight against these superbugs would become an unmanageable problem. The differences in MRSA prevalence could be attributable to differences in study sites and time periods, infection prevention techniques, antibiotic prophylaxis, and therapies practiced in various health centers.

Multidrug resistant *S. aureus* was found to be present in 85.5% of the cases. This is in contrast

to the 44% stated by [24]. Antibiotic usage is driving the evolution of resistance [25]. Antibiotic resistance develops in bacteria due to horizontal gene transfer between various species of bacteria and mutation [26]. As a result, the greater prevalence of MDR could be attributable to antibiotic overuse and abuse, which is frequent in Sokoto, Nigeria. Many phenotypic methods to detect MRSA have been developed but they are slow and vary in sensitivity and specificity [27] (**Datta et al. 2011**). Currently, detection of *mec-A* gene by PCR is the gold standard for MRSA identification [28] (**Pillai et al. 2012**). *mecA* gene is located within chromosome in a structure called Staphylococcal Cassette Chromosome (*SCCmec*) encodes mutant PBP2a or PBP2' of 76 kDa [29] (**Jain et al. 2008**). The presence of *mecA* gene is generally to indicate the potential resistance to beta-lactam group and used as a marker to identify MRSA. In this study 12 MRSA isolates were selected for PCR and 33% possessed *mecA* gene and 67% were *mecA* negative. Similar results were obtained by [16] **Siddiqui et al.** (2018) and [24] (**Gaire et al 2021**) in which 36.5% and 53% were *mecA* positive respectively. Our result is lower compared to a report by [30] **Hadyeh et al. 2019**, [31] **McTavish et al. 2019** who reported 95% *mecA* positive. Absence of *mecA* gene in isolates classified as MRSA phenotypically could be attributed to the *mecC* gene that also confers resistance to methicillin. Other factors that may be responsible for the methicillin resistance is hyper-production of  $\beta$ -lactamase by these isolates [32] (**Adhikari et al., 2017**).

### Conclusion

One of the most pervasive modern pathogens, MRSA can cause various illnesses, from minor skin infections to serious ones including pneumonia, meningitis, and septicemia. Controlling such infections has become more challenging due to the rise in methicillin resistance. Healthcare- and community-related illnesses have a connection to MRSA. Antibiotic usage must be minimized and antibiotic resistance must be considered if we have any chance of defeating these superbugs. This might quickly escalate into an unmanageable situation. According to this study, MRSA is more common than previously believed, with about 80% of isolates being multidrug-resistant. Understanding it is essential for medical therapy and effective utilization and control of infectious sources.

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### Conflicts of interest

The authors declare no conflict of interest.

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### Ethical approval

Permissions for carrying out the study were obtained from the Research Ethics Committee at Specialist Hospital Sokoto.

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