Journal of Wasit for Science and Medicine 2024: 17, (3), 66-78

Molecular Study to Detect MRSA Strains in Different Clinical Cases

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Abstract

Staphylococcus aureus is an opportunist that causes systemic infections in the human being body. This organism increases its resistance to many categories of antibiotics more resistant. Given this fact, a total of one hundred seventy-two (N = 172) samples, and the total number of positive samples for Staphylococcus aureus was seventy-seven (N = 77) samples. The number of positive which included 30 (38.39 %) pus samples, 27 (35.0 %) urine samples, 11 (14.28 %) of nasal swabs, and 9 (11.68 %) blood samples. These isolates were identified according to culture, microscopic examination, biochemical tests, and APIstaph system identification kits. The results showed 34 (44.15 %) of isolated *S. aureus* were MRSA strain. regarding of the molecular identification of MRSA the results showed mecA and Insertion sequence (IS431) is present in all cases in percent 100 % (34/34), (IS127) were 100 %, (34/34). Other gens were gene of ccrC 20/34 (57.14 %) and ccrA2-B gene 6/34 (17.64 %).

Keywords: MRSA Strains, *Staphylococcus Aureus,* and Systemic Infections.

1. Introduction

Staphylococcus aureus is a common opportunistic pathogen. Usually colonizes human skin, and nasal mucosa and causes a variety of infections ranging from mild skin lesions to severe diseases such as endocarditis, osteomyelitis, pneumonia, and even life-threatening septic shock [1]. *S. aureus* infection becomes more troublesome when methicillin-resistant *S. aureus* (MRSA) was isolated [2].

Resistance of *Staphylococcus aureus* to *β*-lactam antibiotics is associated with the expression of penicillin-binding protein 2a (PBP2a). That encoded by the mecA gene, which is located on a mobile genetic element, *staphylococcal cassette* chromosome mec (SCCmec) [3]. According to the gene structure and composition, SCCmec can be classified to different types. Among them, type I, II and III are associated with healthcare-

associated MRSA (HA-MRSA) because they are more likely to carry resistance genes.

While type IV and V are thought to be associated with community-associated MRSA (CA-MRSA) [4]. In addition to antibiotics resistant *S. aureus* is a versatile pathogen that can produce various kinds of virulence factors to destroy host cells and cause infections by multiple ways [5]. The existence of some traditionally important virulence factors genes of *S. aureus* is including some adhesion-associated genes which are crucial in biofilm formation such as icaA, icaD, fib, fnbA, fnbB, clfA, clfB, and extra.

Superantigen genes including some enterotoxin genes and the tst gene, and some other important exotoxin genes such as pvl, hla, and hld, which are closely related to severe *S. aureus* infections and tissues destruction [6-7].

2. Materials and Methods

Cross-sectional study was conducted at General Hospital of Al-Suweira in Wasit province in Iraq during the period from December 2023 to May 2024. Approval was obtained from Wasit Health Director to conduct the study using simple random sampling technique.

Patients of either gender were enrolled for obtaining specimens from different clinical cases. Also, the sample size was calculated using the formula [8].

$$Z_{1-\alpha/2}{}^2 P(1-P) / d^2$$

Where, the standard normal variate $Z_{1-\alpha/2}$ was 1.96 at 5 % type 1 error ($p = 0.05$), and 2.58 at 1 % type 1 error ($p = 0.01$). As $p < 0.05$ are typically regarded as significant in research, 1.96 was utilised in the formula in which p was the population expected proportion based on previous studies [9], and d was the absolute inaccuracy or precision determined by the researchers.

Briefly, sample was collected from one or both eyes based on the nature of the infection. Sterile cotton swab that had been premoistened with sterile physiological saline was used gently to collect eye discharge. The Eswab was rubbed softly over the lower conjunctival sac from medial to lateral side and back again [10]. Then, collected simple was cultured on several different media, such as blood agar, MacConkey agar, and mannitol salt agar.

The latter being a selective medium for *S. aureus*. Moreover, samples were incubated at 37 °C for 24 hours after which cultured bacteria were isolated and identified according to colony morphology, shape, size, colour and pigment production. Deoxyribonucleic acid (DNA) extractions were carried out using a commercial kit (Presto™ Mini gDNA Bacteria Kit, Geneaid, Thailand) to obtain DNA

templates for use in PCR assays. The DNA of P. aeruginosa isolates were extracted as per the manufacturer's instructions.

Cell harvesting pre-lyses, the bacterial strains were cultured on mannitol salt agar for 18 hours at 37 °C. Then, they were harvested by centrifugation for 1 minute at a speed of 14,000 rpm, with the supernatant being discarded. Furthermore, a 20 µL of proteinase potassium (K) (solution and 180 µL of buffer guanidinium thiocyanate (GT) were added to the pellet and mixed, with the sample tubes inverted every three minutes for the duration of the incubation period.

After mixing for 10 seconds with 200 µL of buffer guanidine brochloride (GB), the cell lysate was incubated for 10 minutes at 70 °C, with sample tubes mixed by inversion every three minutes to induce lysis. The elution buffer was pre-heated 200 L/sample at 70 °C for DNA elution. DNA binding, the lysate samples were treated with 200 µL of 100 % ethanol and thoroughly mixed by shaking. The mixture was transferred to a spin column in a 2 mL collection tube and placed in a new 2 mL collection tube for the genome DNA (GD) column.

DNA elution, the spin column was placed in a 1.5 microcentrifuge tube, and 100 µL of pre-heated elution buffer was added to the middle of the column matrix. After letting the mixture stand for three minutes to ensure that all elution buffers had been absorbed. The spin column was centrifuged for 30 seconds at 14,000 rpm to elute the purified DNA. The extracted DNA was stored in the freezer at -20 °C until use.

The concentration and purity of the DNA was measured by using an instrument (Nano Drop) and agarose gel electrophoresis. During the process, 1 µL of the extracted DNA was added to the instrument to detect DNA concentration and purity by analysing the optimal degree (OD) 260/280 ratio to verify the protein and DNA concentration.

Agarose gel electrophoresis, 1x Tris-borate-EDT(TBE) buffer was placed in the electrophoresis tank, after which the agarose tray was immersed in the electrophoresis tank. It was ensured that the buffer was roughly several millilitres above the agarose surface. Each well was filled with 5 µL of the sample and 2 µL of dye, and the tank was then filled and closed. Electrophoresis was performed using 70 volt/cm of gel run swat electrophoresis.

With the use of gel paper, the agarose was extracted from the tank and visualised. The optimization of the primers used, 2.5µL of the master mix was mixed with 5-6 µL of DNA, along with 1 µL of the forward and revers primers. Optimisation was programmed for SeA, Seb, Sec, Sed, Tst, eta, mecA, and Etb gens. Primer of gene grades were chosen, and the annealing

temperature of PCR were set at 55 °C, 58 °C and 52 °C. Detection of SeA, Seb, Sec, Sed, Tst, eta, mecA, and Etb. genes were carried out by mixing 12.5 mL master mix, 5-6 mL DNA, 1 mL each of forward and reverse primers.

Nuclease-free deionised water to a final volume of 20 mL, as per the manufacturer's instructions. PCR cycling programme parameters used in the reaction for the detection of the genes of interest were noted (table 1). Resulted data were analysed using SPSS 20. Chi-square test was used to analyse the data. $P < 0.001$ was considered statistically significant [11].

Table 1: The sequence and source of the gene primers used in the study [3].

	Gene		Primer sequence	Size(pb)
SCCmec Typing	Ba3	F	ATTGCCTTGATAATAGCCYTCT	937 pb
		R	TAAAGGCATCAATGCACAAACACT	
	ccrC	F	CGTCTATTACAAGATGTTAAGGATAAT	518 pb
		R	CCTTTATAGACTGGATTATTCAAAATAT	
	1272	F	GCCACTCATAACATATGGAA	415 pb
		R	CATCCGAGTGAAACCCAAA	
	5R*mec*A 5R431	F	TATACCAAACCCGACAACTAC	359 pb
		R	CGGCTACAGTGATAACATCC	

2.1 Antimicrobial Susceptibility Test

For antibiotic resistance phenotypes methicillin / oxacillin sensitivity tests. All isolates of *S. aureus* were checked for the sensitivity to 1 µg oxacillin disc and 5 µg methicillin disc (Difco) by the disk diffusion method that instructed by NCCLS. The resistance breakpoints were ≥ 12 mm to ≤ 10 mm for 1 µg oxacillin and ≥ 14 mm to ≤ 10 mm for 5 µg methicillin. The capacity of extra antibiotic discs to inhibit MRSA or MSSA was estimated according to the instructions provided by NCCLS using commercially available discs that include.

Augmenitin (AC 30 µg), tetracycline (T,30 µg), erythromycin (E,15 µg), ceftizoxime (CEF 20 µg), ciprofloxacin (Ci 5 µg), clindamycin (CC, 2 µg), clarithromycin (Cl 15 µg), and vancomycin (V, 30 µg). The zone of inhibition produced by *S. aureus* against each antibiotic was measured and interpreted as resistant and susceptible according to standards of Clinical Laboratory and Standards Institute [12].

3. Results and Discussion

3.1 Prevalence of *S. aureus* Among Various Clinical Samples

The current study included one hundred seventy-two samples. The total number of positive samples for *Staphylococcus aureus* was seventy-seven samples. The number of positive which included 30 (38.39 %) pus samples, 27 (35.0 %) urine samples, 11 (14.28%) of nasal swabs, and 9 (11.68 %) blood samples as shown in (figure 1). The study was conducted at the General Hospital of Al-

Suweira during the period from December 2023 to May 2024.

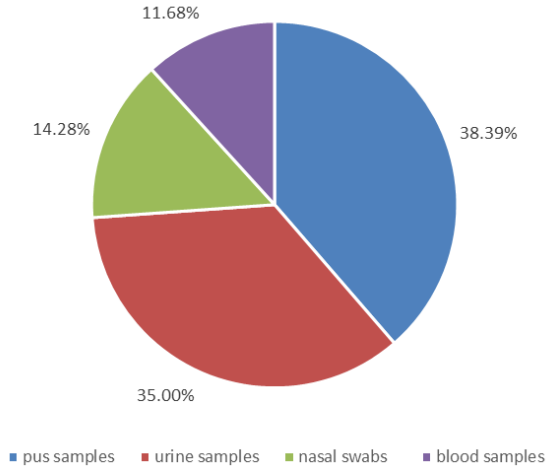

Figure 1: Prevalence of *S. aureus* among various clinical samples.

The present study findings revealed that *S. aureus* isolated from nosocomial infection. Maybe caused by different disease, can operate as a reservoir for opportunistic microorganisms. If antibiotics are used to treat periodontal disease or other infections. Antibiotics can lead to an increase in *Staphylococcus spp* strains. Also, can cause antibiotic resistance is widespread and can periodontitis develop because of antibiotic therapy.

The fact that *S. aureus* is more prevalent in nosocomial infection. The current percentages of isolated *S. aureus* are consistent with those reported by Jahanshahi and co-workers [13]. Jahanshahi found that pus samples were 36 (33.8 %), followed by urine samples and nasal swabs at 19 (26.8 %), and 12 (16.9 %) respectively. According to Kirmusaolu [14] finds a prevalence of *S.aureus* in the pus samples of 21 % and gingival swabs of 11 % in 110 patients attending a dental hospital with a variety of oral illnesses. Salivary carriages of *S. aureus* were detected in 41 % of patients with decreased salivary flow rates attending an oral medicine clinic, with concentrations ranging from 3.7 × 101 to 5.2 × 103 cfu mL [15].

Because of the variety of the normal oral flora and the healthy carriage of *S. aureus* in specific patient groups, the case for *S. aureus* in the etiologic of nosocomial infection. However, given the high rates of *S. aureus* recovery in patients with oral mucosal symptoms such as pain, burning, erythema, and swelling, physicians should consider the potential of this pathogen playing a role in oral mucosal illness [16].

3.2 Prevalence of Methicillin Resistant S. aureus (MRSA)

All 77 coagulase positive isolates of *S. aureus* were subjected to disc diffusion method to 5 µg methicillin disc and 1 µg oxacillin disc to determine MRSA. The test results discovered that 34 (44.15 %) of isolated *S. aureus* were MRSA strain (figure 2).

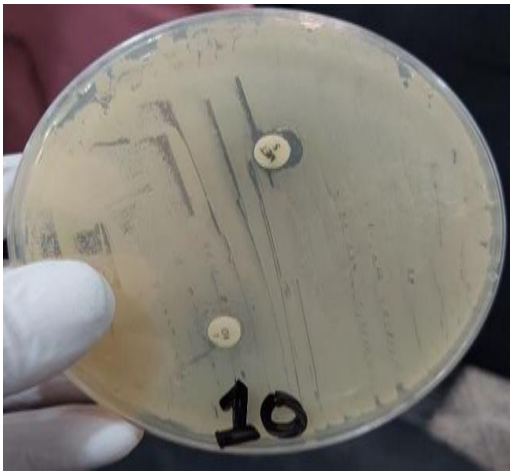

Figure 2: Detection of MRSA.

3.3 The Susceptibility of MRSA and MSSA Isolates toward Antimicrobial Agents

The Susceptibility of MRSA and MSSA isolates toward antimicrobial agents are shown in (figure 3), and (figure 4). The results of current study showed that the rate of MRSA was 34 (44.15 %) from nosocomial infection is lower than the rate reported from Iraq in previous reports in which MRSA was isolated from 85 % of health workers in Basrah city [17].

Also, it was lower than that reported by Hussein [18]. Among health care workers in Kurdistan region of Iraq in 2015 where the MRSA prevalence was 53 %. On the other hand, studies in Iran were 69 % by Jahanshahi et al., [13]. While in a study conducted in India, the percentage was much lower 16.6 % [19].

MRSA prevalence 51.4 % at the Korean hospital from the *Staph aureus* collected from blood and nasal colonizers [20]. In general MRSA was highly prevalent in Asian countries [12]. Moreover, in Germany the study shows a decrease in MRSA rate [21].

In Turkey high rates of *Staph aureus* highly resisted to penicillin and ampicillin. Another study in Isfahan Iran, in 2018 showed that MRSA was 51.9 % among oral infection patients and 16 % among health workers [14]. HA-MRSA occurred at a higher rate than CA-MRSA in the world, but in Iraq the rates were similar for the HA-MRSA and CA-MRSA 19.4 %, and 17 %.

This result can be explained by long hospitalization, random use of antibiotics, lack of awareness, and receiving antibiotics before coming to hospital. That are some of potential predisposing factors for the appearance of MRSA in the hospital and community. Results of current study differs from that reported in the United States of America where a high incidence of MRSA occurred in a hospital-acquired *S. aureus* infection (HA-MRSA) (59 %), compared to a community-acquired infection of *S. aureus* (17 %) 19.

This difference can be explained by the CA-MRSA biology appearing to be different from the HA-MRSA and the MSSA, which may allow CA-MRSA to cause diseases other than those expected from MSSA [22]. Regarding susceptibility

of MRSA and MSSA isolates to antimicrobial agents the results showed that MRSA appeared more resistant to antibiotic than MSSA. Wang also found higher antibiotic resistance rate in MRSA compared to MSSA except with Trimethoprim/Sulfamethoxazole [23]. Multi-Drug Resistance (MDR) was more evident among the MRSA than MSSA [21].

MRSA in this study were Multi Drug Resistant (MDR). The result was like previous research by Arora et al., [4]. Like the study results by a high resistance to Cifoxitin (100 %) amoung MRSA isolates. Hghest susceptibility for Vancomycin and Genatmycin MRSA is resistant to all types of antibiotics containing β -lactam [24].

The resistance is conducted with low affinity for *β*-lactam antibiotics resulting in resistance to all *β*-lactams antibiotics or due enzymes that hydrolytically destroy *β*-lactams, MRSA may contain one or both mechanisms [23].

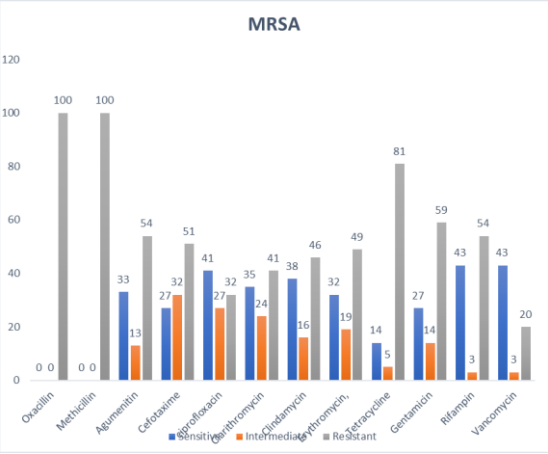

Figure 3: Susceptibility of MRSA isolates to antimicrobial agents.

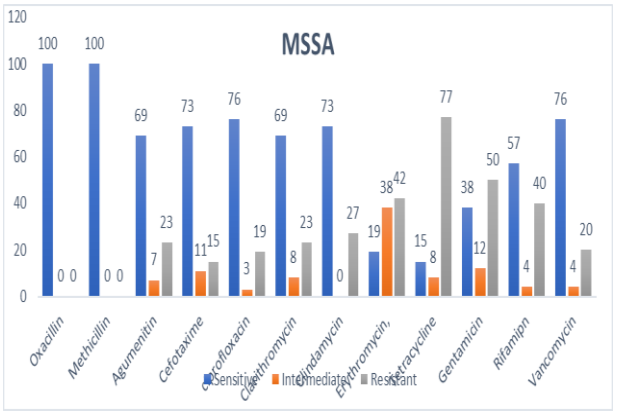

Figure 4: Susceptibility of MSSA isolates to antimicrobial agents.

3.4 Conventional PCR Screening for mecA and IS431 Genes of MRSA

The amplification gene of mecA, and insertion sequence (IS431) by designed primer sequences was used in this investigation for the molecular identification of MRSA. Genes were present in 34/34 (100 %) of MRSA with a PCR product of bp (figure 5). The mecA gene is the key determinant of methicillin resistance in MRSA strains.

The mecA gene, Encodes the penicillin-binding protein 2a (PBP2a), which has a lower affinity for *β*-lactam antibiotics. The production of PBP2a allows MRSA to continue cell wall synthesis and replication in the presence of β-lactam antibiotics [25].

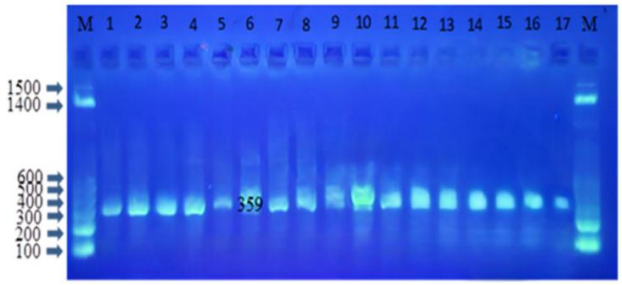

Figure 5: Agarose gel electrophoresis image shows PCR product of MRSA. Lane (M) Marker ladder (100-1500 bp), lane (1-17): mecA-IS431 gene of MRSA isolates with 359 bp.

3.5 Conventional PCR Screening Insertion Gene IS1272 of MRSA

The amplification gene of insertion sequence 1272 (IS127) by designed primer sequences was used in this investigation for the molecular identification of MRSA. Genes were present in 34/34 (100 %) of MRSA with a PCR product of bp as shown in (figure 6). The gene is horizontal transfer from coagulase-negative *Staphyloccci* (CONS) like *S. haemolyticus* have important role in mobility of SCCmec and ability to capture resistant genes may have facilitated the evolution of MRSA [26].

A high number of genes studies detected this gene in class B mec complex that mean these MRSA isolates belong to type I or IV of SCCmec [27]. While Wan detected this gene in SCCmec type III, IV, V and VII [26].

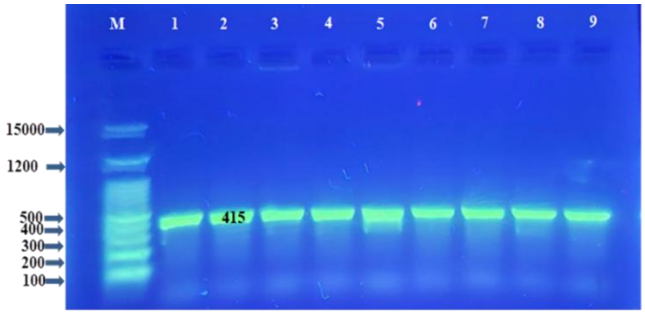

Figure 6: Agarose gel electrophoresis image shows PCR product of MR SA. Lane (M) Marker ladder (100-1500 bp), lane (1-9): 1272 gene of MRSA isolates with 415 bp.

3.6 Conventional PCR Screening ccrC of MRSA

The amplification gene of ccrC by designed primer sequences was used in this investigation for the molecular identification of MRSA. Thes genes were presented in 20/34 (57.14 %) of MRSA with a PCR product of bp as shown in (figure 7). The presence of ccrC indicates that the MRSA isolate likely carries a specific type of SCCmec, most associated with SCCmec type V [28].

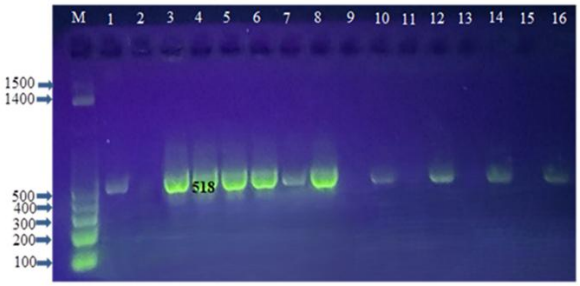

Figure 7: Agarose gel electrophoresis image shows PCR product of MR SA. Lane (M) Marker ladder (100-1500 bp), lane (1-16), and ccrC gene of MRSA isolates with 518 bp.

3.7 Conventional PCR Screening ccrA2-B of MRSA

Primer designed (β, α3) is target to ccrA2-B genes. Play a crucial role in the excision and integration of the *staphylococcal cassette* chromosome mec (SCCmec) in MRSA [27]. Found in multiple SCCmec types, including types I, II, and III, in addition to type IV [29].

The presence of the ccrA2-B gene in class V of SCCmec is not universally reported. While some studies have identified ccrA2-B genes in SCCmec type V elements, others have not detected these genes in similar elements. This suggests that the ccrA2-B genes may not be a consistent feature of all SCCmec type V elements [30-32]. Those genes were presented in 6/34 (17.64 %) of MRSA with a PCR prduct of bp in (figure 8).

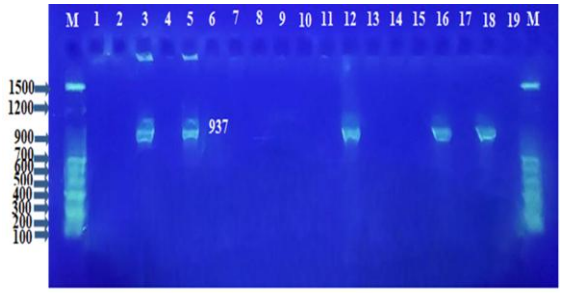

Figure 8: Agarose gel electrophoresis image shows PCR product of MR SA. Lane (M) marker ladder (100-1500 bp), lane (1-9), and ccrA2-B gene of MRSA isolates with 937 bp.

4. Conclusion

The study's most critical finding is the comprehensive identification and characterization of MRSA strains in different clinical samples. Revealing a high prevalence and genetic diversity of methicillin-resistant *Staphylococcus aureus* in the Wasit region of Iraq. This underscores the urgent need for enhanced infection control practices and targeted public health strategies to mitigate the spread of MRSA in healthcare settings.

The molecular analysis, particularly the universal presence of the mecA gene and insertion sequences IS431 and IS127 in all MRSA isolates. Along with the varying presence of other resistance genes, highlights the complexity of MRSA's resistance mechanisms. These insights are pivotal for guiding effective treatment protocols and developing robust diagnostic tools to manage and control MRSA infections more efficiently.

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