

Detection of SCCmec Gene in Methicillin - Staphylococcal Aureus

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Annotation: Objective: to detect SCCmec types I to III in Hospital-associated (HA) Methicillin-resistant *Staphylococcus aureus* (MRSA). **Method:** a total of one hundred seventy-two (N = 172) samples from different clinical cases and Health Care Workers: skin infection 46 (26.7%), surgical wounds 35(20.3%), pneumonia 27 (15.6%), septicemia 22 (12.7%), urinary tract infections 17 (9.8%) and Health Care Workers 25 (14.5%). This study was conducted at the General Hospital of Wasit during the period from December 2023 to May 2024. A combination of biochemical testing, microscopic analysis, culture, and the APIstaph system's identification kits allowed for the confirmation of these isolates. **Results:** showed 34(44.15 %) of isolated *S. aureus* were MRSA including: in Surgical wounds 8/27 (29.6%) followed by skin infections 7/27 (25.9 %) from patients most of them were already on an antibiotic, septicemia was 6/27 (22.2%), samples of Urinary tract infection 4/27 (14.8%) while, in pneumonia, cases were 2/27 (7.4 %). The prevalence of MRSA among Health Care

Workers is the highest rate were in Lab technicians at 4/7 (57.1%) followed by Nears staff at 2/7 (28.7 %) and Doctors were 1/7 (14.2 %). regarding the molecular identification of MRSA, the results showed that *mecA* and Insertion sequence (IS431) exist in every instance at a rate of 100% (34/34), (IS127) were 100 %, (34/34). The other genes were genes of *ccrC* 20/34(57.14 %) and *ccrA2-B* gene 6/34 (17.64 %). **Conclusion:** the study 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.

Keywords: SCCmec, Hospital-acquired infection, MRSA.

INTRODUCTION

The opportunistic pathogen *Staphylococcus aureus* can colonise the nasal mucosa and skin, leading to a host of infections that can range from relatively harmless skin blemishes to potentially fatal conditions like septic shock, endocarditis, osteomyelitis, and pneumonia (Powers and Bubeck 2020). The identification of methicillin-resistant *staph aureus* (MRSA) increases the severity of the *S. aureus* infection. *Staphylococcus aureus* has developed resistance to methicillin by the transfer of *MecA* genes between individual bacteria. This transfer is facilitated by mobile genetic elements (MGE) (Mitiku 2020).This resistance is commonly referred to as methicillin resistance. The bacterium *Staphylococcus aureus* has the ability to withstand the effects of a modified form of penicillin-binding protein 2a, which is produced by the *MecA* gene (Al-Talib *et al.*, 2018). depicts the resistance mechanisms present in MRSA. β -lactam resistance is attributed to two

Distinct primary mechanisms: the first the initial mechanism involves the formation of the β -lactamase enzyme, which degrades penicillin and hydrolyzes the antibiotic (Rocchetti *et al.*, 2018). The second is The process involves the alteration of the β -lactams site of action by generating a novel penicillin-binding protein that exhibits reduced affinity for antibiotics *Staphylococcal aureus* antibiotic resistance is linked to the production of penicillin-binding proteins 2a (PBP2a), which is encoded by the *mecA* gene. This gene is found on a mobile genetic element called staphylococcal cassette chromosomal *mec* (SCC*mec*) (Ito *et al.*, 2004). SCC*mec* can be categorised into many categories based on its gene structure and makeup. According to research (Algammal and Elkelish 2020) types I, II, and III are more likely to have resistance genes, which makes them connected with healthcare-associated MRSA (HA-MRSA). On the other hand, types IV and V are believed to be related to community-associated MRSA (CA-MRSA). Furthermore, *S. aureus*, which is resistant to antibiotics, is a highly adaptable pathogen capable of producing a wide range of virulence factors

that can kill host cells and induce infections through multiple mechanisms (Ito *et al.*, 2004). The presence of certain virulence factors genes of *S. aureus*, including adhesion-associated genes that are essential for biofilm formation (e.g., *icaA*, *icaD*, *fib*, *fnbB*, *fnbA*, *clfB*, *clfA*), superantigen genes (e.g., some genes for enterotoxin and the *tst* gene), and other significant exotoxin genes (e.g., *pvl*, *hla*, *hld*, etc., which are closely associated with severe *S. aureus* infections and tissue destruction), Polymerase chain reaction (PCR) virulence factor detection yields information regarding virulence factor profiles, which facilitates the analysis of the relationship between a particular their virulence factor and clinical characteristics. This process may also aid in the identification of targets for effective anti-infection medication (Rasmi *et al.*, 2022). The two most common types of methicillin-resistant *Staphylococcus aureus* (MRSA) strains are those found in hospitals and those in the community. HA- MRSA infections have been linked to longer hospital stays and higher healthcare expenses, A high morbidity and mortality rate is related to infections attributed to HA-MRSA strains, according to clinical research. These strains typically exhibit resistance to multiple drugs, which can restrict the choice of an appropriate antibiotic for treating staphylococcal infections (Kong *et al.*, 2019) Panton-Valentine leukocidin is one of the virulence factors that an increasing number of CA-MRSA strains are expressing; this protein is linked to devastating conditions including severe necrotic infections, CA-MRSA bacteria typically exhibit reduced susceptibility to non- β -lactam antimicrobials in comparison to other classes of antimicrobials , HA-MRSA isolates are commonly classified as SCCmec types I to III, whereas types IV and V are typically linked to CA-MRSA isolates(Rodrigues *et al.*, 2020). HA-MRSA isolates in the United States predominantly possess SCCmec type II, but in other countries, these isolates typically possess SCCmec type III, SCCmec typing has yielded compelling evidence supporting the existence of a different genesis of HA-MRSA strains apart from CAMRSA strains(Kadam and Bhalerao 2019).

MATERIALS AND METHODS

The cross-sectional study took place between December 2023 to May 2024 at the Teaching Hospital of Alsuweira in Iraq's Wasit governorate. The study can proceed with the green light from the Wasit Health Director. To collect specimens from various clinical instances, a simple random sample procedure was used to recruit patients of either gender. The following formula was used to determine the sample size: (Kadam, and Bhalerao, 2020)

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

Using the level of significance of 5% ($p=0.05$), the $Z_{1-\alpha/2}$ value of A standard ordinary variate was 1.96, and with a level of significance of 1% ($p=0.01$), it was 2.58. The algorithm uses a p-value of 1.96 because, as is customary in research, p-values below 0.05 are assumed to be statistically significant. In this equation, p stood for the predicted population proportion according to earlier studies (Fearon, *et al.*, 2019) for the researchers' documented absolute inaccuracy or precision. To summarise, depending on the type of illness, a sample was taken from either eye by having the patient look upward while lowering their eyelid. To delicately remove discharge from the eye, a sterile cotton swab was dampened with sterile normal saline before usage. Using gentle pressure, the lower conjunctiva sac was massaged from side to side and back again with the swab (Rodrigues, *et al.*, 2019) Next, the bacteria were cultivated on several media, including blood agar, MacConkey agar, and mannitol salt agar, which is specific to *Staphylococcus aureus*. Isolated bacteria were identified based on colony morphological shape, size, color, and pigment synthesis after incubating the samples at 37 degrees Celsius for 24 hours. A commercial kit (Presto™ Mini gDNA Bacterial Kit, Geneaid, Thailand) was used to extract deoxyribonucleic acid (DNA) for use in polymerase chain reaction (PCR) experiments. We followed the manufacturer's instructions to extract DNA of the *P. aeruginosa* isolates

Various bacterial strains were cultivated on mannitol salt for 18 hours at 37°C in preparation for cell harvesting pre-lyses. After that, they were collected by spinning a centrifuge at 14,000 rpm for

one minute, and the liquid above was thrown out. Additionally, 20µL of the proteinase potassium (K) solution with 180µL of buffered Guanidinium thiocyanate (GT) were combined with the pellet and stirred. Throughout the incubation period, the sample tubes were turned upside down every three minutes. The bacterial cell lysis was incubated at 70°C for 10 minutes after being mixed for ten seconds adding 200µL of buffered Guanidine Brochloride (GB). In order to induce lysis, the sample tubes were mixed by invert every 3 minutes. For DNA elution, the elution buffered was preheated to 70°C and 200 l/sample.

In order to facilitate DNA binding, 200µL from 100% ethanol was added to the lysate samples and mixed thoroughly by shaking. Next, the mixture was moved to the spin column in a separate 2ml collect tube, and then to the genomic DNA (GD) column in an additional 2ml collection tube. The 1.5 microcentrifuge tube was used to hold the spin column while 100µL of pre-heated the elution was added to the column matrix in the centre for DNA elution. Centrifuging for thirty seconds at 14,000 rpm, the pure DNA was eluted after leaving the mixture remain for three minutes in order to make sure the buffer used for elution had been fully absorbed. Until needed, DNA that was extracted was kept at -20°C in the freezer. Nano Drop and electrophoresis on agarose gels were used to determine the DNA content and purity.

The equipment was calibrated to identify the purity and concentration of DNA by measuring the optimal degree OD (260/280 ratio) during the process, which involved adding 1µl of the DNA that was extracted.

The electrophoresis tank was prepared for electrophoresis on agarose gel by adding 1x Tris-borate-EDT (TBE) buffer. The agarose tray was then submerged in the tank. The buffer was made to sit a few millilitres above the surface of the agarose. The tank was filled and sealed after 5µl of specimen and 2µl dye fluorescence were added to each well. A gel run under electrophoresis gradient of 70 volts/cm was used for the experiment. The agarose was removed off the tank and shown using gel paper.

Primers were optimised by mixing 2.5µl of master mix along with 5-6µl DNA molecules and 1µl of forward along with revers primers. Primers from different gene grades were selected, and the PCR annealing temperatures have been set at 55°C, 58°C, and 52°C, respectively, for the *SeA*, *Seb*, *Sec*, *Sed*, *Tst*, *eta*, *mecA*, and *Etb* gens.

Following the manufacturer's recommendations, a mixture of 12.5 ml master mix, 5-6 ml DNA, 1 ml reverse and forward primers, and 20 ml of nuclease-free deionised water was used to detect the *SeA*, *Seb*, *Sec*, *Sed*, *Tst*, *eta*, *mecA*, and *Etb* genes. In order to identify the target genes, we recorded the PCR cycle program parameters (Table 1). The data was examined by means of SPSS 20. To examine the data, a chi-square test was employed. The significance level was deemed to be $P < 0.001$ (Sharpe, 2018).

Table 1. The sequence and source of the gene primers used in the study.

Gene		Primer sequence	Size (pb)	Reference
B a3	F	ATTGCCTTGATAATAGCCYTCT	937pb	(Arciola <i>et al.</i> , 2005)
	R	TAAAGGCATCAATGCACAAACACT		
<i>ccrC</i>	F	CGTCTATTACAAGATGTTAAGGATAAT	518pb	(Arciola <i>et al.</i> , 2005)
	R	CCTTTATAGACTGGATTATTCAAAATAT		
1272	F	GCCACTCATAACATATGGAA	415pb	(Arciola <i>et al.</i> , 2005)
	R	CATCCGAGTGAAACCCAAA		
5R <i>mecA</i> 5R431	F	TATACCAAACCCGACAACACTAC	359pb	(Arciola <i>et al.</i> , 2005)
	R	CGGCTACAGTGATAACATCC		

RESULT AND DISCUSSION

Prevalence of *S. aureus* among various clinical cases

This is the outcome shown in Table (2). Showed a prevalence of *S. aureus* infections among various medical cases. Surgical wounds had the second-highest the occurrence of *S. aureus* at 13 (25%), followed by pneumonia at 17.3% and septicaemia at 7.4%; urinary tract infections had the lowest incidence at 3 (5.7%). Skin infections accounted for 20 (38.4%) of the *S. aureus* infections in the present study.

Table (2): Prevalence of *S. aureus* among various clinical cases

clinical cases	Number	%
skin infection	20	38.4
Surgical wounds	13	25.0
pneumonia	9	17.3
Septicemia	7	12.4
Urinary tract infection	3	5.7
Total	52	100
X2	47.7*	
P value	0	

Highly significant difference ($P < 0.01$)

The bacteria causing the wound infection may be come from autoinfection's or transferred from one patient to another during bandages changing, especially when the patient stays for a long time in the hospital (Powers and Bubeck ,2018) Wounds infection may be initial in the operating room before wound closure or after the patient has been returned to the ward. This study is designed to uncover potential sources of wound infection. The most common bacterial pathogen of surgical site infection is *Staph. aureus* 25.0% that agree with many studies. (Ito *et al.*, 2018). In another study in Iran, *Staph aureus* was common Pathogens in burn wound infections after *Pseudomonas aeruginosa*, at a rate of 20.2% (AL-Akwa *et al.*, 2020) In another local study by Alsaimary *Staph aureus* isolate was (4.5%) from diabetic foot. (Al-Shamahy *et al.*, 2020) The difference in results was due to the difference in the targeted patients in the studies, *Staph. aureus* colonizes human skin & mucous membranes and can transmiited from hands and nose to skin.

Prevalence of *S. aureus* among Health Care Workers

According to the data in Table (3). The results demonstrated that *S. aureus* is common among healthcare workers. The current study found that lab technicians had the greatest incidence of *S. aureus* infections at 20 (38.4%). This bacterium is known to be a major agent of hospital-acquired *S. aureus* infections in health care workers. Nears staff had the second-highest frequency of *S. aureus* infections at 13 (25%), while doctors had the lowest: 3 (5.7%).

Table 3. Prevalence of *S. aureus* among various Health Care Workers

Health Care Workers	Number	%
Lab technician	6	38.4
Nears staff	4	25.0
Doctors	2	17.3
Total	12	100
X2	9.510*	
P value	0.00 9	

* Highly significant difference ($P < 0.01$)

The current study found that healthcare workers were a major cause of *S. aureus* infection in hospitals (Yu *et al.*, 2018) and that the rate of *S. aureus* in their hands was 13.8% and in their noses

it was 22%. This is much lower than previous reports, which indicated that healthcare workers in Baghdad had a rate of 47.6% (Arciola *et al.*, 2018) of *Staph. aureus* carriers in their nasal passages and that other studies found that 34% of healthcare workers in Baghdad had *Staph. aureus* in their nasal swabs. The presence of *Staph. aureus* in the hands of H.C.Ws was similar to that observed in previous studies (Congdon, *et al.*, 2024). A significantly higher rate of *Staph. aureus* isolation from medical intensive care unit staff hands was achieved in 1990 by Bauer and colleagues, at 20.5% (Tong, *et al.*, 2018) Compared to this investigation, Pessoa-Silva *et al.* only managed to recover 2.5% of *S. aureus* from H.C.Ws hands in the neonatal ICU (Alkhafaji, *et al.*, 2020). The difference between this study and those obtained might be due to different sampling techniques or sterilization methods and the selection of appropriate disinfectants. Risk of developing *Staph. aureus* infection in nasal carriers was 5.8 times higher in compared with non-carriers (Diriba, *et al.*, 2020) In a study conducted in Baghdad, the percentage of *Staph. aureus* appeared in nasal swab for patients 45%. (Hussain, *et al.*, 2018)) Another study that included children in primary school was the proportion of *Staph. aureus* nasal carrier was 47.3%, among prisoner 50%, while the proportion of nasal colonization with *Staph. aureus* in adult were 32.7% (Lukowski, *et al.*, 2018), This study shows alower rate than the above study and this may be due to that most patients in this study were under antibiotic cover. Nasal carriers are also colonized at extranasal sites, which is the primary source of *Staph. aureus* surgical site infection (Goud, *et al.*, 2019), *Staph. aures* isolates from operating theaters and hospital wards about 5% and this result is close to what Alsaimary found in a previous study. (Hessen *et al.*, 2021) *Staph. aureus* was the third commonest microorganism reported by health directorates' common bacteria contaminates operating theaters of 16 health directorates in 14 Iraqi governorates from January to June 2023 (Hami, *et al.*, 2023).

Identification of Methicillin Resistant *S. aureus* (MRSA)

A total of 34 out of 64 *Staphylococcus aureus* isolates were found to be MRSA. The HiCrome MeReSa Agar Base medium was used to culture the *Staphylococcus aureus* isolates. When mixed with the cefoxitin supplement (FD259) and the MeReSa Selective Supplement (FD229), the medium becomes a selective medium for isolating MRSA. The bluish-green colour of colonies that test positive for MRSA allows for their identification and selection for further assays (Schubert *et al.*, 2022). Figure (1) illustrates the growth of MRSA isolate on this medium.



Figure 1. Methicillin-Resistant *Staphylococcus aureus* (MRSA) on HiCrome MeReSa Agar Base medium

Prevalence MRSA in different clinical samples

The Prevalence of (MRSA) among various infections the highest rate were in Surgical wounds 8/27 (29.6%) followed by skin infection 7/27 (25.9 %) from patient most of them were already on antibiotic, septicemia were 6/27 (22.2%), samples of Urinary tract infection 4/27 (14.8%) while, in pneumonia cases were 2/27 (7.4 %) as shown in Table (4).

Table (4): Prevalence of MRSA among various clinical cases

clinical cases	Number	%
Surgical wounds	8	29.6
skin infection	7	25.9
Septicemia	6	22.2
Urinary tract infection	4	14.8
Pneumonia	2	7.4
Total	27	100
X2	36.8*	
P value	0	

* Highly significant difference ($P < 0.01$)

In this study our founding the prevalence of MRSA Surgical wounds were (29.6 %). This conclusion was consistent with the findings of conducted by (Yılmaz, Schubert *et al.*, 2020) documented the presence of MRSA isolates in various clinical samples in Iraq, with Surgical wounds samples demonstrating the highest prevalence at 32.8%. Another study by (Moshtagheian, *et al.* 2021), conducted in a rural medical college in North India, discovered that pus samples had the highest proportion of MRSA isolates at 61.7%, compared to other clinical specimens. A study conducted in Mosul, Iraq, by (M'Aiber *et al.* 2021) revealed a significant prevalence of multidrug-resistant organisms (MDROs) at 86%, 52.8% of isolates most of these MRSA isolates were obtained from wounds samples. The high prevalence in pus is likely due to the exposure of wounds to microorganisms in the environment and the presence of *S. aureus* as a skin commensal, making wounds prone to MRSA infection. Also the prevalence of MRSA in urine was (14.8 %) This conclusion was consistent with the findings of conducted by Mitiku *et al.*, (28) in Ethiopia found that out of 422 urine samples from urinary tract infection (UTI) suspected patients, 53 (12.6%) cultured *S. aureus*. Of these *S. aureus* isolates, 43.4% (23/53) were MRSA. Another study by (Wang, *et al.* 2019) in a tertiary care hospital in Northern India, out of 27 *S. aureus* isolates from urine samples, 13 (48.1%) were MRSA, but there are study conducted by (Hami and Ibrahim, 2023) in Zakho City, Kurdistan Region, Iraq found a high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among patients with urinary tract infections (UTIs), out of 37 *S. aureus* isolates, 28 (75.7%) was MRSA. The prevalence is influenced by factors such as previous antibiotic usage, hospitalization, chronic illnesses, and catheterization, which contribute to an increased risk of MRSA in urine samples.

Prevalence MRSA in Health Care Workers

The Prevalence of (MRSA) among **Health Care Workers** the highest rate were in Lab technician 4/7 (57.1%) followed by Nears staff 2/7 (28.7 %) and in Doctors were 1/7 (14.2 %) as shown in **Table (5)**. In this study our founding the prevalence of MRSA in nasal swab was high, This conclusion was consistent with the findings of conducted in Iraq have reported varying rates. Hantoosh (Walana, *et al.*, 2020) found a prevalence of 41.2% among healthy children in Basrah City. Among intermediate students in Muthanna Province, (Jamalludeen *et al.*, 2019) reported a prevalence of 24%, while (Arora *et al.*, 2019). Prevalence of staphylococcus aureus in dental infections and the occurrence of mrsa in isolates. Universal journal of pharmaceutical research. 10.22270/ujpr.v5i2.384) identified a prevalence of 16% among restaurant workers in Kirkuk City. The rates of MRSA nasal carriage in Iraq range from 2% to 42%, with higher rates typically observed among healthcare workers, students, and rural populations compared to the general urban population (Fuda *et al.*, 2018). Regular screening and decolonization of carriers are recommended strategies to reduce the transmission of MRSA in these various clinical settings. A higher colonization rate of methicillin-resistant *Staphylococcus aureus* increases the likelihood of drug-resistant wound infections. Hence, it's crucial to stay as healthy as possible. Because of the high

incidence of cross-contamination among healthcare workers, previous studies have indicated a high infection rate (Chen *et al.*, 2020).

Table (5): Prevalence of MRSA among Health Care Workers

clinical cases	Number	%
Lab technician	4	57.1
Nears staff	2	28.7
Doctors	1	14.2
Total	7	100
X2		
P value		

* Highly significant difference ($P < 0.01$)

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. This genes was present in 34/34(100%) of MRSA with a PCR product of bp, Figure (2). In methicillin-resistant *Staphylococcus aureus* (MRSA), the *mecA* gene plays a pivotal role. One protein that the *mecA* gene codes for is PBP2a, and it binds to β -lactam antibiotics with a lesser affinity. Despite the existence of β -lactam antibiotics, MRSA is able to maintain cell wall construction and replication through the formation of PBP2a (Kirmusaolu, 2020).

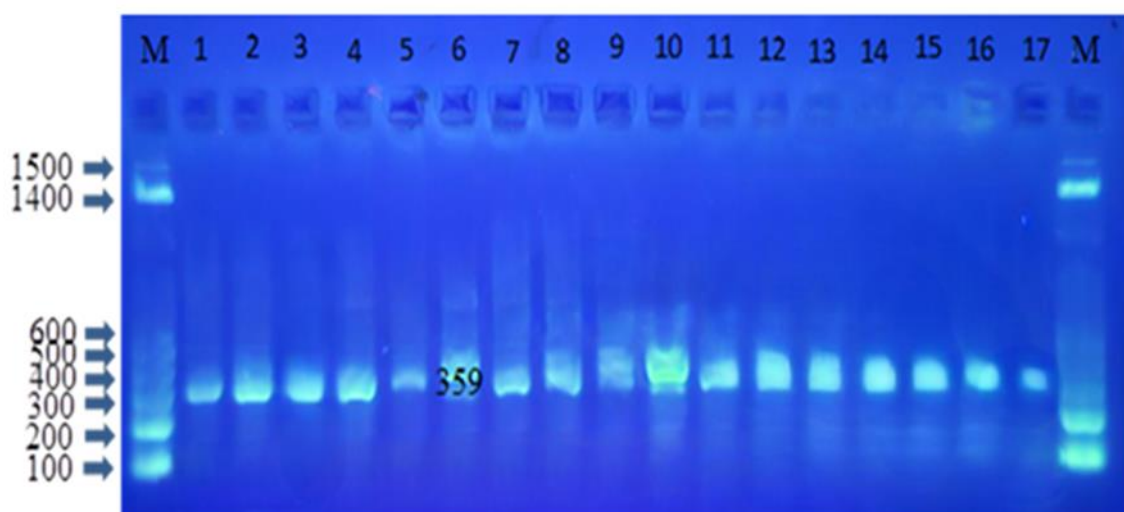


Figure (2): Agarose gel electrophoresis image shows PCR product of MRSA. Lane (M) Marker ladder (100-1500 bp), lane (1-17): *mecA*-IS431 gene of MRSA isolates with 359 bp

Conventional PCR screening insertion gene *IS1272* of MRSA

The amplification gene of Insertion sequence1272 (*IS127*) by designed primer sequences was used in this investigation for the molecular identification of MRSA. This genes was present in 34/34(100%) of MRSA with a PCR product of bp, Figure(3).This gene is horizontal transfer from coagulase-negative *Staphylococci* (CONS) like *S. haemolyticus* have important role in mobility of SCCmec and ability to capture resistant genes may have facilitated the evolution of MRSA(Yoon *et al.*, 2019).Many studies about this genes, (Kondo *et al.* ,2020)detected this gene in class B *mec* complex that mean these MRSA isolates belong to type I or IV of SCCmec. While (Youssef *et al.*, 2020) has been detected this gene in SCCmec type III,IV,V and VII.

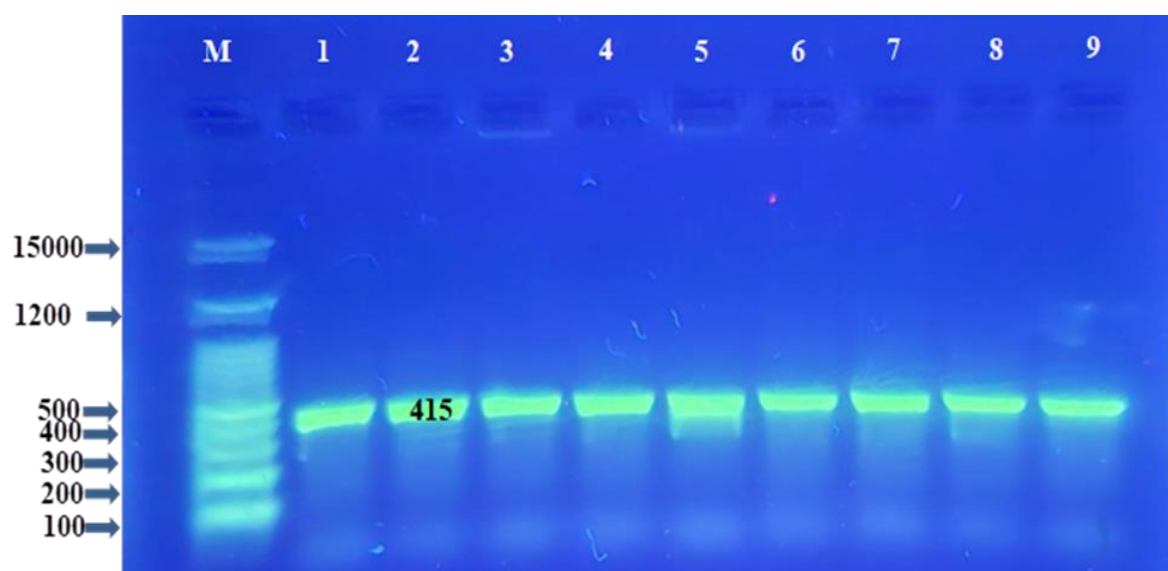


Figure (3): Agarose gel electrophoresis image shows PCR product of MR SA. Lane (M) Marker ladder (100-1500 bp), lane (1-9): **1272** gene of MRSA isolates with 415 bp.

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. This genes was present in 20/34(57.14%) of MRSA with a PCR product of bp, Figure (4). The presence of *ccrC* indicates that the MRSA isolate likely carries a specific type of SCCmec, most commonly associated with SCCmec type V (Moosavian *et al.*, 2019).

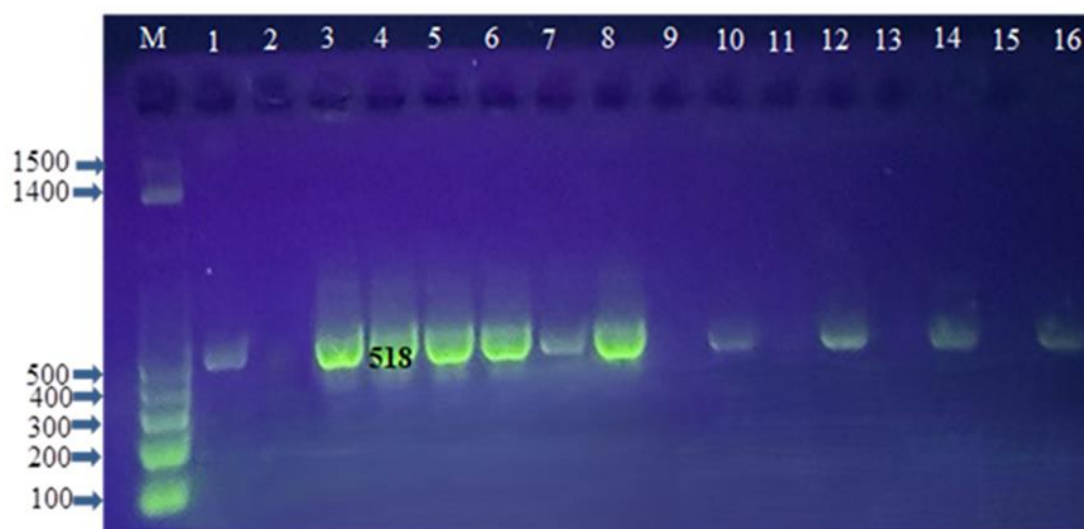


Figure 4. Agarose gel electrophoresis image shows PCR product of MR SA. Lane (M) Marker ladder (100-1500 bp), lane (1-16): *ccrC* gene of MRSA isolates with 518 bp.

Conventional PCR screening *ccrA2-B* of MRSA

The primer designed ($\beta, \alpha 3$) is target to *ccrA2-B* genes, play a crucial role in the excision and integration of the staphylococcal cassette chromosome mec (SCCmec) in MRSA (Masaisa *et al.*, 2018). And found in multiple SCCmec types, including types I, II, and III, in addition to type IV (Moosavian *et al.*, 2019). 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 (Fearon, *et al.*, 2018). This genes was present in 6/34(17.64%) of MRSA with a PCR product of bp, Figure (5).

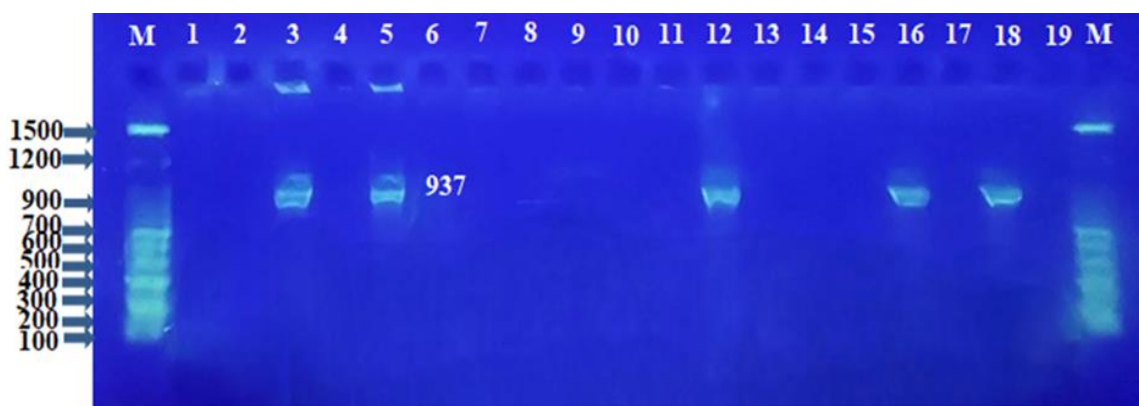


Figure (5): Agarose gel electrophoresis image shows PCR product of MR SA. Lane (M) Marker ladder (100-1500 bp), lane (1-9): **ccrA2-B** gene of MRSA isolates with 937 bp.

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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