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DETECTION OF RESISTANCE STAPHLOCOCCUS AUREUS (MRSA) AMONG VARIOUS CLINICAL CASES AND HEALTH CARE WORKERS IN WASIT PROVINCE

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Abstract:

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. Given this fact, a total of two hundred seventy five specimens had been collected from patients (Skin swab, Nasal swab and Wound swab), Health Care Workers (Skin swab and Nasal swab). During the period from August to December 2024. The current study showed among the 172 Staphylococcus aureus isolates from various clinical cases and healthcare workers , 64 isolates identified Methicillin Resistant S. aureus (MRSA) (42 from clinical cases and 22 from Health Care Workers) . Isolates that were identified as Staphylococcus aureus were cultured on HiCrome MeReSa Agar Base medium. The Prevalence of (MRSA) among various infections was a variant rate were in Burns 16/42 (28.0%) followed by Abscesses 13/42 (20.9 %)from patients most of them were already on antibiotics, Endocarditis was 6/42 (14.2%), samples of Otitis 5/42 (11.9%) while, pneumonia cases were 2/42 (7.4 %).regarding of The Prevalence of (MRSA) among among health care workers was highest among lab Nursing 10/22 (45.4%), followed by Dentist 7/22 (31.8%), Lab Staff 4/22, (18.1%) and Surgery 1/22 (4.5%). twelve antibiotics used in Antimicrobial susceptibility test against MRSA most of them showed high resistance. regarding of virulence factors to MRSA the results were (icaA) gene in 28/64 (43.7%) , hla gene in 20/64(31.2%), hlb gene 42/64(65.6%) and pvl gene in 15/64(23.4%). The study 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.

Keywords: Clinical Cases, Resistance Staphlococcus aureus, Health Care Workers.

Introduction

The identification of methicillin-resistant staph aureus (MRSA) increases the severity of the S. aureus infection (1). *Staphylococcal aureus* antibiotic resistance is linked to the production of penicillin-binding protein 2a (PBP2a), which is encoded by the mecA gene. This gene is found

on a mobile genetic element called staphylococcal cassette chromosomal mec (SCCmec) (Ito et al., 2004). SCCmec can be categorised into many categories based on its gene structure and makeup. According to research (2), 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 (3). 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) (4). Polymeraschain reaction (PCR) virulence factor detection yields information regarding virulence factor profiles, which facilitates the analysis of the relationship between a particular virulence factor and clinical characteristics. This process may also aid in the identification of targets for effective anti-infection medication .A developing disease, methicillin-resistant *Staphylococcus aureus* (MRSA) may cause infections ranging from mild to severe in animals and people alike. The most common skin and soft tissue infections in humans include staphylococcal scalded skin syndrome (SSSS), pustules, impetigo contagiosa, abscesses, and papules; the most dangerous infections in humans include pneumonia, or a disease similar to newborn skin and soft tissue syndrome (TSS-like exanthematous disease) (5).

1. Materials and Methods

Sample collection

A total of 275 samples were collected; from patients (Skin swab, Nasal swab and Wound swab), Health Care Workers (Skin swab and Nasal swab). during the period from August to December 2024. approval has been obtained from the Wasit health Director to conduct the study using a simple random sampling technique, patients of either gender were enrolled to obtain specimens from different clinical cases. The sample size was calculated using the formula (6).

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

Using the level of significance of 5% (p=0.05), the Z1-α/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 (7), and d 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 conjunctive sac was massaged from side to side and back again with the swab (8).

Isolation of bacteria

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, colour, and pigment synthesis after incubating the samples at 37 degrees Celsius for 24 hours.

DNA Extraction

A commercial kit (PrestoTM 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 *Staphylococcus aureus* isolates. 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 (Tables. 1). (9).

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

Gen	Primer sequence		Size pb	Reference
Ica	F	TCAGACACTTGCTGGCGCAGTC	936	
	R	TCACGATTCTCTCCCTCTCTGCCATT		
Hlb	F	GTGCACTTACTGACAATAGTGC	309	
	R	GTTGATGAGTAGCTACCTTCAGT		
Hla	F	CTGATTACTATCCAAGAAATTCGATTG	209	
	R	CTTTCCAGCCTACTTTTTTATCAGT		
Pvl	F	ATCATTAGGTAAAATGTCTGGACATGATCC	433	
	R	GCATCAASTGTATTGGATAGCAAAAG		

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Susceptibility testing for antibiotics

The sensitivity pattern of the isolates to antimicrobial drugs was evaluated by Kirby-Bauer method , 0.5 McFarland preparation, and a swab dipped in the inoculum and disseminated on Mueller-Hinton agar plates. Antibiotic disks were employed, and the plate was then incubated in an upright posture at 35 C° for 24 hours (16-18 hours) Antibiotic susceptibility testing (Antibiogram). The sensitivity pattern of the isolates to antimicrobial agents was evaluated by Kirby Force propagation, 0.5 McFarland preparation, and the swab was dipped into the inoculum and spread on the agar plates of Mueller-Hinton. Antibiotic disks were used, and then the plate was incubated at 35 C° in Standards for Region diameter understanding.

Statistical Analysis

Design of the study: Cross sequential comparative study.Statistical research carried out using the Statistical Kit of Social Science (SPSS) software V. 20 analyzed descriptive statistics and the exact test of Chi-square ($\chi 2$) or Fisher (typically used where sample sizes are small) used to evaluate the relationship between the variables, P-value < 0.05 was deemed statistically important.

Result and Discussion

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

Among the 172 *Staphylococcus aureus* isolates from various clinical cases and healthcare workers , 64 isolates identified Methicillin Resistant *S. aureus* (MRSA) (42 from clinical cases and 22 from Health Care Workers) . Isolates that were identified as *Staphylococcus aureus* were cultured on HiCrome MeReSa Agar Base medium. The medium is used as a selective medium for MRSA isolation by combining it with cefoxitin supplement (FD259) and MeReSa Selective Supplement (FD229). Positive colonies are detected by their bluish green color and identified as MRSA and selected for future assays (10). As illustrated in figure (1).

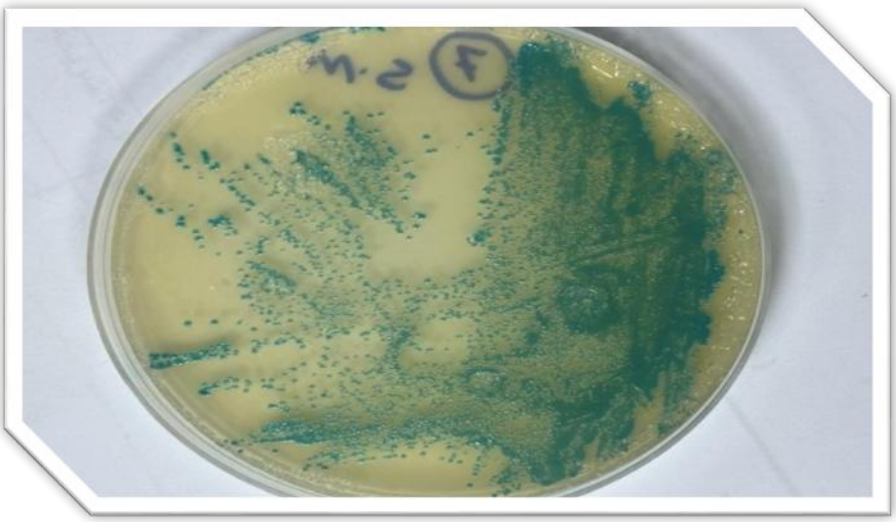

Fig.1 (MRSA) on HiCrome MeReSa Agar Base medium.

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Prevalence MRSA in different clinical samples

The Prevalence of (MRSA) *among* various infections was a variant rate were in Burns 16/42 (28.0%) followed by Abscesses 13/42 (20.9 %)from patients most of them were already on antibiotics, Endocarditis was 6/42 (14.2%), samples of Otitis 5/42 (11.9%) while, pneumonia cases were 2/42 (7.4 %) as shown in table (2).

Table (2): Prevalence of *MRSA among* various clinical cases

Clinical Cases	Number	%
Burns	16	38.0
Abscesses	13	30.9
Endocarditis	6	14.2
Otitis	5	11.9
Pneumonia	2	7.4
Total	42	100
X2	35.4*	
P value	<0.01	

* Highly significant difference (P<0.01)

In this study our founding the prevalence of MRSA Burns were (38.0%).This conclusion was consistent with the findings of conducted by (11) documented the presence of MRSA isolates in various clinical samples in Iraq, with burns samples demonstrating the highest prevalence at 32.8%. Another study by (12), 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 (13) revealed a significant prevalence of multidrug-resistant organisms (MDROs) at 86%, 52.8% of isolates most of these MRSA isolates were obtained from wound samples. The high prevalence of 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 wound prone to MRSA infection. Also the prevalence of MRSA in urine was (14.8 %) This conclusion was not consistent with the findings of conducted by (14) 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 (Arora *et al*,.2010) in a tertiary care hospital in Northern India, out of 27 *S. aureus* isolates from urine samples, 13 (48.1%) were MRSA , but there is study conducted by Hami and Ibrahim,(15) in Zakho City, Kurdistan Region, Iraq found a high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among patients with Endocarditis ,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 of MRSA among Health Care Workers

Table (3) shows that among health care workers, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) was highest among lab Nursing 10/22 (45.4%), followed by Dentist 7/22 (31.8%), Lab Staff 4/22, (18.1%) and Surgery 1/22 (4.5%). Our results showed a significant incidence of MRSA in nasal swabs, which is in line with previous research that has shown variable rates across Iraq. Among otherwise healthy kids in Basrah City, (16) discovered a prevalence of 41.2%. The prevalence was found to be 24% amongst secondary students in Muthanna Governorate according to (17), and 16% in workers at restaurants in Kirkuk City according to(18). Iraq has a range of nasal carriage rates for methicillin-resistant *Staphylococcus aureus* (MRSA) from 2% to 42%; however, rural areas, students, and healthcare personnel tend to have greater rates than the overall urban populace (19). Reducing the spreading of MRSA in these different clinical settings can be achieved through regular screening and the decolonization of carriers. The prevalence of drug-resistant wound infections is directly correlated to the rate of methicillin-resistant *Staphylococcus aureus* colonization.

Table (3): Prevalence of *MRSA among* Health Care Workers

Health Care Workers	Number	%
Nursing	10	45.4
Dentist	7	31.8
Lab Staff	4	18.1
Surgery	1	4.5
Total	22	100
X2	1.91*	
P value	<0.01	

* Highly significant difference (P<0.01)

In the current study all isolates of *methicillin-resistant Staphylococcus aureus* were subjected to an Antimicrobial susceptibility test against twelve antibiotics (Oxacillin& Methicillin, Agumenitin, Erythromycin, Ciprofloxacin, Tetracycline, Cefotaxime, Clindamycin, Clarithromycin, Gentamicin, Rifampin and Vancomycin) was determined by using the Antibiotic disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) Now Clinical Laboratory Standards Institute (CLSI) guidelines (2023) .To investigate the multidrug resistance pattern that usually associated with Methicillin resistance Staphylococci and the most effective therapy for this type of bacteria figure (4) , illustrate the distribution of antibiotic susceptibility to Staph. aureus isolated from various infections where Tetracycline Cefoxitin and Methicillin exhibit the highest resistance among

the antibiotics at the resistance rate (79.36%, 58.73%, 58.73%) respectively, Gentamicin resistance rate was 52.38% and Erythromycin 46.03% .The highest sensitivity rate was 61.90% in Vancomycin followed by Ciprofloxacin 55.55% then Clindamycin, Clarithromycin and Rifampin (52.38% 49.20% and 49.20%) respectively. Agumenitin sensitivity rate was 47.61%% and for Cefotaxime 46.3%. All Staph. aureus isolates were tested for antibiotic susceptibility in this study. Among the drugs, Tetracycline, cefoxitin, and methicillin showed the highest resistance rate, while vancomycin had the highest sensitivity rate. The findings corroborated those of previous local research (20), which found 76% of strains to be sensitive to the antibiotic vancomycin.Resistance to ciprofloxacin is very low at 9%, according to another study (21).Those findings don't add up with what (22) found, which indicated that 25.5% of the staphylococcus aureus isolates were resistant to erythromycin.Consistent with this study, research conducted by (23)indicated that the bacteria Staph. aureus seemed more responsive to therapies with Vancomycin, Rifampin, and Ciprofloxacin; 89.5% were susceptible to Imipenem, 79% to Gentamicin, 44.8% to Ciprofloxacin, 95.2% to Rifampicin, and 36.2% to Erythromycin. The percentage of Staph. aureus isolates showing resistance to vancomycin (VRSA) was 23.8 % in this investigation. In contrast, a vancomycin resistance rate of less than 10% was seen in Asian nations. In addition to the 3.3% reported in Pakistan by (24), Sonavane and Mathur2007)reported 6% in India, (25) 7.5% in Iran, and(26) 9% in Jordan, VRSA is also found in other Asian nations. Antimicrobial resistance in S. aureus as well as invasive Staphylococcal bacterial infections, overuse of antibiotics, mutations within the bacteria, and the role of virulence factors in human antibiotic resistance are all factors contributing to Iraq's higher resistance rates. Poor hygiene and non-compliance with infection control policies also play a role. Specifically in MRSA isolates exhibiting intermediate vancomycin resistance, transcription factors have been demonstrated to control antibiotic resistance genes (27).

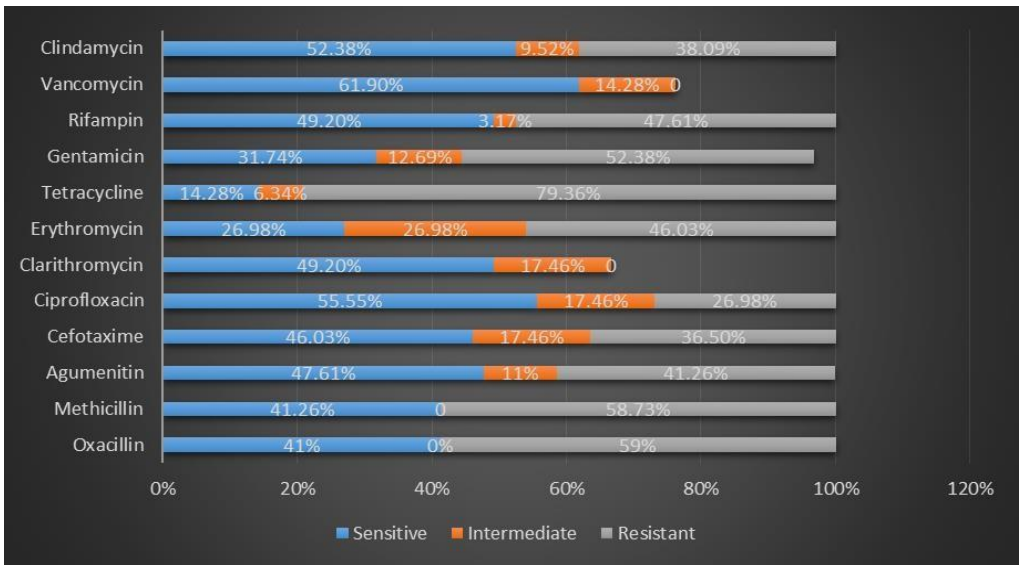

Figure (2) : The distribution of antibiotic susceptibility to *MRSA*

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Molecular detection of virulence factors to MRSA

Conventional PCR Screening for icaA gene

Fourteen isolates of MRSA were positive for(*ica*A) gene 28/64(43.7%).PCR product of this gene was 930 bp, as shown in the figure (3).

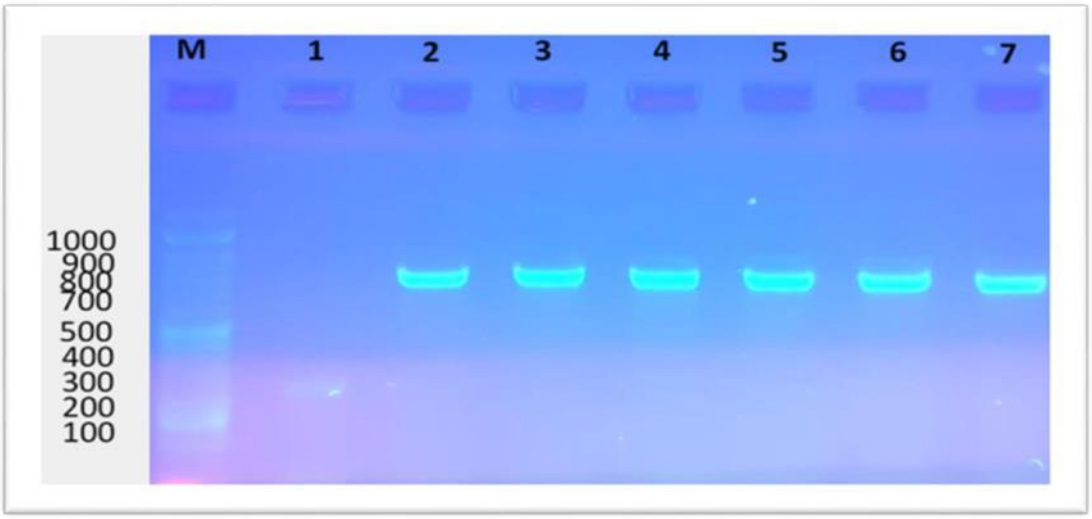

Figure (3): PCR product gel electrophoresis of icaA gene. M lane is standard DNA sized from 1500-100 bp. Lanes 1, is a negative sample for the icaA gene. Lanes 2-7 represent positive samples for 930 bp *ica*A gene. Electrophoresis performed on 100V for 55 minutes on 1.2% agarose.

Conventional PCR Screening for *hla* gene

Ten isolates belong MRSA were positive for *hla* gene 20/64(31.2%), PCR product of this gene was 209 bp, as shown in the figure (4).

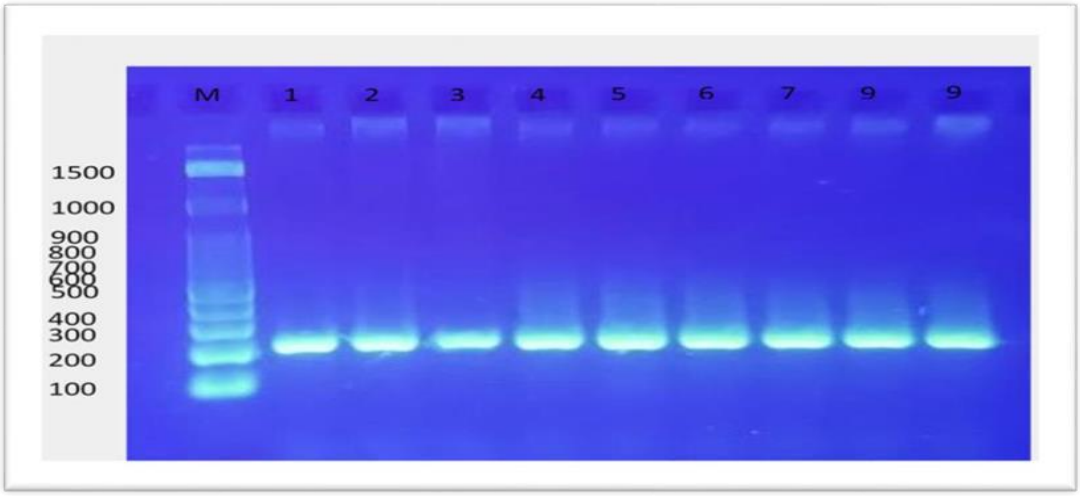

Figure (4): PCR product gel electrophoresis of *hla* gene. M lane is standard DNA sized form 1500-100 bp.(1-9)lane represent positive samples for 209 bp hla gene, Electrophoresis was performed on 100V for 55 minutes on 1.5% agarose.

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Conventional PCR Screening for hlb gene

Twenty isolates belong MRSA were positive for hlb gene 42/64(65.6%). PCR product of this gene was 309 bp, as shown in the figure (5).

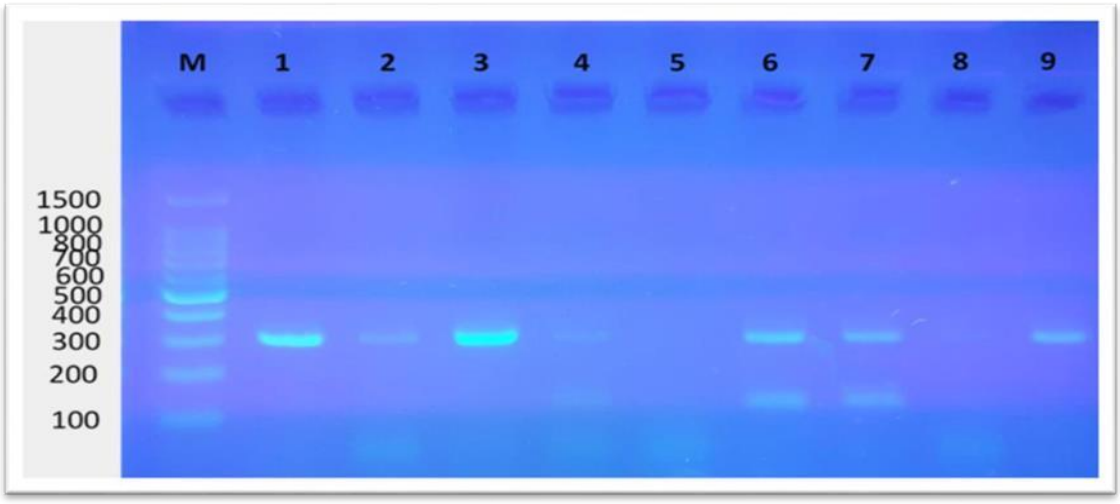

Figure (5): PCR product gel electrophoresis of hlb gene. M lane is standard DNA sized from 1500-100 bp. Lanes 1, 2, 3, 4, 6, 7 and 9 were positive samples. Lanes 5 and 8 represent positive samples for 309 bp hlb gene. Electrophoresis was performed on 100V for 55 minutes on 1.5% agarose.

Conventional PCR Screening for PVL gene

Seven isolates belong to MRSA were positive for pvl gene 15/64(23.4%), PCR product of this gene was 433 bp, as shown in the figure (6).

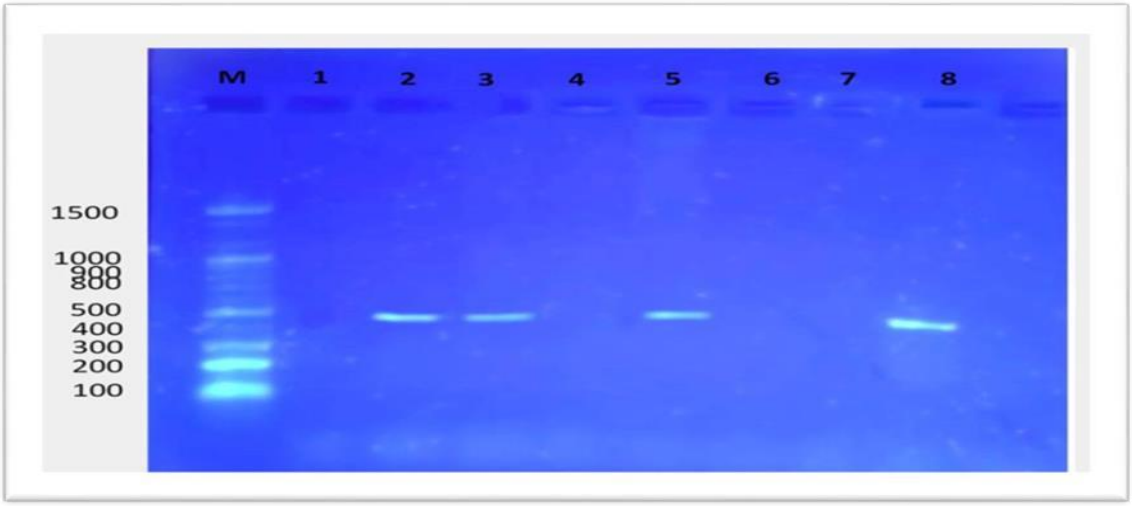

Figure (6): PCR product gel electrophoresis of *PVL* (panto valantine) gene. M lane is standard DNA sized form 1500-100 bp. Lanes 1, 4, 5, 6 and 7were negative samples. Lanes 2, 3, 5 and 8 represent positive samples for 433 bp PVL gene. Electrophoresis was performed on 100V for 55 minutes on 1.5% agarose.

Conclusion

The study's most critical finding is the comprehensive identification and characterization of MRSA strains in different clinical samples and Health Care Workers , 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 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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