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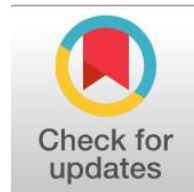
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## Identification of *mecA* Gene in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Using Molecular Method from Clinically Isolated Samples in Babylon Province, Iraq

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### Abstract

**General Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant cause of hospital- and community-acquired infections due to its antibiotic resistance. **Specific Background:** Molecular diagnosis using polymerase chain reaction (PCR) is widely used to identify MRSA strains carrying the *mecA* gene. **Knowledge Gap:** Data on *mecA*-positive MRSA isolates and antibiotic resistance patterns in Babylon Governorate, Iraq, remain limited. **Aims:** This study aimed to isolate and identify MRSA from clinical samples and evaluate antibiotic susceptibility patterns. **Results:** Among 100 clinical samples, 60% were positive for *Staphylococcus aureus*, and 58.3% of the isolates were confirmed as MRSA by *mecA* gene detection. Wound and pus samples showed the highest MRSA prevalence. The isolates exhibited high resistance to penicillin, methicillin, and erythromycin, while vancomycin and linezolid showed high sensitivity rates. **Novelty:** The study combines molecular detection of *mecA*-positive MRSA with antibiotic susceptibility profiling in clinical isolates from Babylon hospitals. **Implications:** The findings support the use of PCR-based diagnosis, antibiotic stewardship, and infection control programs to reduce the spread of resistant bacterial strains.

### Highlights:

- Molecular PCR analysis confirmed *mecA*-positive MRSA strains in more than half of the *Staphylococcus aureus* isolates.
- Wound and pus specimens represented the primary clinical source of resistant bacterial isolates.
- Vancomycin and linezolid demonstrated high susceptibility rates against multidrug-resistant strains.

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## Introduction

*Staphylococcus aureus* is one of the most important pathogens for humans and causes infections of varying severity, ranging from uncomplicated skin infections to critical illness. These include bacteremia, pneumonia, and deep wound infections. Its chief danger is having an extremely high tendency to develop resistance against many antibiotics, thereby complicating treatment. (Tong et al., 2015; Abdulazeem, and Jassim, 2018. ). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prominent resistant strains characterized by resistance to several types of beta-lactam antibiotics., including methicillin, penicillin, and cephalosporins. This strain is a worldwide public health concern as it occurs in hospitals (hospital-acquired MRSA) and in the community (community-acquired MRSA) (Chambers & DeLeo, 2009; Jassim et al,2026). MRSA spreads easily in hospitals and puts patients, especially vulnerable ones, at higher risk of healthcare-related infections. or those undergoing surgery or receiving medical device implants. Irrational antibiotic use has also played a large part in the increasing resistance of bacteria. (WHO, 2020; Al-Sultany, and Jassim, 2016). Hospital studies show high levels of antibiotic resistance in Iraq. This shows the need for specific resistance studies in each governorate. Therefore, this study in Babylon Governorate is crucial for determining the prevalence of MRSA in various clinical samples and assessing its antibiotic susceptibility, which will contribute to improving treatment strategies and infection control.

**Study Objectives:** This study aims to Isolate and identify methicillin-resistant *Staphylococcus aureus* (MRSA) from various clinical samples in hospitals in Babylon Governorate. Determine the prevalence of MRSA strains among the bacterial isolates. Evaluate the susceptibility and resistance patterns of commonly used antibiotics. Compare the effectiveness of different antibiotics in controlling the bacterial isolates.

## Methods and Materials

### 1. Study Design and Sample Collection

A cross-sectional laboratory study was conducted in the laboratories of hospitals in Babylon Governorate. Various clinical samples, including blood, wounds, urine, and sputum, were collected from patients with bacterial infections after obtaining the necessary consents.

### 2. Bacterial Culture and Isolation

Samples were cultured on standard media such as: Blood agar and Mannitol salt agar. The plates were then incubated at 37°C for 24 hours. Initial identification of *S. aureus* colonies was based on their phenotype (golden color and fermentation on mannitol agar) (Forbes et al., 2017; Hassan et al,2026).

### 3. Microscopic and Biochemical Diagnosis

The diagnosis was confirmed using: (Tille, 2017; Hassan et al,2025)

- Gram staining to show Gram-positive staphylococci
- Catalase test
- Coagulase test to confirm *S. aureus* identity.

### 4. MRSA Strain Identification

Methicillin sensitivity testing was performed using: Cefoxetine disc diffusion test on Mueller-Hinton agar. According to CLSI guidelines, mefoxitin resistance is indicative of MRSA (CLSI, 2023; Naji et al,2024). And genetically detection *mecA* gene by used Primers which provided by Macrogen company from Korea (5'-CCT AGT AAA GCT CCG GAA-3' , 5'-CTA GTC CAT TCG GTC CA-3') 314 bp size. (Ardic et al.,2006).

### 5. Antibiotic Susceptibility Testing

Susceptibility testing was performed using the Kirby-Bauer disk diffusion method with a range of common antibiotics, including: Vancomycin, Clindamycin, Erythromycin, Gentamicin and Ciprofloxacin. The results were interpreted according to CLSI criteria for determining sensitivity or resistance (Khdhair et al,2024; Mohi AL-kahfaji et al,2023).

## Results

### Distribution of bacterial samples.

One hundred clinical samples collected from patients in hospitals in Babylon Governorate as cleared in table 1 were examined, revealing the following: Out of the 100 samples: 60 samples (60%) showed growth of *S. aureus* bacteria. 40 samples (40%) did not show growth of this bacterium or were for other bacteria as cleared in table 2.

Table 1 shows the distribution of clinical samples collected in the study, with the highest percentage being wound and pus samples (35%), followed by urine samples (30%), then blood (20%), and finally sputum (15%). This distribution demonstrates the diversity of sample sources used in the study, with a greater focus on cases of wound infections.

**Table 1: Distribution of Clinical Samples (n = 100)**

Sample Source	Number of Samples	Percentage (%)
Wound & Pus	35	35%
Urine	30	30%
Blood	20	20%
Sputum	15	15%
<b>Total</b>	<b>100</b>	<b>100%</b>

Isolation of Staphylococcus aureus

Table 2 shows the results of bacterial isolation. *S. aureus* was isolated from 60% of the samples, while 40% of the samples showed either the absence of this bacterium or the presence of other bacterial species. These results indicate a significant prevalence of this bacterium in the clinical samples studied.

**Table 2: Isolation of Staphylococcus aureus**

Result	Number of Samples	Percentage (%)
<i>S. aureus</i> Positive	60	60%
Other bacteria / No growth	40	40%
<b>Total</b>	<b>100</b>	<b>100%</b>

Distribution of *S. aureus* Isolates by Sample Source

Table 3 shows the distribution of *S. aureus* isolates by sample source. The highest percentage was recorded in wound and pus samples (41.7%), followed by urine samples (25%), and then blood and sputum at an equal percentage (16.7%). This indicates that wounds are among the most suitable environments for the spread of this bacterium.

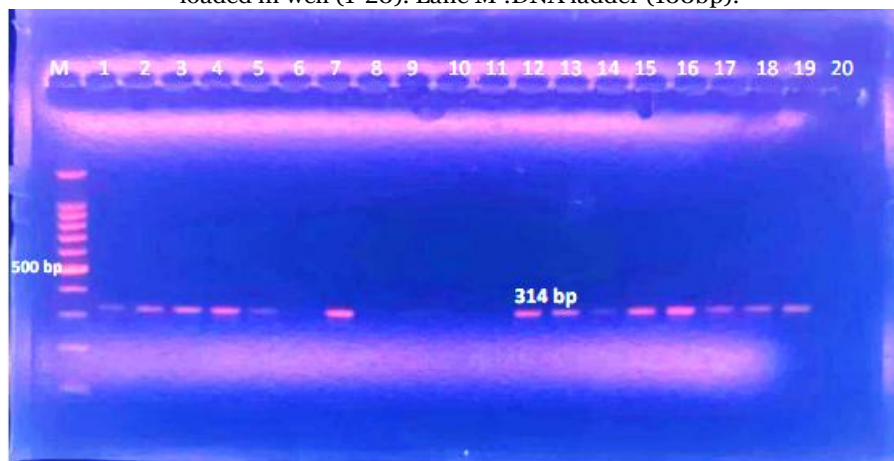
**Table 3: Distribution of *S. aureus* Isolates by Sample Source (n = 60)**

Sample Source	Number of Isolates	Percentage (%)
Wound & Pus	25	41.7%
Urine	15	25%
Blood	10	16.7%
Sputum	10	16.7%
<b>Total</b>	<b>60</b>	<b>100%</b>

Detection of MRSA Using the *mecA* Gene (PCR)

The results of genetic diagnosis of *S. aureus* isolates using PCR. The *mecA* gene was detected in 58.3% of the isolates, confirming their MRSA status, while 41.7% of the isolates were negative for the gene (MSSA). These results indicate a high prevalence of methicillin-resistant strains as shown in table 4 and figure 1.

**Figure 1 :** Gel electrophoresis of *mecA* gene for *S. aureus* bacteria in voltage (85V) time (70 minute) and 5µL of PCR product loaded in well (1-20). Lane M :DNA ladder (100bp).



**Table 4: Detection of MRSA Using *mecA* Gene (PCR).**

Type	Number of Isolates	Percentage (%)
MRSA ( <i>mecA</i> positive)	35	58.3%
MSSA ( <i>mecA</i> negative)	25	41.7%
<b>Total</b>	<b>60</b>	<b>100%</b>

Distribution of MRSA Isolates by Sample Source

Table 5 shows the distribution of MRSA isolates by sample source. The highest percentage was recorded in wound and pus samples (72%), followed by blood samples (60%), then urine (53.3%), and the lowest percentage in sputum (30%). These results indicate that wounds are the most common source of MRSA isolates.

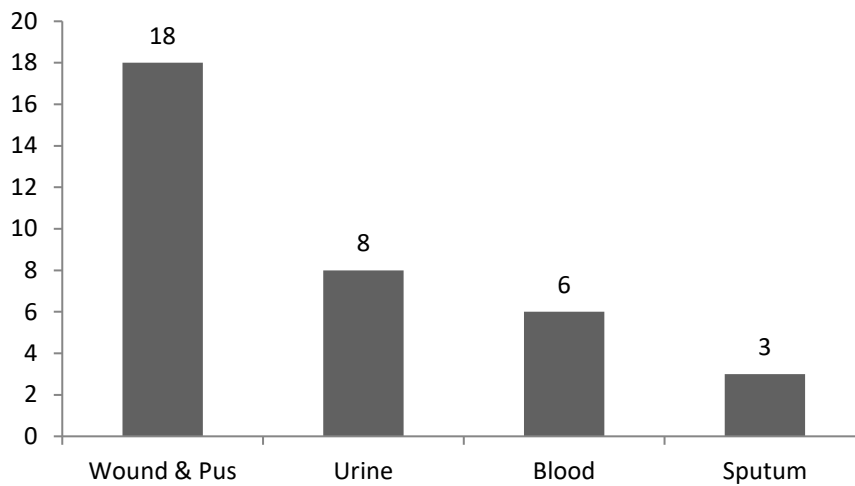
Table 5: Distribution of MRSA Isolates by Sample Source.

Sample Source	MRSA Isolates	Percentage (%)
Wound & Pus	18	72%
Urine	8	53.3%
Blood	6	60%
Sputum	3	30%
Total	35	100%

Distribution of MRSA Isolates by Sample Source

Figure 2 shows the distribution of MRSA isolates by clinical sample source. The highest number of isolates was recorded in wound and pus samples (18 isolates), followed by urine samples (8 isolates), then blood samples (6 isolates), while the lowest number was recorded in sputum samples (3 isolates). This distribution demonstrates that wounds are the most common source of MRSA isolates, reflecting their close association with wound infections and healthcare-associated infections.

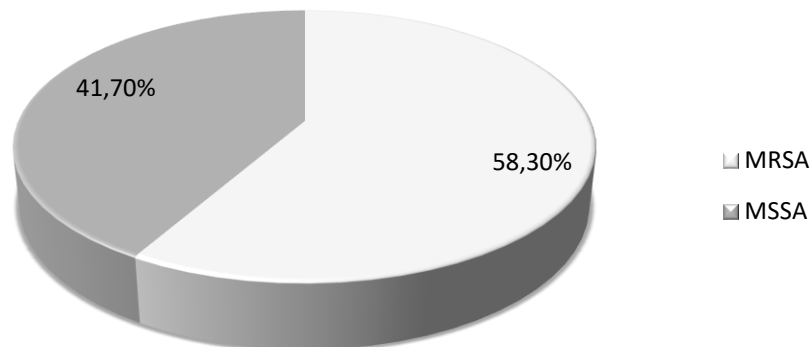
Figure 2: Distribution of MRSA isolates according to clinical sample sources



### MRSA vs. MSSA Distribution

Figure 3 shows the Methicillin Resistance Distribution among Staphylococcus aureus within the sample population. 58.3% of the total isolates were Methicillin resistant Staphylococcus aureus isolates, while methicillin-susceptible (MSSA) isolates comprised 41.7%. The sampling for the study indicated a concentrated presence of resistant strains, posing a major threat to public health especially in addressing concerns of growing antibiotic resistance in the clinical setting

Figure 3: Percentage distribution of MRSA and MSSA isolates detected using mecA gene



### Antibiotic Susceptibility Pattern of MRSA Isolates

Table 6 shows the susceptibility and resistance pattern of MRSA isolates to a range of antibiotics. The isolates exhibited high resistance to penicillin and methicillin (100%), high resistance to erythromycin (80%), and moderate resistance to ciprofloxacin (55%) and gentamicin (45%). Conversely, the isolates showed high susceptibility to vancomycin (95%) and linezolid (90%). These results confirm the effectiveness of some antibiotics against these strains despite the prevalence of resistance.

**Table 6:** Antibiotic Susceptibility Pattern of MRSA Isolates

Antibiotic	Resistance (%)	Sensitivity (%)
Penicillin	100%	0%
Methicillin	100%	0%
Erythromycin	80%	20%
Ciprofloxacin	55%	45%
Gentamicin	45%	55%
Vancomycin	5%	95%
Linezolid	10%	90%

## Discussion

The results of this study showed that *S. aureus* was isolated in 60% of the total clinical samples, a relatively high percentage, confirming the important role of this bacterium as a major cause of infection in clinical settings. This finding is consistent with several studies that have indicated the widespread prevalence of this bacterium, particularly in cases of wound infections (Tong et al., 2015; Jassim et al,2022). The results also showed that MRSA isolates comprised 58.3% of all *S. aureus* isolates, a high percentage reflecting the growing problem of antibiotic resistance (Jassim, and Ridah,2018; Aniz, and Jassim 2024). This finding is consistent with global reports indicating high rates of MRSA in developing countries due to the uncontrolled use of antibiotics and weak infection control measures (WHO, 2020; Hassoun et al., 2017). The study also showed that the highest rate of MRSA isolation was from wound and pus samples, which can be explained by the fact that this bacterium is strongly associated with hospital-acquired infections, particularly in cases of burns and surgical wounds (Jassim et al,2023). This finding supports the findings of Lee et al. (2018) and Jassim et al,2024 that MRSA is one of the most common causes of wound infections in healthcare settings. Regarding genetic diagnosis, the *mecA* gene was confirmed in 58.3% of the isolates using PCR, significantly improving diagnostic accuracy compared to traditional methods. The *mecA* gene is primarily responsible for methicillin resistance through the production of the PBP2a protein, which reduces the effectiveness of beta-lactam antibiotics (Lakhundi & Zhang, 2018). Therefore, the use of molecular techniques is a crucial step towards accurate and rapid diagnosis. Regarding antibiotic susceptibility patterns, the results showed high resistance to penicillin and methicillin, which is expected given the widespread presence of beta-lactamase enzymes and resistance mechanisms associated with the *mecA* gene. Instead, isolates demonstrated that the efficacy of these antibiotics in treating MRSA cases showed confirmatory results with several recent studies, as the isolates had considerable sensitivity. studies (Turner et al., 2019). These findings identify a particular concern regarding control of MRSA infection in the Babylon Governorate, and suggest the strengthening of antibiotic stewardship programs, as well as implement strict infection control measures within health institutions.

## Conclusion

The findings of this study indicate that there is a high occurrence of *S. aureus* bacteria in clinical samples from Babylon Governorate hospitals, with a high percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Using the polymerase chain reaction (PCR) approach for the *mecA* gene detection showed promising results for the diagnosis of these strains with high specificity. The results also indicated that MRSA is most frequently recovered from neither swabs, purulent material, nor pus, demonstrating the link between this bacterium and anecdotal evidence of hospital-acquired infections. Some antibiotics, including vancomycin and linezolid, remained effective against MRSA isolates despite these isolates showing significant resistance to many common antibiotics. Thus, this study elaborates on the significance of using molecular approaches for diagnostics, the importance of rationalizing the use of antibiotics, and strengthening the infection control system. procedures within health institutions to reduce the spread of resistant strains.

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