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Molecular identification of MRSA-MDR *Staphylococcus aureus* isolated from raw milk samples in Baghdad province

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Abstract

Objective

This study was conducted with the aim of isolating and identifying methicillin-resistant MRSA-MDR *Staphylococcus aureus* a bacterium that is known to be resistant to methicillin, an antibiotic used to treat many infections caused by *Staphylococcus aureus*. It is believed that methicillin resistance resulted from the bacteria acquiring a gene that encodes a protein capable of conferring resistance. This strain is known as methicillin-resistant *Staphylococcus aureus* (MRSA-MDR). The sensitivity of this bacterium to some antibiotics was studied by collecting samples of raw milk in Baghdad Governorate after its biochemical diagnosis using the API Staph System and verifying some of the genes responsible for resistance to the methicillin.

Materials and methods

Fifty raw milk samples were sampled from random popular markets spread throughout Baghdad (unlicensed points of sale) where raw milk and its derivatives are sold for the year 2025. Diagnostic tests were conducted using the API Staph System test to confirm their affiliation to *Staph.aureu*s specifically. The antibiotic susceptibility test was conducted to all identified *Staph.aureus* isolates by Kirby-Bauer Disk Diffusion technique employing 7 kinds of antibiotics Ciproflxacin, Erythromycin, Azithromycin Trimethoprim, Levofloxacin, Methicillin and Colistin. The genetic study included the detection of different identification and antibiotic-resistance genes (*nuc*, *mec*A, *lmr*S, and *mep*A) using the polymerase chain reaction (PCR) technique. For amplifying fragments of 276 bp for *nuc*, 147 bp for *mec*A, 718 bp for *mep*A, and 241 bp for *lmr*S, gene-specific primers were used.

Results

The findings of antibiotics susceptibility test showed significant degree of resistance toward Colistin (CL) 100%, Erythromycin (E) 82.50%, Azithromycin (AZM) 80%, The percentage of resistance isolates to fluoroquinolones group (Ciprofloxacin (CIP), Levofloxacin (LE)) was 25.80%. The *Staph.aureus* isolates recorded highly sensitivity to Trimethoprim (TR) 93.3%. The genetic study included the detection of different identification and antibiotic-resistance genes (*nuc*, *mec*A, *lmr*S, and *mep*A) using the polymerase chain reaction (PCR) technique revealed that 32 isolates were *Staphylococcus aureus* based on the appearance of a 276-bp band corresponding to the *nuc* identification gene after gel electrophoresis. Meanwhile, the results of molecular detection for *mecA* gene showed that it was found in 24 (75%) of *Staphylococcus aureus* isolates with amplicon size of 147bp. Moreover, the result of PCR amplification for efflux pump genes disclosed that *mep*A and *lmr*S were found in 30 (93.75%) and 31 (96.9%) of the studied isolates respectively.

Conclusions

The result of this study revealed that among 50 studied samples 32 (64%) isolated showed the typical biochemical and morphological characteristics that is specific to *Staphylococcus aureus*. The widespread of the antibiotics uses resulted in emergence of the MRSA and other multidrug resistant bacteria. The genetic study also included different identification and antibiotics resistance genes (*nuc*, *mecA*, *lmrS*, *mepA*).

Keywords: antibiotic resistance, API staph system, raw milk, MRSA-MDR

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Introduction

Staphylococcus aureus is one of the most important species of the genus *Staphylococcus* from a human health perspective, as it possesses numerous virulence factors, multiple antibiotic resistance mechanisms, and its ability to form biofilms. It also has the ability to acquire new resistance genes via integrons, which contributes to severe infections that lead to death. It is also a cause of milk and dairy product contamination, causing food poisoning, primarily due to the production of enterotoxins. *Staphylococcus aureus* has a high capacity to spread within the body and penetrate tissues easily due to the production of numerous extracellular substances, such as enzymes and toxins (Abu-Almaaly et al., 2023; Khairullah et al., 2024). Important factors that have contributed to the spread of these bacterial strains and the increase in infection are their ease of transmission among medical personnel, their natural presence in the external respiratory tract of hospital workers, and their resistance to a wide range of antibiotics (Al- Musawi et al., 2025a). The genus *Staphylococcus* belongs to the *Micrococcaceae* family, which also includes, the genera *Micrococcus* and *Stomatococcus*, each with its own specific characteristics. It is considered one of the most important types of bacteria in its genus, among more than 50 identified species. It is difficult to identify the strains of this bacterium due to the differences in their biochemical activities. Therefore, some special systems have been used to diagnose staphylococci, which are used to diagnose all strains and species of this genus, including the Rapidic STAPH system, the Sensititer system, and the AIP Staph system, which are distinguished by their reliable and rapid diagnostic results (Collee et al.,1996). It is worth noting that the majority of *Staphylococcus aureus* strains produce beta-lactamase enzymes, making them resistant to a wide range of beta-lactam antibiotics. The first methicillin-resistant *Staphylococcus aureus* (MRSA) isolate was recorded in England in 1961, causing a major health disaster that spread to various regions of the world (Suzuki et al.,1997; Al-Musawi et al., 2025a). Methicillin belongs to the group of anti-staphylococcal penicillins. It is a semi-synthetic penicillin, as it contains a beta-lactam ring and two methyl groups on the side. This antibiotic is characterized by Methicillin-sensitive *Staphylococcus aureus* (MRSA-MDR) infection can be divided into different types, including hospital-associated (HA-MRSA) infections, which can be distinguished according to the site of infection. There is also community-associated (CA-MRSA) infections, while methicillin-sensitive (CA-MRSA) infections can be divided into different types, including hospital-associated (HA-MRSA) infections, which can be distinguished according to the site of infection. *Staphylococcus aureus* (MSSA) refers to *Staphylococcus aureus* that is sensitive to methicillin and other beta-lactam antibiotics, while Vancomycin-Resistant *Staphylococcus aureus* (VRSA) refers to *Staphylococcus aureus* that is resistant to vancomycin, and Oxacillin-Resistant *Staphylococcus aureus*

(ORSA) refers to *Staphylococcus aureus* that is resistant to oxacillin. Livestock-Associated (LA-MRSA) infections also occur (Schindler et al., 2015; Lakhundi et al., 2018). The proteins NorA, NorB, NorC, MdeA, and SdrM are members of the Major Facilitator Superfamily (MFS). But, MepA belongs to the Multidrug and Toxic Compound Extrusion (MATE) family. Antibiotic (particularly tetracycline) biocide and dye resistance can be linked to over-expression of the pump in an indirect way (Daka et al., 2012). Milk may be contaminated at different stages of production, whether on farms, livestock fields, factories, or even consumers' homes, regardless of the type of animal or contamination of the raw materials used. In order to increase knowledge and educate the community about the importance of awareness of the danger resulting from the use (repeated, random, or inappropriate) of the antibiotic methicillin, the aim of this study was to isolate and diagnose *Staphylococcus aureus* bacteria, including methicillin-resistant and multidrug-resistant (MRSA-MDR) strains. The other goal of this study was to assess *Staphylococcus aureus'* susceptibility to some antibiotics and investigate some genes responsible for methicillin (*nuc*, *mecA*, *lmr*S, *mep*A) and other antibiotic resistance in the samples of raw milk without sterilization, collected from Baghdad Governorate.

Materials and methods

Sample collection: In 2025, fifty raw milk samples were collected from randomly selected popular markets spread throughout Baghdad (unlicensed points of sale) where raw milk and its derivatives are sold. The samples were collected in sterile 25 mL glass bottles, taking into account that the samples were not subjected to sterilization and were not obtained from approved companies or factories. The samples were quickly transported in a refrigerated container prepared for this purpose until they transported to the laboratory.

***Staphylococcus aureus* isolation and identification:** The culture media used in the study were ready to use media, except for blood agar formulated according to the manufacturer's Company. All used media were sterilized by autoclaving at 121ºC for 15min and poured in sterile Petri dishes. Moreover, blood agar was prepared by adding 5% of blood to the medium for the routine mixture after autoclaving (Al-Musawi, 2022). All samples were inoculated aerobically for 24 hours at 35°C-37°C onto blood agar and mannitol salt agar. The isolated bacteria were identified according to colonial morphology examination, size, shape and Gram reaction, followed by biochemical test (MacFaddin, 2000; Markey et al., 2013; Al- Musawi et al., 2025b). Then, all isolates were identified depending on their morphological and the biochemical tests and according to API Staph System test (AL-Samaraey and Jaafar, 2021).

Antimicrobial susceptibility test: The susceptibility of the bacterial isolates under study to a group of commonly used antibiotics (Bio-Analyze) was assessed. The Ciproflxacin (10 µg), Erythromycin (5 µg), Azithromycin (30 µg), Trimethoprim (10 µg), Levofloxacin (5 µg), Methicillin (10 µg), and Colistin (5 µg) antibiotics (HiMedia) were applied. This method was performed based on the Clinical Laboratory Standards Institute (CLSI) guideline (CLSI, 2020; Mohammed et al., 2022). The surface of the Muller-Hinton agar medium was inoculated with the colonies of isolated bacteria using a sterile swab dipped in a bacterial suspension adjusted to 1.5×10^{8} CFU/mL. Antibiotic discs were placed on the inoculated agar surface, and each disc of the antibiotics under study was placed individually in three replicates. Then the plates were left at room temperature for 30 minutes to permit diffusion, then the plates were incubated at 37°C for 24 ± 2 hours. Incubation was then performed. To determine antimicrobial susceptibility, the diameter of the zones of inhibition (in millimeters) was measured according to the interpretative standards provided by the antibiotic manufacturers.

Genomic DNA extraction: The Presto™ Mini gDNA Bacteria Kit based on the manufacturer's quick protocol and instructions provided in the user manual was used to extract genomic DNA from *Staphylococcus aureus* isolates.

Estimating DNA concentration and purity: To determine the concentration and purity of the extracted DNA, a NanoDrop spectrophotometer (Avans Biotechnology, Taiwan) was used. For measuring the optical density (OD) at 260/280 nm, representing the ratio of nucleic acid to protein content, a 2 µL aliquot of each DNA sample was analyzed. Agarose gel electrophoresis, as described by Sambrook et al. (1989) was also used to evaluate the integrity of the DNA.

Primers for Detection of *nuc*, *mecA*, *mepA*, and *lmrS* Genes in *Staphylococcus aureus*: To determine species-specific and antibiotic-resistance genes (*nuc*, *mecA*, *lmrS*, *mepA*) for *Staphylococcus aureus* isolates, a genetic analysis using the PCR technique was performed. For PCR amplification four different pairs primers were used. The primer sequences and amplicon sizes foe *nuc*, *mecA*, *lmrS*, and *mepA* were Forward: 5′-GCGATTGATGGTGATACGGTT-3′, Reverse: 5′-AGCCAAGCCTTGACGAACTAAAGC-3′, Amplicon size: 276 bp; Forward: 5′-GTGAAGATATACCAAGTGATT-3′, Reverse: 5′-ATGCGCTATAGATTGAAAGGAT-3′, Amplicon size: 147 bp; Forward: 5′-TAACAACCCGGAGACGAAAC-3′, Reverse: 5′-CCCATCGCCGCTAAGTATAA-3′, Amplicon size: 241 bp; and Forward: 5′-ATGTTGCTGCTGCTCTGTTC-3′, Reverse: 5′-TCAACTGTCAAACGATCACG-3′, Amplicon size: 718 bp, respectively. The methods described by Zhang et al. (2005) and Couto et al. (2008) were used for selecting primers and amplification conditions.

Primer preparation: The DNA primers (Promega, USA) were supplied in lyophilized form and dissolved in deionized water according to the manufacturer's instructions to reach a final concentration of 10 pmol/µL.

PCR technique for detection of *nuc*, *mecA*, *mepA*, and *lmrS* genes: Polymerase chain reaction (PCR) was performed to amplify the *nuc*, *mecA*, *mepA*, and *lmrS* genes using their corresponding specific primers. All PCR components were assembled in PCR tubes and thoroughly mixed on ice under sterile conditions (Table 1).

Conditions of PCR thermocycling: The PCR tubes were placed in a thermocycler, and several trials were performed to optimize the conditions for amplifying the target genes. The final optimized amplification parameters achieved for the reactions are shown in Tables 2, 3, and 4.

Table 1. Characteristics of used protocol for PCR reactions

Components	Promega Protocol (final volume 25 µL)
Nuclease free water	5.5
Go Taq green master mix (x2)	12.5
Forward primer	1
Reverse Primer	1
Template DNA	5

Table 2. The final optimized amplification parameters for *mec*A gene

Steps	No. of cycle	Temp.	Time
Initial denaturation	1 cycle	94	5 min.
Denaturation		94	45 sec.
Annealing	35 cycles	54	45 sec.
Extension		72	45 sec.
Final extension	1 cycle	72	7 min.

Table 3. The final optimized amplification parameters for *lmr*S gene

Steps	No. of cycle	Temp.	Time
Initial denaturation	1 cycle	94	5 min.
Denaturation		94	45 sec.
Annealing	35 cycles	60	45 sec.
Extension		72	1 min.
Final extension	1 cycle	72	7 min.

Agarose gel electrophoresis: Standard laboratory procedures as described by Voytas (1992) were used to analysis DNA on agarose gel electrophoresis. A 1.5% agarose gel was applied to separate genomic DNA and PCR products. Then, ethidium bromide for 15–20 minutes was used to stain gels. A gel documentation system was used to visualize genomic DNA and PCR products.

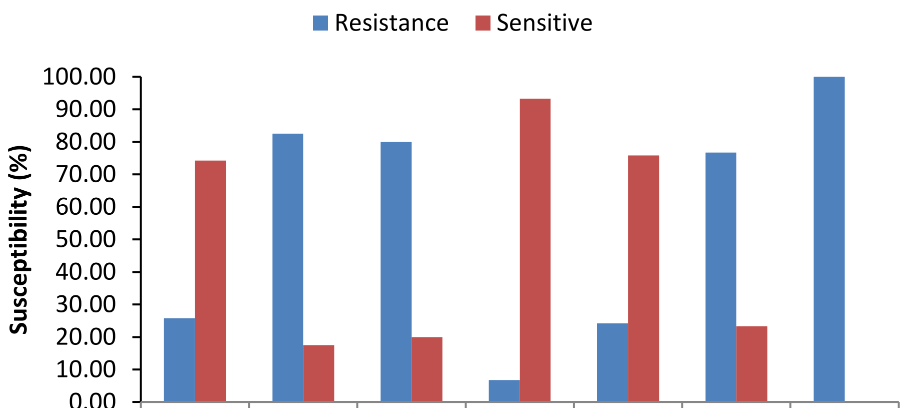
Table 4. The final optimized amplification parameters for *mep*A gene

Steps	No. of cycle	Temp.	Time
Initial denaturation	1 cycle	94	5 min.
Denaturation		94	30 sec.
Annealing	35 cycles	53	30 sec.
Extension		72	30 sec.
Final extension	1 cycle	72	7 min.

Results and discussion

Several methods, such as cultural characteristics, Gram staining, biochemical tests, the API Staph system, and molecular identification genes were used to primary identify all samples. From all considered isolates, 32 (64%) showed the characteristic morphology and biochemical profiles, which are special for *Staphylococcus aureus*. *Staphylococcus aureus* was found to be the most common cause of clinical ovine mastitis in Norwegian research, where it was found in the mammary secretions of 64 to 87% of the ewes (Leijon et al., 2021). The southern region was determined to have the highest concentration of *Staphylococcus aureus* in this research (76%). Indrebø and Mosdøl (1991) in the previous study considered cases from a nearby municipality and reported 75% that is very similar to results of current study. According to research conducted in various countries (Bocklisch and Wetzstein,1994), the prevalence of *Staphylococcus aureus* infections in clinical mastitis of meat sheep varies, but is typically quite high (20-60%). Lafi et al. (1998) studied clinical mastitis in Jordanian dairy sheep and demonstrated that *Staphylococcus aureus* is the causative agent in 22% of cases. *Staphylococcus aureus* colonies were identified by a golden yellow color when culturing on mannitol salt agar media at 37˚C for 24 hours. This change occurs because the organism has aerobically fermented the mannitol, causing the red color to change to yellow (Kaddumukasa et al., 2019). Culturing clinical isolates on blood agar revealed the formation of clear halos around the colonies. This is indicative of beta hemolysis, the lysis of red blood cells. This type of hemolysis is also known as complete hemolysis. In this hemolysis, the red blood cells in the medium below and around the colonies are completely broken down, changing the color of the agar to a clear and bright (often yellow) state (Sum et al.,2017). The positive coagulase reaction was the result of biochemical testing of the isolates. This indicates that they have the ability to convert fibrinogen to fibrin and form visible clots. The isolates were also catalase positive, as demonstrated by the decomposition of hydrogen peroxide into water and oxygen and the production of gas bubbles. Urease activity was positive for clinical isolates. This is because the enzyme urease hydrolyzes urea, releasing ammonia and carbon dioxide. This alkalizes the medium and changes its color to pink-red. The isolates were oxidase negative. This helps to distinguish *Staphylococcus aureus* from Micrococcus species (Becker et al., 2015). The API Staph system was used for further biochemical confirmation and showed that all *Staphylococcus aureus* isolates produce the same result.

Antibiotics susceptibility test of *Staphylococcus aureus* isolates: The emergence of strains antibiotic resistance is considered a major therapeutic challenge that can be explained by several theories, such as the effect of excessive or improper use of antibiotics. Antimicrobial resistance in bacterial pathogens is a significant challenge that has a high morbidity and mortality (Frieri et al., 2017). The results showed highly resistance noted among *Staphylococcus aureus* isolates to Methicillin (76.70%) and among *Staphylococcus aureus* isolates to Methicillin (76.70%). It also showed a high resistance to Colistin (100%), Erythromycin (82.5%) and Azithromycin (80%). The isolates ciprofloxacin and levofloxacin showed moderate resistance toward fluoroquinolones group (25.80%), while these isolates showed highly sensitivity (93.30%) toward Trimethoprim (Figure 1).



	CIP	E	AZM	TR	LE	ME	CL
Resistance	25.8	82.5	80	6.7	24.2	76.7	100
Sensitive	74.2	17.5	20	93.3	75.8	23.3	0

Figure 1. Antimicrobial Susceptibilities of the *Staphylococcus aureus* isolates

In a study Hamilton et al. (2017) showed that *Staphylococcus aureus* isolates were highly resistance to β lactam antibiotics, while Santos et al. (2018) demonstrated that *Staphylococcus aureus* isolates are highly sensitively to β lactam antibiotics. In this study, the percentage resistant of *Staphylococcus aureus* to methicillin was 76.70%. This result is in line with the results of local studies that demonstrated that the MRSA covered 68% and 80%, respectively of all *Staphylococcus aureus* isolates. The current study showed highly percentage of Erythromycin and Azithromycin resistance which is in line with the results of Zulkeflle et al. (2019) who showed all *Staphylococcus aureus* resistance to erythromycin and also with the results of Si et al. (2018) who found 66.67% among *Staphylococcus aureus* tested isolates resistance to azithromycin. In another study Pantosti et al. (2007) also showed highly resistance to colistin. They used colistin in treatment of *Staphylococcus aureus* infection. In the current study, the *Staphylococcus aureus* shows very high sensitivity (100%) to Amikacin, Trimethoprim, and Trimethoprim–Sulfamethoxazole. These results were in line with the results of Zulkeflle et al. (2019). They demonstrated that *Staphylococcus aureus* isolates are 100% resistant to Trimethoprim–Sulfamethoxazole. Also, Peacock and Paterson (2015) showed that all isolates were resistant to this antibiotic. Pantosti et al. (2007) using MRSA testing disclosed that isolates are resistant to all β-lactam antibiotics, such as cephalosporins, carbapenems, and penicillins. The mec operon is part of the SCCmec element and plays an important role in methicillin resistance. This element contains the *mecA* gene and encodes PBP2a protein. The low affinity of this protein for beta-lactam antibiotics allows the bacteria to build their cell walls in the presence of antibiotics. Therefore, beta-lactam antibiotics, including penicillin, are not used to treat MRSA infections (Mah et al., 2014). Because *Staphylococcus aureus* bacteria produce penicillinase, they become resistant to penicillin. This enzyme inactivates penicillin by breaking the beta-lactam ring. Therefore, new penicillinase-resistant antibiotics such as methicillin were developed and marketed (Peacock and Paterson, 2015). When *Staphylococcus aureus* is exposed to low doses of antibiotics, it may develop cross-resistance to different antibiotic groups, because of β-lactamase activity and efflux pumps. We need to monitor antibiotic susceptibility of patients taking antibiotics so that we can treat resistant infections appropriately. Combination therapy is best used to treat infections caused by bacteria that have shown varying levels of resistance (Jo and Ahn, 2016).

Genomic DNA extraction: Total genomic DNA was extracted from all 32 isolates of *Staphylococcus aureus*. The purity of all isolates varies between 1.8–2 and the concentration was between 50–360 ng/µL. This DNA was used to perform molecular identification and to detect the efflux pump genes.

Genetically identification of *S. aureus* by the *nuc* gene: Only *Staphylococcus aureus* can be identified by the *nuc* primer and separated from other staphylococci. The PCR products of all *Staphylococcus aureus* isolates showed the specific gene sequence. It confirms that these isolates were *Staphylococcus aureus*, as shown in Figure 2.

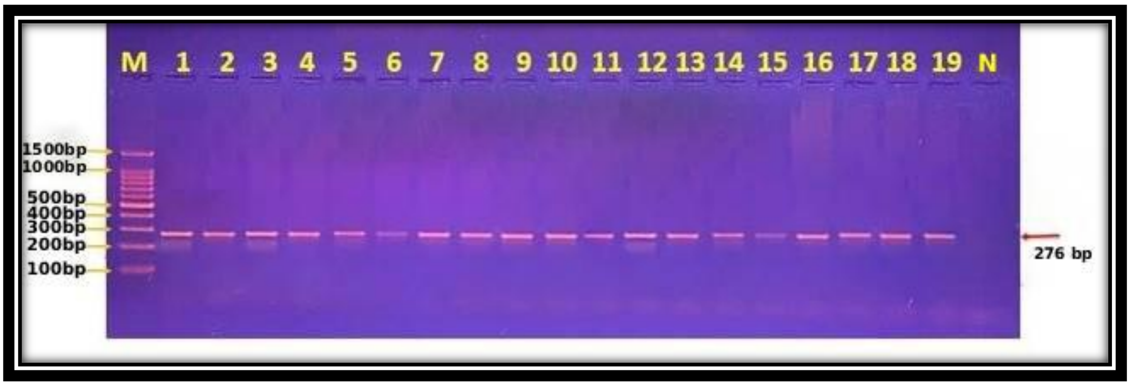


Figure 2. Gel electrophoresis for the PCR amplification product of the *nuc* gene on 1.5% agarose (70 V for 90 minutes). Lane L: 1500-bp DNA ladder. Lanes 1–19: positive samples for *Staphylococcus aureus* isolates (276 bp). N: Negative control

The PCR products of the bacterial isolates showed as single DNA bands (276 bp). These results were in line with the results of Rushdy et al. (2007). They also observed a 276 bp DNA fragment in all studied *Staphylococcus aureus* isolates. The findings confirm the validity of the morphological and biochemical tests used to identify the isolates at the species level (Chakraborty et al., 2011; Al-Ugaili, 2013).

Genetic identification of MRSA by the *mecA* Gene: Conventional methods are considered the most reliable for identifying the methicillin resistance gene (*mecA*) in *Staphylococcus aureus*, due to the presence of various staphylococcal species with different resistance levels. In this study, PCR was used for correct detection of the *mecA* gene in *Staphylococcus aureus* isolates, using the primers defined in the methodology. All isolates that tested positive for methicillin by disc diffusion and were characterized as MRSA were subjected to PCR detection of the *mecA* gene, as shown in Figure 3.

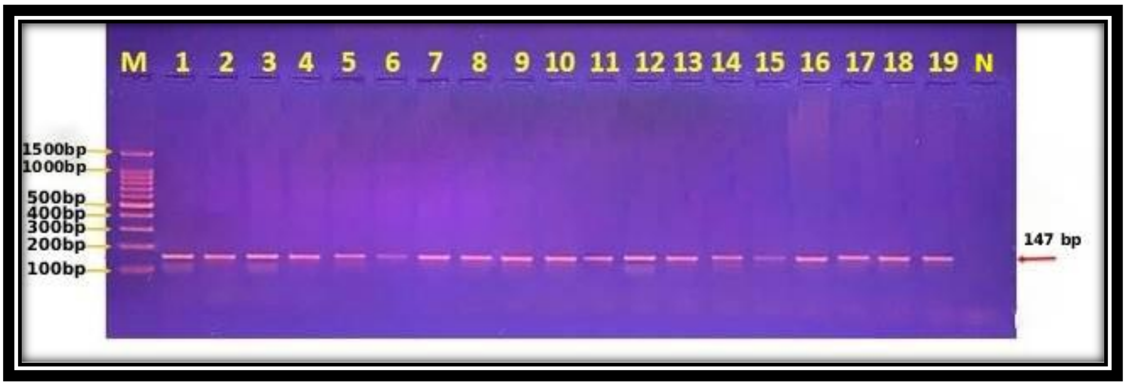


Figure 3. Gel electrophoresis for the PCR amplification product of the *mecA* gene on 1.5% agarose (70 V for 90 minutes). Lane L: 1500-bp DNA ladder. Lanes 1–19: positive samples for *Staphylococcus aureus* isolates (147 bp). N: Negative control

The molecular size of the *mecA* PCR product was 147 bp. This gene was seen in 24 isolates (75%). Detection of MRSA using PCR amplification of the *mecA* gene is considered the gold standard (Alipour et al., 2014). This finding is consistent with the results of Rasheed et al. (2020). They showed that 98.7% of MRSA isolates were positive for the *mecA* gene. The presence of the *mecA* gene indicates potential resistance to β-lactam antibiotics and can be used as a marker for MRSA identification.

Detection of the *mepA* gene: Molecular detection of the *mepA* gene by PCR was done on 32 *Staphylococcus aureus* isolates using specific primers. The results showed that the *mepA* gene was detected in 30 isolates (93.75%) with amplicon size of 718 bp, as shown in Figure 4. This result is in line with the results of other studies that reported the *mepA* gene as having the second highest prevalence (97.9%) among *Staphylococcus aureus* isolates. Other studies also found *mepA* prevalence of 92.71% and 89.4% in *Staphylococcus aureus* isolates (Antiabong et al., 2017; Shamkhi et al., 2019). In contrast, Hassanzadeh et al. (2017) reported a lower prevalence of 60% for the *mepA* gene in *S. aureus* isolates.

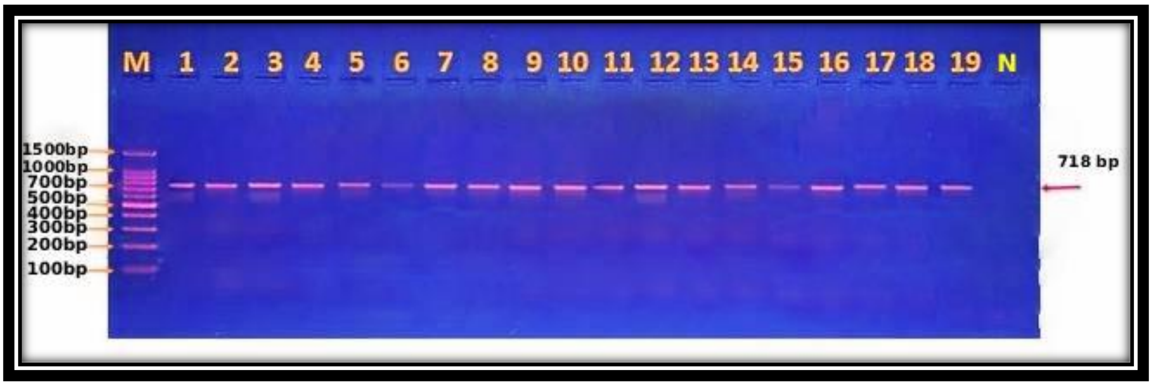


Figure 4. Gel electrophoresis for the PCR amplification product of the *mepA* gene on 1.5% agarose (70 V for 90 minutes). Lane L: 1500-bp DNA ladder. Lanes 1–19: positive samples for *Staphylococcus aureus* isolates (718 bp). N: Negative control

Detection of the *lmrS* gene: The molecular detection of the *lmrS* gene was performed using PCR with specific primers. 31 isolates (96.9%) from 32 isolates, gave a positive result. They showed a band with a molecular size of 241 bp on the agarose gel electrophoresis (Figure 5). This result is supported by Antiabong et al. (2017). They reported a *lmrS* gene prevalence of 88.7% among *Staphylococcus aureus* isolates. Other studies have reported variable prevalence rates, ranging from 60.8% to 68.42% (Conceição et al., 2016; Lekshmi et al., 2018).

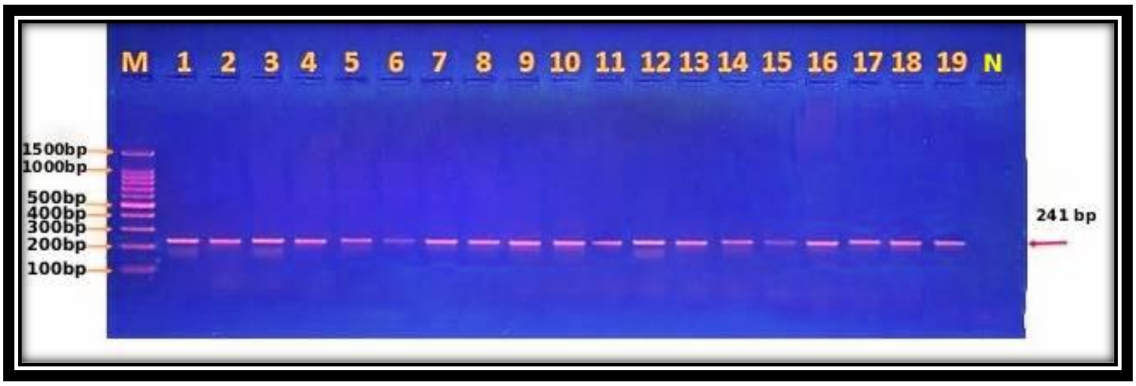


Figure 5. Gel electrophoresis for the PCR amplification product of the *lmrS* gene on 1.5% agarose (70 V for 90 minutes). Lane L: 1500-bp DNA ladder. Lanes 1–19: positive samples for *Staphylococcus aureus* isolates (241 bp). N: Negative control

Conclusions: The results of our study showed that out of 50 samples cultured and identified, 32 isolates (64%) showed the typical biochemical reactions and morphological characteristics that are specific to *Staphylococcus aureus*. The widespread use of antibiotics has contributed to the emergence of MRSA and other multidrug-resistant bacteria. In addition, the genetic analysis included the detection of several identification and antibiotic-resistance genes, including nuc, mecA, lmrS, and mepA.

Author contributions

Adil Turki Al-Musawi: Investigation, Project administration, Data curation, analysis of the data Writing-original draft, Writing-review and editing.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical considerations

The author avoided data fabrication, falsification, plagiarism, and misconduct.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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شناسایی مولکولی *Staphylococcus aureus* MRSA-MDR جداشده از نمونه‌های شیر خام در استان بغداد

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چکیده

هدف: این مطالعه با هدف جداسازی و شناسایی باکتری *Staphylococcus aureus* مقاوم به متی‌سیلین (MRSA-MDR) انجام شد. این باکتری به متی‌سیلین، که آنتی‌بیوتیکی برای درمان بسیاری از عفونت‌های ناشی از *Staphylococcus aureus* استفاده می‌شود مقاوم است. تصور می‌شود این مقاومت از طریق کسب ژنی حاصل شده باشد که پروتئینی را رمزگذاری می‌کند که توانایی ایجاد مقاومت را دارد. این سویه به‌عنوان *Staphylococcus aureus* مقاوم به متی‌سیلین (MRSA-MDR) شناخته می‌شود. حساسیت این باکتری به برخی آنتی‌بیوتیک‌ها از طریق جمع‌آوری نمونه‌های شیر خام در استان بغداد و انجام تشخیص بیوشیمیایی با استفاده از سیستم API Staph و نیز بررسی برخی از ژن‌های مسئول مقاومت به متی‌سیلین مطالعه شد.

مواد و روش‌ها: پنجاه نمونه شیر خام از بازارهای مردمی و پراکنده در سراسر بغداد (نقاط فروش غیرمجاز) که شیر خام و مشتقات آن عرضه می‌شود، در سال ۲۰۲۵ جمع‌آوری گردید. برای تأیید وابستگی نمونه‌ها به *Staphylococcus aureus* از آزمون تشخیصی API Staph System استفاده شد. آزمون حساسیت آنتی‌بیوتیکی برای تمامی جدایه‌های شناسایی‌شده *Staphylococcus aureus* با روش Kirby-Bauer Disk Diffusion انجام شد که شامل ۷ نوع آنتی‌بیوتیک سیپروفلوکساسین، اریترومایسین، آزیترومایسین، تری‌متوپریم، لووفلوکساسین، متی‌سیلین و کولیستین بود. مطالعه ژنتیکی شامل شناسایی ژن‌های تشخیصی و مقاومت آنتی‌بیوتیکی (*nuc*، *mecA*، *lmrS* و *mepA*) با استفاده از واکنش زنجیره پلیمراز (PCR) بود. برای تکثیر قطعات ژنی ۲۷۶ جفت‌باز برای *nuc*، ۱۴۷ جفت‌باز برای *mecA*، ۷۱۸ جفت‌باز برای *mepA* و ۲۴۱ جفت‌باز برای *lmrS* از پرایمرهای اختصاصی استفاده شد.

نتایج: یافته‌های آزمون حساسیت آنتی‌بیوتیکی نشان داد مقاومت قابل توجهی نسبت به کولیستین (CL) با ۱۰۰٪، اریترومایسین (E) با ۸۲/۵٪ و آزیترومایسین (AZM) با ۸۰٪ مقاومت وجود دارد. درصد جدایه‌های مقاوم به گروه فلوروکینولون‌ها

(سیپروفلوکساسین (CIP) و لووفلوکساسین (LE)) برابر با ۲۵/۸٪ بود. جدایه‌های *S. aureus* حساسیت بالایی به تری‌متوپریم (TR)با بازدهی ۹۳/۳٪ نشان دادند. مطالعه ژنتیکی نشان داد ۳۲ جدایه بر اساس مشاهده نوار ۲۷۶ جفت‌بازی مربوط به ژن تشخیصی *nuc* پس از الکتروفورز روی ژل، *Staphylococcus aureus* بودند. همچنین، نتایج شناسایی مولکولی ژن *mecA* نشان داد که این ژن در ۲۴ مورد (۷۵٪) از جدایه‌های *Staphylococcus aureus* با اندازه تکثیر ۱۴۷ جفت‌باز وجود داشت. علاوه بر این، نتایج PCR ژن‌های پمپ دفعی نشان داد که ژن‌های *mep*A و *lmr*S به‌ترتیب در ۳۰ (۹۳/۷۵٪) و ۳۱ (۹۶/۹٪) از جدایه‌ها مشاهده شدند.

نتیجه‌گیری: نتایج این مطالعه نشان داد که از میان ۵۰ نمونه بررسی‌شده، ۳۲ نمونه (۶۴٪) دارای ویژگی‌های بیوشیمیایی و مورفولوژیک اختصاصی *Staphylococcus aureus* بودند. مصرف گسترده آنتی‌بیوتیک‌ها منجر به بروز MRSA و دیگر باکتری‌های مقاوم چنددارویی شده است. مطالعه ژنتیکی نیز شامل شناسایی ژن‌های تشخیصی و مقاومت آنتی‌بیوتیکی (*nuc*، *mecA*، *lmrS* و *mepA*) بود.

کلمات کلیدی: سیستم API staph، شیر خام، مقاومت آنتی‌بیوتیکی، MRSA-MDR

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