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Molecular identification of antibiotic resistance profile and virulence genes in *Staphylococcus aureus* from human and animal sources

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Abstract

Staphylococcus aureus is a common cause of infection in both animals and human. It often resists many antibiotics. This resistance is partly due to efflux pumps and genes like *mecA*. This study aimed to detect *S. aureus* in humans and animals, test its antibiotic resistance, and identify some important virulence genes. A total of 14 samples were collected between December 2022 and August 2023. Seven came from humans in Dhi Qar, Iraq. The other seven came from animals in Al-Najaf. Five isolates were confirmed as *S. aureus*. where. Diagnosis used blood agar and mannitol salt agar, followed by Gram stain and biochemical tests. VITEK 2 was used for confirmation. Antibiotic sensitivity was tested using Muller Hinton agar and VITEK 2 Ast card. PCR was used to detect virulence and resistance genes, including *mecA* (60.0%), nuclease (80.0%), lipase (100.0%), Saphylokinase (100.0%), invasive protease (60.0%), and Enterotoxin A (100.0%). A phylogenetic tree was done. Five isolates (35.7%) were identified as *S. aureus*. All five carried lipase, Enterotoxin A, and staphylokinase genes. Most had *mecA* and *nuc* genes. Three isolates carried invasive protease. Resistance was highest to penicillin, oxacillin, and cefoxitin. No resistance was found to gentamicin 0(0%), ciprofloxacin 0(0%), or linezolid. Phylogenetic analysis showed genetic diversity among strains. This study showed that *S. aureus* strains from humans and animals can carry multiple virulence genes and resist many antibiotics. The high rate of *mecA* gene indicates MRSA is present in both sources.

Keywords: 16S rRNA, Enterotoxin A, Invasive protease, Lipase, *mecA*, nuclease, Phylogenetic tree, *Staphylococcus aureus*, Staphylokinase, VITEK compacted 2 system

1. Introduction

Staphylococcus aureus is a Gram-positive coccus. It does not move and can survive in harsh environments (Al-Daraghi, 2022) ^[1]. It grows well in both oxygen and low-oxygen conditions. This makes it a common pathogen in many places (Al-Daraghi, 2022) ^[1]. *S. aureus* lives in both humans and animals. It is often found in animals used for food. These bacteria can enter the food chain during slaughter, handling, or packaging (Alkuraythi *et al.*, 2023) ^[4].

This bacterium causes many infections. These range from mild skin infections to serious diseases like pneumonia, bone infections, endocarditis, and blood infections (Guo *et al.*, 2020) ^[7]. It also lives silently in about 30% of healthy people, mostly in the nose (Liu *et al.*, 2022) ^[15]. *S. aureus* is one of the most widespread and dangerous bacteria. It causes millions of infections worldwide each year (Liu *et al.*, 2022) ^[15]. A major concern is its resistance to antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major health concern. It is hard to treat because it does not respond to most penicillin-based antibiotics (Abdulrazziq *et al.*, 2023) ^[2]. MRSA is found in both humans and animals. It can pass easily from one to the other. This makes control and prevention more difficult.

MRSA carries a gene called *mecA*. This gene helps the bacteria survive many antibiotics. It produces a protein called PBP2a, which does not bind well to beta-lactam drugs (Palmieri *et al.*, 2023) ^[20]. Because of this, antibiotics like methicillin and oxacillin cannot kill it. The *mecA* gene sits on a mobile DNA piece called SCCmec. This element can move between bacteria, spreading resistance quickly (Leseigneur *et al.*, 2024) ^[13].

S. aureus also produces many harmful molecules. These are called virulence factors. Some of them are toxins and enzymes. Examples include enterotoxins, lipases, nucleases, proteases, and staphylokinase (Yamanashi *et al.*, 2022) ^[26]. These help the bacteria invade the body and avoid the immune system.

Enterotoxin A (SEA) is one of the most dangerous toxins. It can cause food poisoning if the bacteria grow in food. SEA is made when a special virus, called a phage, becomes active inside the bacteria. Its production is not linked to how fast the bacteria grow or how many are present (Yamanashi *et al.*, 2022) ^[26].

The nuc gene helps the bacteria escape immune traps by breaking down DNA and RNA. It is very stable and is often used to identify *S. aureus* in the lab (Alkhafajy & Al-Mathkhury, 2023) ^[3]. This gene also helps the bacteria destroy tissues and spread through the body.

Lipase is another enzyme that *S. aureus* uses to survive. It breaks down fats in human cells. This damages tissues and helps the bacteria spread. High amounts of lipase are often found in serious MRSA infections (Kitadokoro *et al.*, 2024) ^[11]. Lipase also lowers the body's immune response by destroying protective lipids.

Staphylokinase plays a different role. It turns plasminogen into plasmin, a protein that breaks down blood clots. This action helps the bacteria move through tissues more easily. It also helps them avoid being trapped by immune cells (Noori & Aziz, 2020) ^[19]. It is also used as a drug to treat blood clots. Proteases are enzymes that cut proteins. They help bacteria damage host tissues and avoid immune responses (Marquart, 2021) ^[16]. In summary, *S. aureus* can resist many drugs and cause many kinds of disease. This study focuses on its resistance patterns and key virulence genes.

Materials and Methods

Sample Collection and Bacterial Isolation

A total of 14 samples were collected between December 1, 2022, and August 31, 2023. Seven samples came from humans through the central laboratory in Dhi Qar, and seven came from animals at a private slaughterhouse in Al-Najaf Al-Ashraf. The samples were kept in Cary-Blair transport medium (Himedia) and transported in cold boxes to the central laboratory in Dhi Qar within 24 hours. Isolation and identification began by culturing the samples on blood agar and mannitol salt agar. Both media were prepared following the manufacturer's instructions. The plates were incubated and examined using aseptic techniques. Colonies suspected to be *Staphylococcus* species were subjected to Gram staining using a Himedia kit, followed by biochemical tests including catalase, coagulase, and others for confirmation. Final identification was carried out using the VITEK 2 compact

system at the College of Health and Medical Technologies, Islamic University, Najaf, based on instructions from the Biomerieux company.

Antibiotic Susceptibility Testing

Antibiotic resistance was tested using the disc diffusion method on Muller-Hinton agar (Himedia, India). The test followed the Clinical and Laboratory Standards Institute (CLSI) guidelines. The VITEK 2 Ast card compact system was used for additional confirmation using AST cards for Gram-positive cocci. A total of 19 antibiotics were tested, including cefoxitin, oxacillin, benzylpenicillin, erythromycin, clindamycin, gentamicin, ciprofloxacin, moxifloxacin, vancomycin, linezolid, tigecycline, teicoplanin, tetracycline, fosfomycin, fusidic acid, rifampicin, and trimethoprim/sulfamethoxazole. The results were recorded and interpreted according to the latest CLSI standards to determine resistance, intermediate, or susceptibility levels.

DNA Extraction and PCR Amplification

DNA was extracted using the Favorgene kit (Korea), following the manufacturer's instructions. The DNA purity and concentration were measured using a Chimadzu double-beam spectrophotometer at the Al-Amin Research Center. The 16S rRNA gene specific for *Staphylococcus* was targeted using two primer sets: TStaG422/TStaG765 F (GGC CGT GTT GAA CGT GGT CAA ATC A) and R (TIA CCA TTT CAG TAC CTT CTG GTA A) for *Staphylococcus* genus-specific detection with 370 bp amplicon and universal 27F/1492R primers F(AGAGTTTGATCCTGGCTCAG) and R (TACGGYTACCTTGTACGACT) for *Staphylococcus* genus-universal detection with 1468 bp amplicon. PCR was done using 2 µL of extracted DNA with Promega Green Master Mix on an Agilent thermocycler. The thermal cycling conditions were: 94 °C for 2 minutes (initial denaturation), followed by 30 cycles of 94 °C (Denaturation) for 30 seconds, 57 °C (Annealing) for 30 seconds, and 72 °C(extension) for 1 minute, then (a final extension) at 72 °C for 5 minutes. Products were visualized using 1.5% agarose gel electrophoresis with RedSafe dye and photographed under UV light.

Virulence Gene Detection

PCR was also used to detect specific virulence genes in *S. aureus* isolates. The genes included mecA (533 bp), nuc (537 bp), lipase (921 bp), staphylokinase (492 bp), invasive protease (723 bp), and enterotoxin A (521 bp). The amplification protocol was similar to the one used for 16S rRNA. PCR products were run on 1% agarose gel and visualized using gel documentation systems. The presence or absence of these virulence genes was recorded for each isolate.

Table 1: Forward and reverse primers used in study

No.	Primer code	Nucleotide sequences	PCR product	Annealing temperature	References
1.	MecA- F	AAAATCGATGGTAAAGGTTGGC	533	54 °C	(Frey <i>et al.</i> , 2013) ^[6]
	MecA-R	AGTTCGTCAGTACCGGATTGTC			
2.	Nuc- F	AAGAGGTTTTCTTTTCGCTACTAGTTGC	537	54 °C	(Lin <i>et al.</i> , 2021) ^[4]
	Nuc-R	CTCCAAATATTTAATTCTGTTGTTAGCTTT			
3.	Lipase-F	GTAGATTATGGTGCAGCACATGCAGCAAAAATATGG	921	60 °C	(Lin <i>et al.</i> , 2021) ^[4]
	Lipase- R	AGCTTTTCAGTTTTCCTAAATCGTCTGCT			
4.	Staphylokinase -F	ATGCTCAAAAAGAGGTTTATTATTTTAACTGTTTT	492	58 °C	(Lin <i>et al.</i> , 2021) ^[4]
	Staphylokinase-. R	ATTTCCTTTCTATAACAACCTTTGTAATTAAGTTG			
5.	Invasive-F	GAACAAAAACGTAGTCATCAAGAGTTTAGCAGC	723	58 °C	(Lin <i>et al.</i> , 2021) ^[4]
	Invasive-R	CTATGTTTTCTGCAATGAATTTTAAATTTCTGGTGT			
6.	sea (Enter A)-F	GCAGGGAACAGCTTTAGGC	521	60 °C	(Hammer <i>et al.</i> , 2019) ^[8]
	sea (EnterA)-R	GTTCTGTAGAAGTATGAAACACG			

Electrophoresis and Visualization

After PCR, gels were prepared by dissolving 1 gram of agarose in 100 mL of 1X TBE buffer. The solution was microwaved for 1 minute and cooled to about 50°C. Then 0.5 µL of RedSafe dye was added. The gel was poured into a tray and combs were inserted to form wells. After solidification, the DNA samples were loaded and run using electrophoresis. Bands were observed using a gel documentation system from Cleaver Scientific (USA) to confirm the size and presence of target genes.

Statistical Analysis

All collected data were analyzed using SPSS software and Microsoft Excel. Data were expressed as mean ± standard deviation error (SDE). A P-value ≤ 0.05 was considered statistically significant, indicating real differences between the observed values.

Phylogenetic Analysis

The evolutionary relationships of the isolates were analyzed using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987) [21] through MEGA11 software (Tamura *et al.*, 2021) [23]. The evolutionary distances were computed with the Maximum Composite Likelihood method (Tamura *et al.*, 2004) [24]. Bootstrap analysis was conducted with 1000 replicates (Felsenstein, 1995) [5]. Poorly aligned regions were removed using the pairwise deletion option. The final dataset included 887 nucleotide positions. This method helped illustrate genetic diversity and clustering patterns among the *S. aureus* isolates.

Results

Phenotypic and Biochemical Identification

Out of 14 collected samples, 5 were confirmed as

Staphylococcus aureus, representing 35.7% of the total. Gram staining showed Gram-positive cocci arranged in irregular grape-like clusters. On Baird-Parker agar, colonies appeared jet-black with clear white halos. Biochemical tests confirmed the identity of the isolates. All five tested positives for catalase, coagulase, DNase, and acetoin production. The oxidase test was negative for all isolates. These results matched the typical characteristics of *S. aureus*. The VITEK 2 system also confirmed these identifications, providing automated validation based on metabolic profiles. Overall, the phenotypic features and biochemical reactions supported the classification of these isolates as *S. aureus*.

Table 2: Distribution of study organism

	Frequency	Percent
<i>Staphylococcus aureus</i>	5	35.7
Other Species	9	64.3
Total	14	100.0

Molecular Confirmation and Gene Detection by PCR

PCR confirmed the presence of the 16S rRNA gene in all five isolates, supporting their identity as *Staphylococcus aureus*. Additional PCR tests were done to detect virulence and resistance genes. The *mecA* gene was detected in 3 out of 5 isolates (60%) as show in (table 3), confirming methicillin resistance. The *nuc* gene, known for being a stable diagnostic marker, was found in 4 isolates (80%) as show in (table 3). All five isolates (100%) tested positive for lipase as show in (table 3), staphylokinase, and Enterotoxin A genes. Invasive protease genes were detected in 3 isolates (60%) as show in (table 3). These results showed that *S. aureus* isolates from both human and animal samples carried multiple virulence genes, some of which are involved in antibiotic resistance and immune evasion.

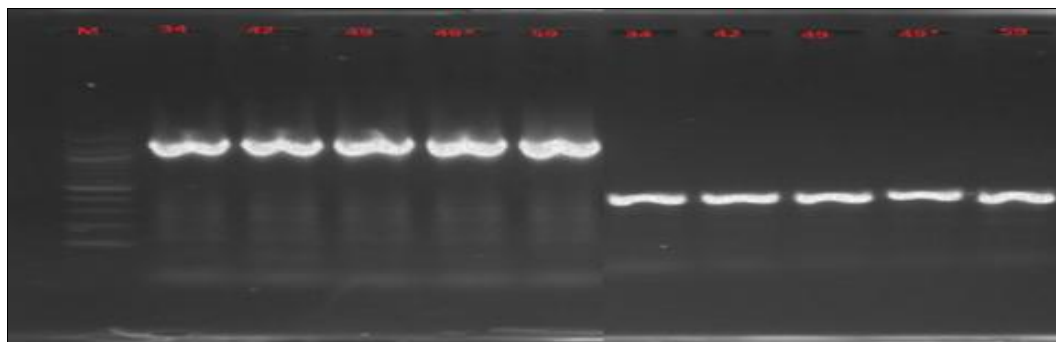


Fig 1: PCR detection of the specific gene in *S. aureus* isolates. Lane M: DNA ladder (100 bp); lanes 34, 42, 49, 49*, 59: *S. aureus* (370 bp) positive isolates and universal primer product size (1468).

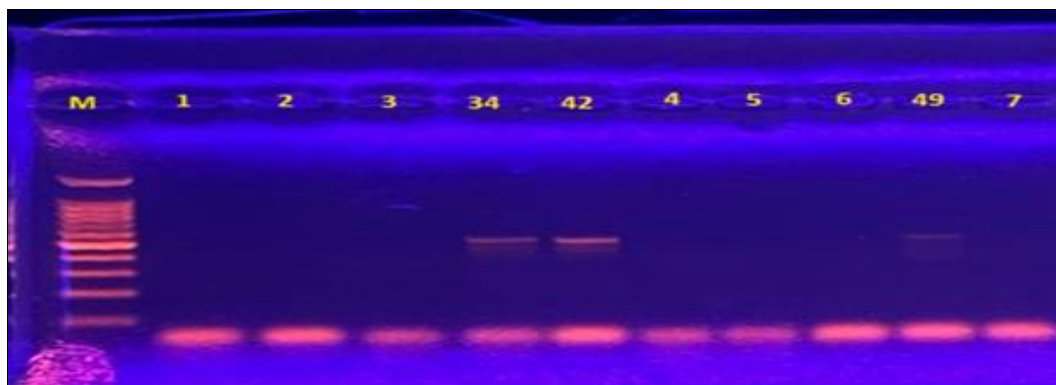


Fig 2: PCR detection of the *mecA* gene in three *S. aureus* isolates. Lane M: DNA ladder (100 bp); lanes 34, 42, 49, *S. aureus* (533 bp) positive isolates and 1,2,3,4,5,6,7 negative isolates negative.

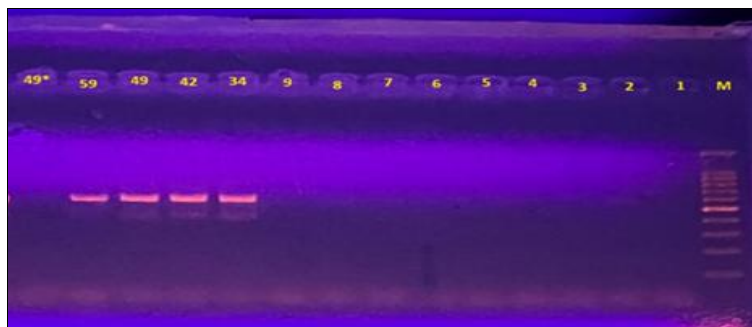


Fig 3: PCR detection of the Nuc gene in four *S. aureus* isolates. Lane M: DNA ladder (100 bp); lanes 34, 42, 49, 59, *S. aureus* (537 bp) positive isolates and 1, 2, 3, 4, 5, 6, 7, 8, 9 negative isolate.

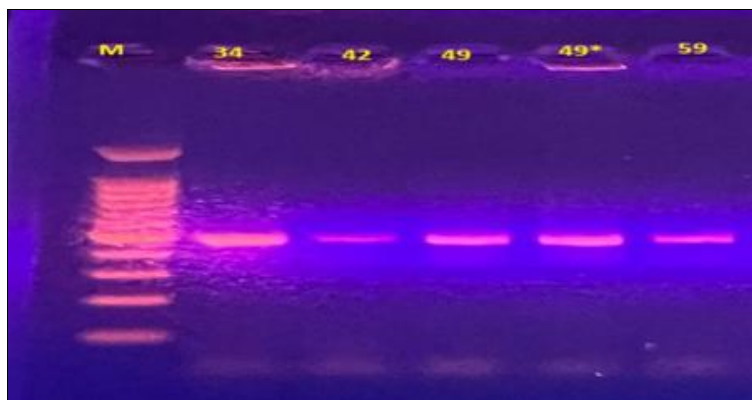


Fig 4: PCR detection of the Staphylokinase gene in five *S. aureus* isolates. Lane M: DNA ladder (100 bp); lanes 34, 42, 49, 49*, 59, *S. aureus* (492bp) positive isolates

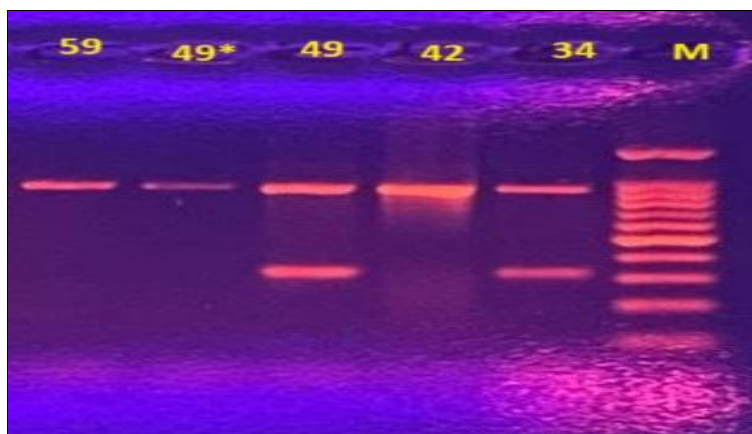


Fig 5: PCR detection of the Lipase gene in five *S. aureus* isolates. Lane M: DNA ladder (100 bp); lanes 34, 42, 49, 49*, 59, *S. aureus* (921 bp) positive isolates

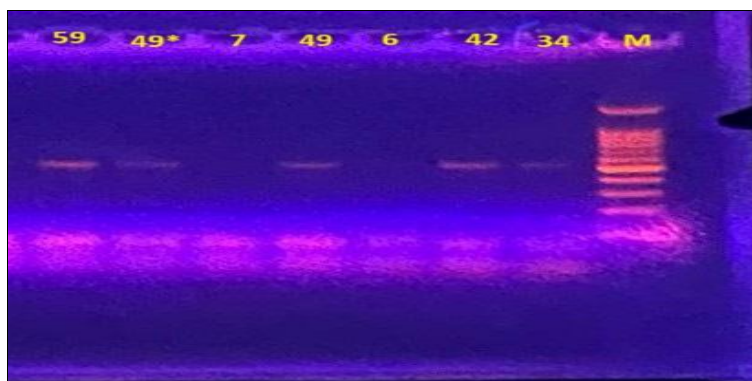


Fig 6: PCR detection of the Enterotoxin A gene in five *S. aureus* isolates. Lane M: DNA ladder (100 bp); lanes 34, 42, 49, 49*, 59, *S. aureus* (521 bp) positive isolates and 6, 7 negative isolates.

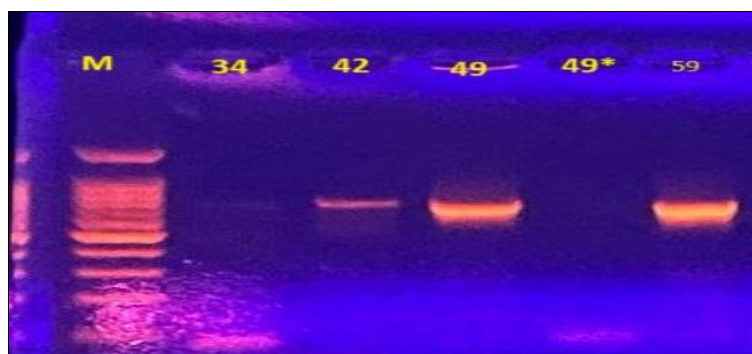


Fig 7: PCR detection of the invasive gene in three *S. aureus* isolates. Lane M: DNA ladder (100 bp); lanes 42, 49, 59, *S. aureus* (723 bp) positive isolates and 34,49* negative isolates.

Table 3: Virulence genes

Sample No	mecA	Lipase	Kinase	Nuclease	Invasive	Enterotoxin A
34	+	+	+	+	—	+
42	+	+	+	+	+	+
49	+	+	+	+	+	+
49*	—	+	+	—	—	+
59	—	+	+	+	+	+
Total	F.	%	F.	%	F.	%
	3	60.0	5	100.0	5	100.0

Note: In Table 3, the symbol F refers to the word Frequency, the symbol % refers to the percentage, the symbol + refers to the word positive, and the symbol - refers to the word negative.

Antibiotic Susceptibility Testing

All five isolates underwent antibiotic sensitivity testing against 19 antibiotics using both disc diffusion and the VITEK 2 system. The results showed high resistance to β -lactam antibiotics. Specifically, resistance to benzylpenicillin was found in 84.2% of isolates, oxacillin and cefoxitin screen each in 73.7%. These findings confirm the presence of MRSA strains. Moderate resistance was observed for clindamycin (31.6%), erythromycin (26.3%), and inducible clindamycin

resistance (26.3%). Low resistance was found for rifampicin (10.5%), tetracycline (5.3%), and trimethoprim/sulfamethoxazole (5.3%). Several antibiotics showed 100% sensitivity, including gentamicin, ciprofloxacin, moxifloxacin, linezolid, tigecycline, teicoplanin, fusidic acid, and fosfomycin. Vancomycin showed 94.7% sensitivity, with one isolate showing intermediate resistance. These results suggest that although MRSA strains are present, several antibiotics remain effective treatment options.

Table 4: Antibiotic Resistance in *Staphylococcus aureus*

Antibiotic	Resistance	Intermediate(I)	Susceptible
Cefoxitin screen	14(73.7%)	0(0%)	5(26.3%)
Benzylpenicillin	16(84.2%)	0(0%)	3(15.8%)
Oxacillin	14(73.7%)	0(0%)	5(26.3%)
Gentamicin	0(0%)	0(0%)	19(100%)
Ciprofloxacin	0(0%)	0(0%)	19(100%)
Moxifloxacin	0(0%)	0(0%)	19(100%)
Inducible Clindamycin Resistance	5(26.3%)	0(0%)	14(73.7%)
Erythromycin	5(26.3%)	1(5.3%)	13(68.4%)
clindamycin	6(31.6%)	0(0%)	13(68.4%)
Linezolid	0(0%)	0(0%)	19(100%)
Teicoplanin	0(0%)	0(0%)	19(100%)
Vancomycin	0(0%)	1(5.3%)	19(100%)
Tetracycline	1(5.3%)	0(0%)	18(94.7%)
Tigecycline	0(0%)	0(0%)	19(100%)
Fosfomycin	0(0%)	0(0%)	19(100%)
Fusidic acid	0(0%)	0(0%)	19(100%)
Rifampicin	2(10.5%)	0(0%)	17(94.5%)
Trimethoprim /Sulfamethoxazole	1(5.3%)	0(0%)	18(94.7%)

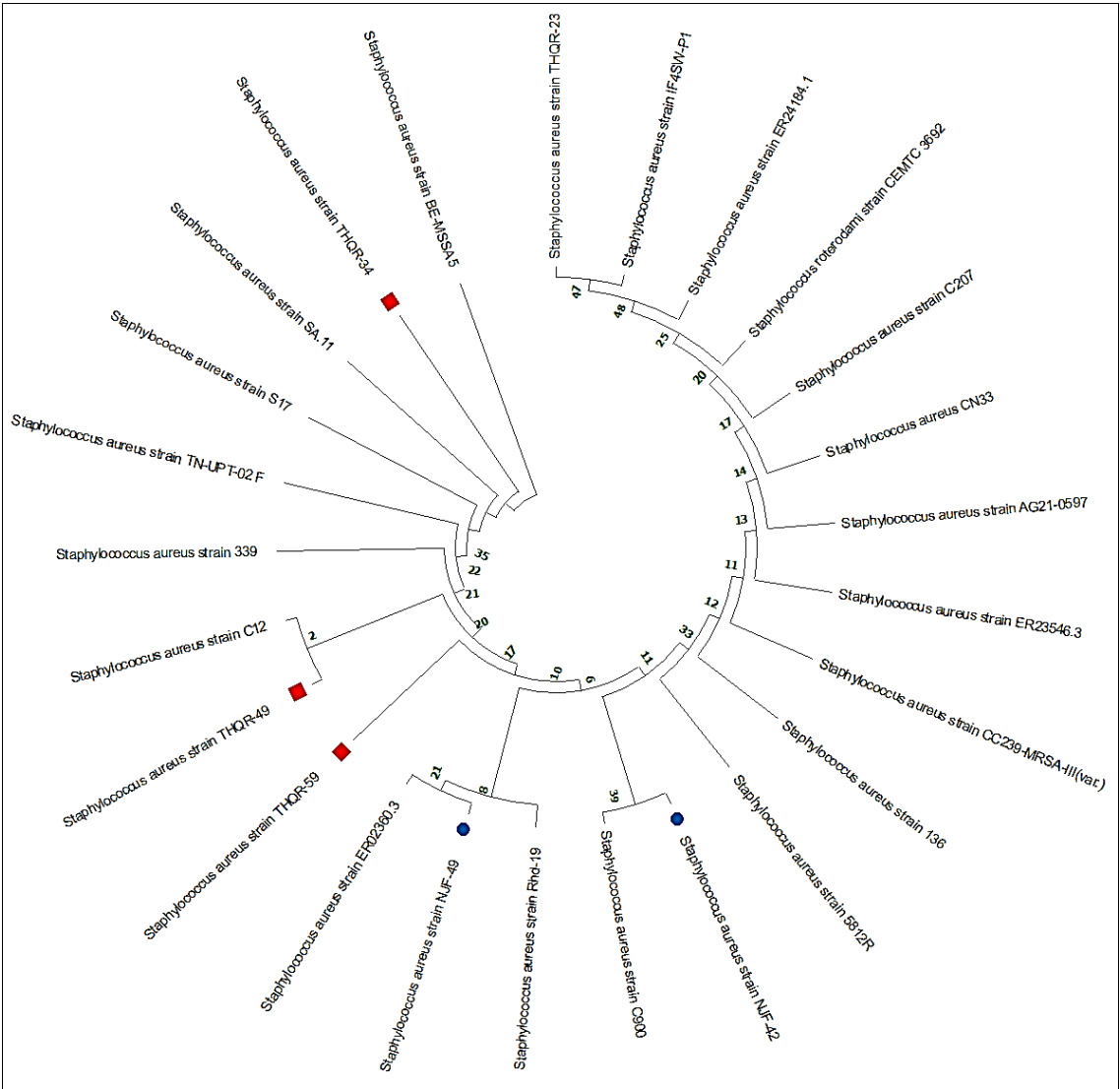
Phylogenetic relation ship

Phylogenetic analysis of the 16S rRNA sequences revealed genetic variation among the five *S. aureus* isolates. Strain NJF-42 clustered closely with strain C900, suggesting recent common ancestry. NJF-49 grouped with strain ER02360.3, forming a distinct clade and showing moderate divergence. THQR-59 was positioned near NJF-49, suggesting a related but separate evolutionary path. THQR-49 grouped with strain

C12, showing very close genetic similarity based on a short branch length. In contrast, THQR-34 formed its own unique branch, far from the others, indicating the highest divergence. These clustering patterns reflect different possible origins or evolutionary paths of the isolates, some possibly linked to hospital-acquired strains (MRSA), while others may represent distinct community-acquired or animal-associated lineages.

Table 5: NCBI related similar sequences to the current study isolates

No.	Sample of organism	strain	Accission N.
1.	<i>Staphylococcus aureus</i>	THQR-49	PQ163697
2.	<i>Staphylococcus aureus</i>	NJF-42	PQ163708
3.	<i>Staphylococcus aureus</i>	NJF-49	PQ163709
4.	<i>Staphylococcus aureus</i>	THQR-59	PQ163696
4.	<i>Staphylococcus aureus</i>	THQR-34	PQ163706



Note: The blue color in Figure 8 indicates the bacteria isolated and identified from the animal, while the blue color indicates the bacteria identified from the human.

Fig 8: Phylogenetic tree of *Staphylococcus aureus* isolates:

Discussion

The current study confirmed the presence of *Staphylococcus aureus* in both human and animal samples collected from Dhi Qar and Al-Najaf. Five isolates were identified through culture, biochemical testing, and confirmed by the VITEK 2 system. The high rate of resistance to β -lactam antibiotics, especially oxacillin and cefoxitin, indicated the presence of methicillin-resistant *S. aureus* (MRSA) strains. This matches previous findings showing the widespread presence of MRSA in clinical and animal sources (Abdulrazziq *et al.*, 2023) [2]. The *mecA* gene was detected in 60% of the *Staphylococcus aureus* isolates. This gene gives the bacteria strong resistance to methicillin and other beta-lactam antibiotics. It works by producing a special protein called PBP2a. This protein has low affinity for these drugs and allows the bacteria to grow even when antibiotics are present (Leseigneur *et al.*, 2024) [13].

These results match the high resistance found in the antibiotic sensitivity tests. The detection of *mecA* supports the presence of MRSA strains in both animal and human samples. MRSA is a serious problem because it spreads easily. It can move between animals and people through contact or contaminated environments. The misuse of antibiotics in livestock and veterinary practice increases this risk. Such settings create pressure that encourages the spread of resistant bacteria (Shahid *et al.*, 2021) [23]. PCR results also showed that all isolates carried three major virulence genes: staphylokinase, lipase, and Enterotoxin A. These genes help the bacteria cause disease in many ways. Staphylokinase helps the bacteria break down blood clots. It turns plasminogen into plasmin, which allows the bacteria to move through tissues and avoid immune cells (Noori & Aziz, 2020) [19]. Lipase breaks down fats in the host's cells. This

damages tissues and weakens immune defenses (Kitadokoro *et al.*, 2020) [12]. Enterotoxin A (SEA) causes food poisoning. It is one of the most common and dangerous toxins made by *S. aureus*. This toxin is active even if the bacteria are not growing quickly, because it is controlled by a phage (Yamanashi *et al.*, 2022) [26]. Finding these three genes in every isolate suggests that they are essential tools for *S. aureus* to survive and spread.

Two other virulence genes, *nuc* and invasive protease, were also detected. The *nuc* gene was found in four isolates. It produces an enzyme that cuts DNA and RNA. This helps the bacteria escape from neutrophil traps in the body. Because of its stability, the *nuc* gene is often used to confirm *S. aureus* in the lab (Alkhafajy & Al-Mathkhury, 2023) [3]. The invasive protease gene was found in three isolates. It helps the bacteria destroy host tissues and weaken the immune system (Marquart, 2021) [16]. The presence of these virulence genes varied among the isolates. Some strains had more, and some had fewer. This difference could be linked to how severe the infections were or where the bacteria came from. In general, isolates with more virulence genes may be more aggressive and dangerous.

The phylogenetic tree showed that the isolates were genetically different. Some isolates, like NJF-42, were closely related to known community strains such as C900. This suggests that they may have come from similar sources. Others, like THQR-34, were very different and formed their own separate group. This might mean they are part of new or uncommon strains. For example, THQR-49 and C12 were very close, while NJF-49 and THQR-59 showed moderate divergence. This diversity means that *S. aureus* in Iraq is not a single type. Some strains may come from hospitals, and others from the community or animal sources. Some may carry more resistance genes, while others carry different toxins. Continued gene sequencing is important to track these changes. It helps detect new threats and control the spread of dangerous strains, especially in places with strong human-animal interaction and high antibiotic use.

Conclusion

This study confirmed the presence of *Staphylococcus aureus* in both humans and animals in southern Iraq. Out of 14 samples, five were identified as *S. aureus*. Most of these showed strong resistance to key antibiotics, especially beta-lactams. The *mecA* gene, which causes methicillin resistance, was found in 60% of the isolates. All five isolates carried important virulence genes like staphylokinase, lipase, and Enterotoxin A. Other genes like *nuc* and invasive protease were also common. These genes help the bacteria enter tissues and avoid the immune system. Phylogenetic analysis showed that the isolates came from different genetic lines. This suggests that they may have different sources and could spread between animals and humans. These results show the need for responsible antibiotic use and regular monitoring of resistant *S. aureus* strains in both public health and veterinary settings.

Conflict of Interest

Not available

Financial Support

Not available

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