

Molecular Detection of the *Sea, seb, Sec, Sed, See, and Tst* Genes and Phylogenetic Tree in Coagulase Positive *Staphylococcus aureus* Isolated from Restaurants

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Submitted: February 12, 2025

Revised: March 23, 2025

Accepted: May 20, 2025

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Abstract Food safety has garnered significant global attention as foodborne illnesses emerge as a major public health threat. *Staphylococcus* (*S.*) *aureus* is a prominent foodborne pathogen worldwide and a common contaminant in various food products. *Staphylococcus aureus* produces a diverse array of toxins, including staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST), all of which exhibit emetic activity. The objectives of this study were to conduct the molecular detection of *S. aureus* isolated from restaurants in Erbil city, identify the genes encoding staphylococcal enterotoxins, and analyze the relationships among the strains using a phylogenetic tree. Three hundred fifty different samples were collected from four streets in Erbil, located in Iraq's Kurdistan Region, between August and November 2024. The results of the current study revealed that the prevalence rate of *S. aureus* was 43.4% (152/350). The prevalence varied by area, with rates of 38.8% on the 30-meter street, 50% on the 40-meter street, 36.7% on the 60-meter street, and 48% on the 100-meter street. Additionally, it was discovered that each *S. aureus* isolate possessed 100% *nuc* and *coa* genes. The prevalence of *S. aureus* isolates carrying the *sea*, *seb*, *sec*, and *tst* genes was 82.5%, 17.5%, 75%, and 32.5%, respectively. However, none of the isolates contained the *sed* or *see* genes. *Staphylococcus aureus* isolates were classified into 11 distinct gene profiles. The most prevalent gene profile, comprising (*nuc* + *coa* + *sea* + *sec*), was observed in 42.5% of the isolates. Based on the *seb* gene, six unique strains of *S. aureus* have been registered in GenBank. Phylogenetic analysis revealed that these newly identified isolates share high similarities with previously characterized *S. aureus* strains from various regions worldwide. Consequently, it is both a significant challenge and an urgent priority to rapidly and accurately detect and identify SEs in *S. aureus* isolated from food. This is crucial for legislative and non-legislative organizations, including martial entities, medical departments, and healthcare facilities. In this context, a comprehensive survey was conducted to provide an overview of SEs detection across various food types available in supermarkets.

Keywords: *S. aureus*, meat, Staphylococcal enterotoxins, phylogenetic tree

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Introduction *Staphylococcus* (*S.*) *aureus* is one of the most common causative agents of foodborne infections in many countries (1, 2), and it causes foodborne bacterial intoxications globally (3). *Staphylococcus aureus* possesses several virulence factors, including thermonuclease, hyaluronidase, lipases, and hemolysin. These factors are important

for host tissue invasion and contribute to pathogenesis in humans (4, 5). The most critical virulence factors in *S. aureus* are the enterotoxins, which are responsible for food poisoning in humans (6). Additionally, *S. aureus* produces various types of enterotoxins, classified into two categories: staphylococcal enterotoxins (SEs) and SE-like toxins



(SEIs). These toxins are considered major virulence factors of *S. aureus*, particularly concerning food safety. Together, they form a superfamily comprising 23 distinct types, including both SEs and SEIs (7). Staphylococcal enterotoxins (SEs) consist of 23 identified enterotoxins, each recognized as a distinct serological entity, including SEA, SEB, SEC, SED, and SEE (8). The staphylococcal enterotoxins SEA and SEB are the most prominent, with SEA being the toxin most frequently associated with food poisoning in humans caused by *S. aureus* (9). SEB, on the other hand, is not only associated with food poisoning but has also been recognized as a potential biological weapon in warfare and terrorism (10). Staphylococcal food poisoning (SFP) is an intoxication caused by the consumption of foods that contain significant amounts of various types of enterotoxins (11). The most notable clinical signs of SFP have a rapid onset, typically within 2–8 hours, and include nausea, severe nausea, abdominal cramping, and sometimes diarrhea (12, 13). *Staphylococcus aureus* is considered a significant bacterium capable of contaminating food through workers' hands, tools, or food scraps during preparation (14). According to numerous earlier studies, *S. aureus* is isolated from different types of foods, including meat (15), fish (16), cow's milk (17), and camel milk (18). All types of food, especially meat and cooked meat products like ham, are linked to the emergence of staphylococcal poisoning outbreaks due to contamination by *S. aureus* (19). In recent decades, food synthesis from animals such as pigs, cattle, chickens, and others has become more exposed to contamination by *S. aureus* (20). Foods with high protein content often require extensive handling, typically involving improper heating and/or storage, which increases the risk of contamination (11). Furthermore, meat is highly susceptible to spoilage and is commonly associated with the transmission of foodborne illnesses. Contamination of meat and its products is considered a major cause of foodborne diseases (21). Meat contamination can begin at the farm stage, where animals come into direct contact with contaminated surfaces. The second stage of contamination occurs in the slaughterhouse during slaughtering, evisceration, and storage. Finally, meat can be further contaminated during transmission, processing, and cooking. Several methods are used for the isolation and detection of *S. aureus*, including traditional techniques and molecular biology-based approaches.

Conventional methods are based on biochemical testing and the morphological characterization of *S. aureus* colonies. In contrast, molecular methods are faster and more accurate, providing results for *S. aureus* detection within three to five hours by targeting the genome of *S. aureus* isolates (22).

The goals of the current study are to identify the pathogen *S. aureus* in various samples collected from restaurants, to detect the *sea*, *seb*, *sec*, *sed*, *see*, and *tst* genes in *S. aureus* isolates using PCR assays, and to investigate the correlation between the *S. aureus* isolates in this research and those isolated from other countries.

Materials and methods

Ethical approval

All samples were collected with the owners' consent and utilized in accordance with the ethical guidelines established by the Institutional Animal Care and Use Committee (IACUC) at Mosul University's College of Veterinary Medicine, under the approved ID UM. Vet.2024.047.

Samples Collection

A total of 350 samples (including knives, meat, tables, hands, and machines) were collected from various locations in Erbil, in the Kurdistan Region of Iraq, specifically from 30, 40, 60, and 100-meter streets. The study commenced in August 2024 and concluded in November 2024. All types of samples were collected using sterile swabs and placed in sanitary containers before being promptly transported to the Health Laboratory at the Faculty of Veterinary Medicine, Mosul University. All peptone water containers were placed in an incubator for a pre-enrichment process at 37°C for 18 to 24 hours.

S. aureus Isolation and Characterization

Phenotypic examination, coagulase and catalase activity tests, and Gram staining were employed to investigate and determine the phenotypic characterizations of the *S. aureus* colonies in accordance with the conventional procedures used for the isolation and identification of *S. aureus* colonies. (23).

Isolation of DNA

To extract the genomic DNA of *S. aureus*, the positive isolates were cultured on Mannitol Salt Agar for over eight hours at 37°C. The DNeasy Blood and Tissue Kit (Addbio, Korea) was used to isolate DNA following the manufacturer's instructions, specifically tailored for Gram-positive bacteria. Subsequently, the DNA concentration was measured using a Nanodrop

spectrophotometer (Jenway, UK). The DNA concentration of *S. aureus* isolates ranged from 18.5 µg/µl to 50 µg/µl. The extracted DNA was stored at -20°C.

Reaction of PCR

The *nuc*, *coa*, *sea*, *seb*, *sec*, *sed*, *see*, and *tst* genes of *S. aureus* were discovered by the PCR technique. The molecular weight of the *nuc* gene was 166 bp (24), *coa* is 674 bp (25), *sea* is 219 bp (26), *seb* is 478 bp (27), *sec* is 257 bp (27), *sed* is 317 bp (27), *see* is 171 bp (28), and *tst* is 559 bp (29). The total volume of the PCR reaction was 25 µl, and the mixture was prepared in a 200 µl tube (Biozym, Germany). The resulting amplicons were analyzed through gel electrophoresis on a 2% agarose gel (Peqlab, Germany), using a 100 bp ladder as a reference. The reaction mixture comprised 6.5 µl of DNeasy-free water (Promega Corporation, USA) and 4 µl of the *S. aureus* DNA template, 12.5 µl of the GoTaq Mix Master (2x) (Addbio company, Korea), 1 µl of each primer (primer 1 and primer 2).

Table 1: Various Primers used in PCR programs for detecting the genes of *S. aureus*

Gene	Primer	Sequence (5'-3')	Amplicon size [bp]	PCR Programme*	Reference
<i>nuc</i>	nuc-1	5-CCTGAAGCAAGTGCATTACGA-3	166	I	(24)
	nuc-2	5-CTTTAGCCAAGCCTTGACGA-3			
<i>coa</i>	coa-1	5-ATAGAGATGCTGGTACAGG-3	674-917	I	(25)
	coa-2	5-GCTTCCGATTGTCGATGC-3			
<i>sea</i>	SEA-1	5-AAAGTCCCGATCAATTTATGGCTA-3	219	I	(26)
	SEA-2	5-GTAATTAACCGAAGTTCTGTAGA-3			
<i>seb</i>	SEB-1	5-TCGCATCAAAGTACAAACG-3	478	I	(27)
	SEB-2	5-GCAGGTACTCTATAAGTGCC-3			
<i>sec</i>	SEC-1	5-GACATAAAAGCTAGGAATTT-3	257	III	(27)
	SEC-2	5-AAATCGGATTAACATTATCC-3			
<i>sed</i>	SED-1	5-CTAGTTTGGTAATATCTCCT-3	317	III	(27)
	SED-2	5-TAATGCTATATCTTATAGGG-3			
<i>see</i>	SEE-1	5-TACCAATTAACCTGTGGATAGAC-3	171	I	(28)
	SEE-2	5-CTCTTTGACCTTACCGC-3			
<i>tst</i>	TSST-1	5-GCTTGCACAAGTCTACAG-3	559	I	(29)

	TSST-2	5-TGGATCCGTCATTTCATTGTTAT-3			
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PCR program: *I: 35 times (94°C – 30s, 55°C – 30s, 72°C – 30s); II: 35 times (94°C – 30s, 60°C – 30s, 72°C – 30s); III: 35 times (94°C – 30s, 53°C – 30s, 72°C – 30s).

DNA sequencing

The isolated DNA was sent to Macrogen, a commercial sequencing company based in South Korea, for purification and sequencing of six PCR amplicons obtained from this study. These amplicons had already been confirmed as positive for *S. aureus* isolates using conventional PCR. The *seb* gene was selected as the target gene for sequencing. The obtained sequences for the *seb* gene were then compared using the NCBI BLASTn software, available at <http://www.ncbi.nlm.nih.gov>, with previously described *S. aureus* sequences available in GenBank. Further alignment and comparison of these sequences were performed using the multiple sequence alignment program CLUSTALW, available in MegAlign program. Phylogenetic trees were generated using the Neighbor-Joining (NJ) method and the CLUSTALW tool from Genome Net. To enhance robustness, 500 duplicate sequences of the *S. aureus seb* gene were included as an outgroup when constructing the phylogenetic tree. Through purification, sequencing, and subsequent bioinformatics analysis, this comprehensive approach aimed to clarify the genetic relationships among the *S. aureus* isolates and deepen the understanding of the phylogenetic context of the isolates from sheep milk.

Results

The present study revealed that *S. aureus* colonies exhibited a golden-yellow color on Mannitol Salt Agar, indicating positive identification. Additionally, specific biochemical tests, including coagulase and catalase assays, confirmed the presence of *S. aureus* with positive results. The concentration of extracted DNA from *S. aureus* isolates ranged from 18.5 mg/µl to 30 mg/µl (Figure 3). PCR analysis further validated that the *nuc* gene found in all *S. aureus* isolates, checking their individuality as *S. aureus* (Figure 4). According to the results, the prevalence of *S. aureus* isolated from restaurants in Erbil city was 43.4% (152/350). The high percentage of *S. aureus* isolated was 50% (40/80) in a 40-meter street. Following, the prevalence rate of *S. aureus* in a 60-meter street, 30-meter street, and 100-meter street was 48% (48/100), 38.8% (31/80), and 36.7% (33/90) respectively, as summarized in Table 2. Additionally, the highest occurrence rate of *S. aureus*

isolated from restaurants was found on hands, tables, and meat, at 44.3% (31/70). In contrast, the lowest incidence rates were observed on knives 41.4% (29/70) and machines 42.9% (30/70), as presented in Table 3.),

Table 2: The percentage rate of *S. aureus* detected in different street in Erbil city

Name of street	No. of samples	No. of positive <i>S. aureus</i>	Percentage (%)
30-Meter Street	80	31	38.8%
40-Meter Street	80	40	50%
60-Meter Street	100	48	48%
100-Meter Street	90	33	36.7%
Total	350	152	43.4%

Table 3: Comparative of percentage of *S. aureus* isolated from different Street in Erbil city

Type of sample	30-meter street	40-meter Street	60-meter Street	100-meter street	Total
Hand	37.5% (6/16)	50% (8/16)	33.3% (6/18)	55% (11/20)	44.3% (31/70)
Table	37.5% (6/16)	62.5% (10/16)	33.3% (6/18)	45% (9/20)	44.3% (31/70)
Machine	31.3% (5/16)	50% (8/16)	38.9% (7/18)	50% (10/20)	42.9% (30/70)
Knife	43.8% (7/16)	50% (8/16)	22.2% (4/18)	50% (10/20)	41.4% (29/70)
Meat	43.8% (7/16)	37.5% (6/16)	55.6% (10/18)	40% (8/20)	44.3% (31/70)
Total					

Figure 1: Comparative prevalence rate of *S. aureus* isolated from restaurants in various areas in Erbil city

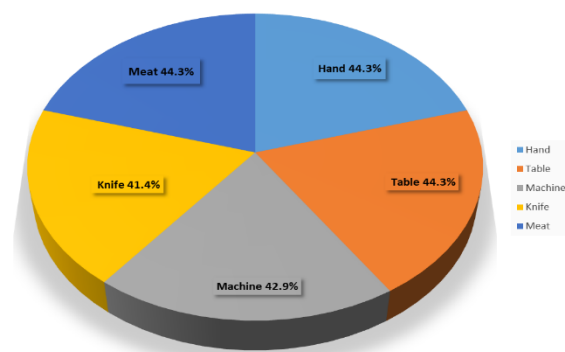


Figure 2: The prevalence rate of *S. aureus* found in difference types of samle

According to Table 4, the results of the PCR assay corroborated the findings of traditional methods, the *nuc* and *coa* genes detected in 100% (40/40) of *S. aureus* isolates (Figures 4 and 5). Additionally, the prevalence of *S. aureus* isolates carrying the *sea*, *seb*, *sec*, and *tst* genes was 82.5% (33/40), 17.5% (7/40), 75% (30/40), and 32.5% (13/40), respectively (Figures 6, 7, 8, and 11). In contrast, the *S. aureus* isolates lacked the *sed* and *see* genes (Figures 9 and 10). Moreover, the results of the current study revealed that *S. aureus* isolates were divided into 11 distinct gene profiles based on the presence of specific genes in each isolate (Table 5). Among these, the most frequently observed gene profile was Profile V (*nuc* + *coa* + *sea* + *sec*), accounting for 42.5% (17/40) of the isolates. Conversely, Profile I, which contained the most genes (*nuc* + *coa* + *sea* + *seb* + *sec* + *tst*), was observed in 7.5% (3/40) of the isolates. Meanwhile, the gene profiles II, VI, VII, and IX were less frequent, each accounting for 2.5% (1/40) of the isolates.

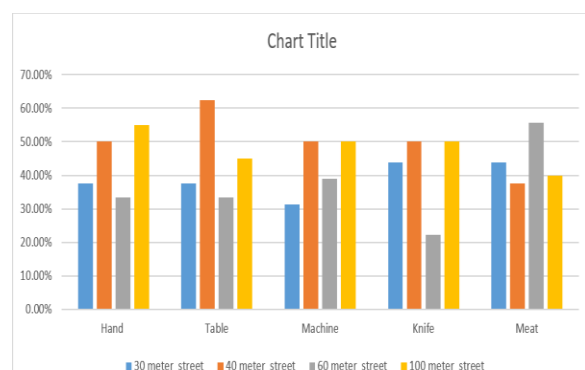


Table 4: The number and percentage of the genes found in *S. aureus* isolates

Gene	<i>S. aureus</i>	
	Number	Percentage (%)
1. <i>nuc</i>	40	100%
2. <i>coa</i>	40	100%

3. sea	33	82.5%
4. seb	7	17.5%
5. sec	30	75%
6. sed	0	0%
7. see	0	0%
8. tst	13	32.5%

Table 5: Types of the gene profiles of *S. aureus* (n = 40) from sheep milk

Genes profile	Staphylococcus genes	Isolates	
		n	%
I	<i>nuc + coa + sea + seb + sec + tst</i>	3	7.5%
II	<i>nuc + coa + sea + seb + sec</i>	1	2.5%
III	<i>nuc + coa + sea + sec + tst</i>	6	15%
IV	<i>nuc + coa + sea + seb + tst</i>	2	5%
V	<i>nuc + coa + sea + sec</i>	17	42.5%
VI	<i>nuc + coa + sea + seb</i>	1	2.5%
VII	<i>nuc + coa + sec + tst</i>	1	2.5%
VIII	<i>nuc + coa + sea</i>	4	10%
IX	<i>nuc + coa + tst</i>	1	2.5%
X	<i>nuc + coa + sea</i>	2	5%
XI	<i>nuc + coa</i>	2	5%

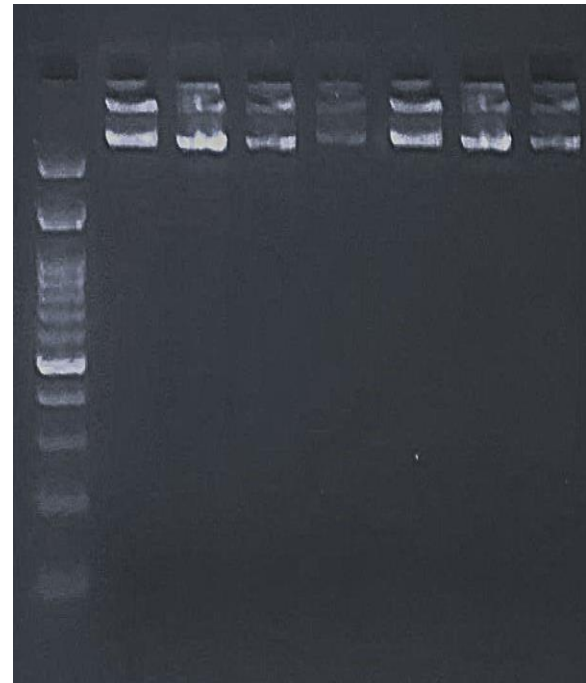


Figure 3: Visualization and comparative concentration of *S. aureus* whole genome DNA using agarose gel electrophoresis

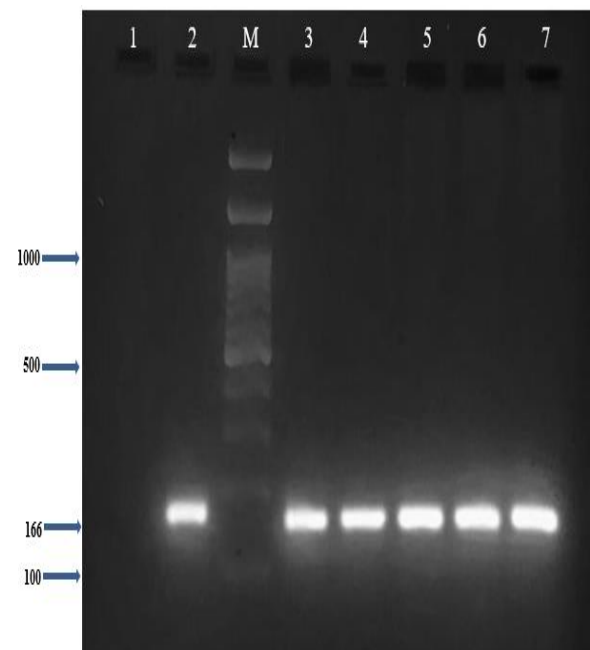


Figure 4: The molecular weight of the *nuc* gene in *S. aureus* was 166 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder.

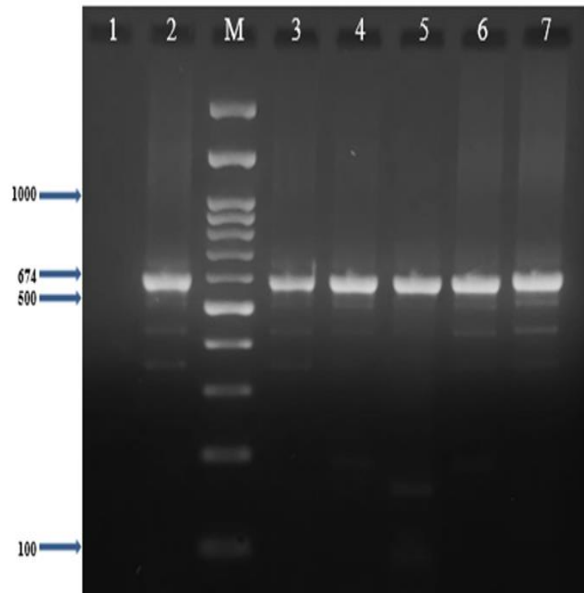


Figure 5: Figure 1: The molecular weight of the *coa* gene in *S. aureus* was 674 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder.

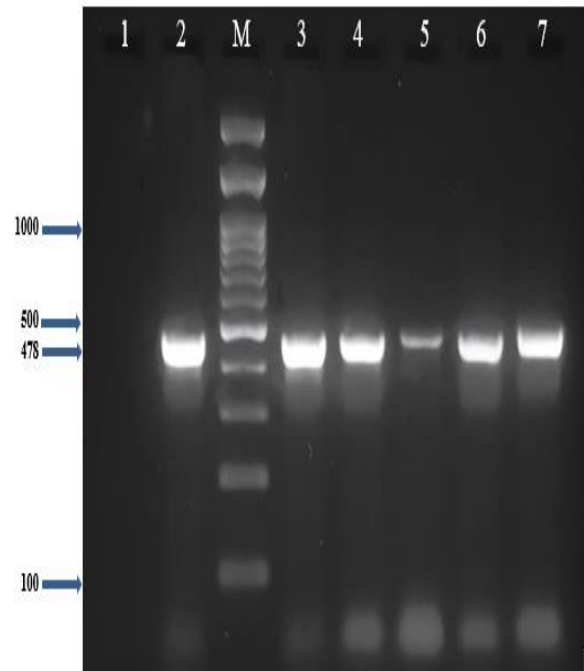


Figure 7: The molecular weight of the *seb* gene in *S. aureus* was 478 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder.

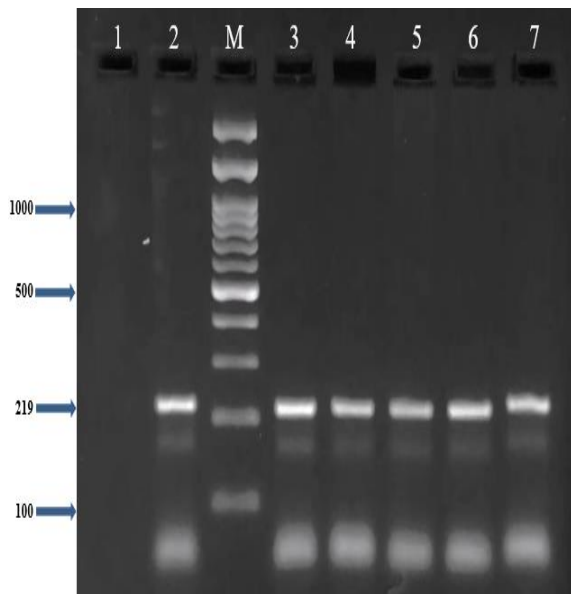


Figure 6: The molecular weight of the *sea* gene in *S. aureus* was 219 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder.

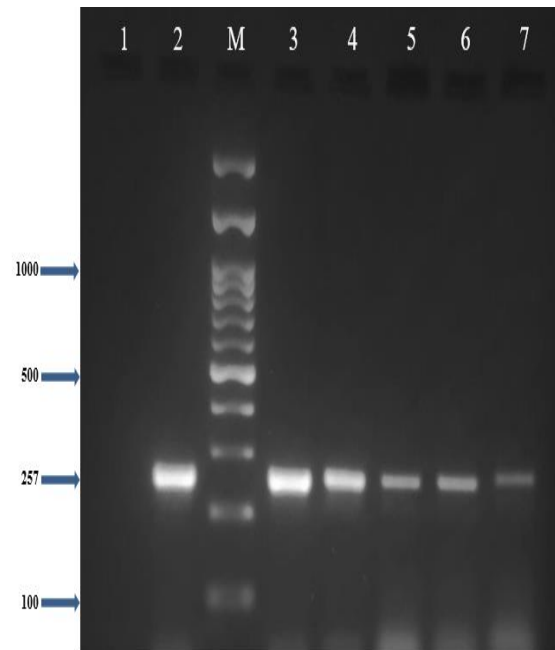


Figure 8: The molecular weight of the *sec* gene in *S. aureus* was 257 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder.

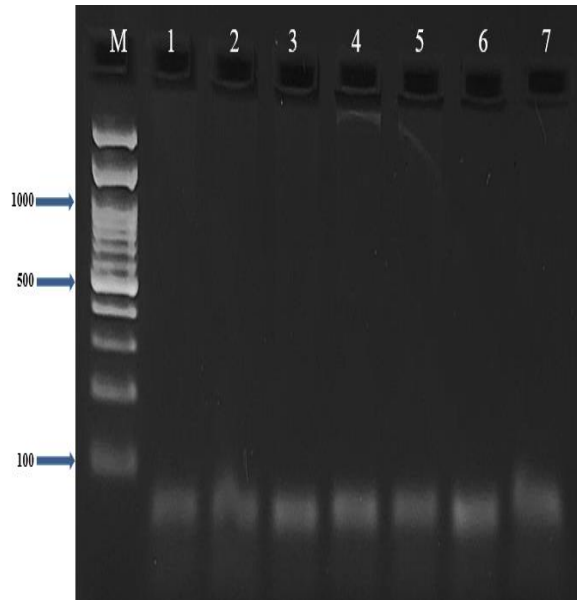


Figure 9: The molecular weight of the sed gene in *S. aureus* was 317 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder. None of *S. aureus* have the sed gene.

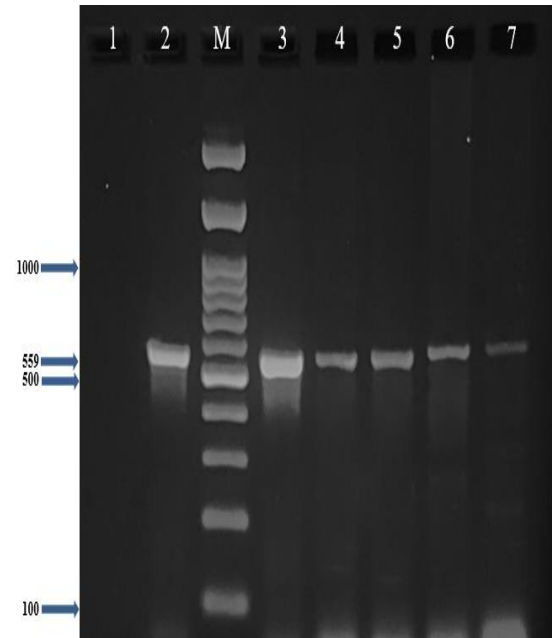


Figure 11: The molecular weight of the tst gene in *S. aureus* was 559 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder

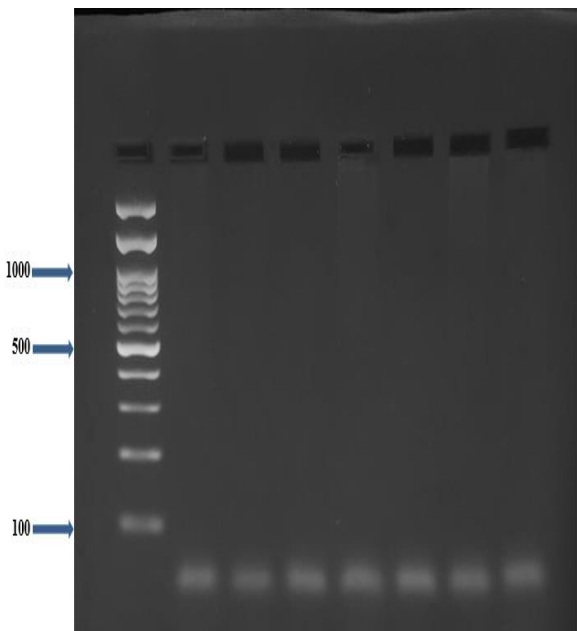


Figure 10: The molecular weight of the see gene in *S. aureus* was 171 bp based on Agarose Gel Electrophoresis (2%). The DNA amplification results in a banding pattern resembling a ladder. None of *S. aureus* isolates possess the see gene

Accession numbers of the gene	Bacteria	Gene	Types of samples
PQ766555	<i>S. aureus</i>	<i>seb</i>	Meat
PQ766556	<i>S. aureus</i>	<i>seb</i>	Meat
PQ766557	<i>S. aureus</i>	<i>seb</i>	Meat
PQ766558	<i>S. aureus</i>	<i>seb</i>	Meat
PQ766559	<i>S. aureus</i>	<i>seb</i>	Meat
PQ766560	<i>S. aureus</i>	<i>seb</i>	Meat

The individual sequencing analysis (BLASTn) was performed on six novel *seb* gene sequences obtained from meat samples collected from restaurants, aligning with the sequencing results of this study. Additionally, table 6 indicates that the NCBI GenBank

contains *S. aureus* sequences indexed under the following accession numbers: PQ766555, PQ766556, PQ766557, PQ766558, PQ766559, and PQ766560. Furthermore, a maximum likelihood phylogenetic tree analysis using the MegAlign program revealed significant variations in the local gene sequences compared to previously identified sequences listed in the GenBank database. As shown in Figure 12, the relationship between the sequences from this study (PQ766555, PQ766556, PQ766557, PQ766558, PQ766559, and PQ766560) and the sequences registered in GenBank from various regions worldwide ranged from 99.3% to 100% (Figure 12 and Table 6).

Table 6: The NCBI GenBank accession numbers for the *seb* gene of *S. aureus* sequences in meat

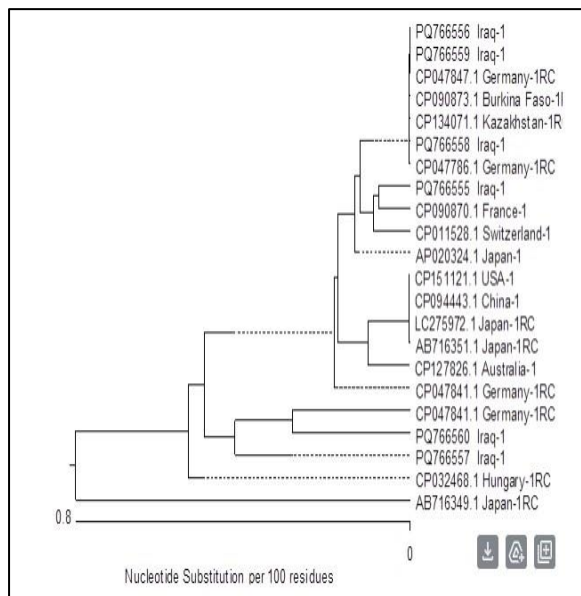


Figure 12: Clustering analysis of *S. aureus* isolates' gene sequences recovered from NCBI GenBank, the NCBI accession number is indicated by the designation in parenthesis

Table 7: DNASTAR's calculation of each pair's gene sequence difference and similarity for *S. aureus*

Sequence Distances of Omar.12.2024.meg ClustalW (Slow/Accurate, IUB)																								
		Percent Identity																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Divergence	1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	CP047841.1 Germany-1RC.seq
	2	0.0	100.0	100.0	100.0	99.9	99.9	99.9	100.0	99.3	99.9	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	CP134071.1 Kazakhstan-1RC.seq
	3	0.0	0.0	100.0	100.0	99.9	99.9	99.9	100.0	99.3	99.9	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	CP090873.1 Burkina Faso-1RC.seq
	4	0.0	0.0	0.0	100.0	99.9	99.9	99.9	100.0	99.3	99.9	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	CP047847.1 Germany-1RC.seq
	5	0.0	0.0	0.0	0.0	100.0	99.9	99.9	100.0	99.3	99.9	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	CP047786.1 Germany-1RC.seq
	6	0.7	1.2	1.2	1.2	1.2	100.0	99.9	99.3	99.3	99.3	99.9	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	AB716349.1 Japan-1RC.seq
	7	0.0	0.2	0.2	0.2	0.2	1.4	100.0	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	LC275972.1 Japan-1RC.seq
	8	0.0	0.2	0.2	0.2	0.2	1.4	0.0	100.0	99.9	99.9	99.9	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	AB716351.1 Japan-1RC.seq
	9	0.0	0.0	0.0	0.0	0.0	0.7	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	CP032468.1 Hungary-1RC.seq
	10	0.0	0.7	0.7	0.7	0.7	1.9	0.9	0.9	0.9	0.9	99.1	99.3	100.0	99.3	99.3	99.1	100.0	99.1	99.3	99.3	100.0	100.0	PQ766560 Iraq-1 seq
	11	0.0	0.2	0.2	0.2	0.2	1.4	0.5	0.5	0.9	0.9	99.6	100.0	99.9	99.9	99.9	99.9	100.0	99.5	100.0	100.0	100.0	100.0	PQ766555 Iraq-1 seq
	12	0.0	0.0	0.0	0.0	0.0	1.2	0.2	0.2	0.7	0.7	0.4	100.0	100.0	100.0	99.9	100.0	99.9	100.0	100.0	100.0	100.0	100.0	PQ766556 Iraq-1 seq
	13	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	PQ766557 Iraq-1 seq
	14	0.0	0.0	0.0	0.0	0.0	1.2	0.2	0.2	0.7	0.7	0.2	0.0	0.0	100.0	99.9	100.0	99.9	100.0	100.0	100.0	100.0	100.0	PQ766558 Iraq-1 seq
	15	0.0	0.0	0.0	0.0	0.0	1.2	0.2	0.2	0.7	0.7	0.2	0.0	0.0	99.9	100.0	99.9	100.0	100.0	100.0	100.0	100.0	100.0	PQ766559 Iraq-1 seq
	16	0.0	0.2	0.2	0.2	0.2	1.4	0.0	0.0	0.9	0.5	0.2	0.0	0.2	0.2	100.0	100.0	99.9	99.9	100.0	100.0	100.0	100.0	CP151121.1 USA-1
	17	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	CP127826.1 Australia-1 seq
	18	0.0	0.2	0.2	0.2	0.2	1.4	0.0	0.0	0.9	0.5	0.2	0.0	0.2	0.0	0.0	99.9	99.9	100.0	100.0	100.0	100.0	100.0	CP094443.1 China-1 seq
	19	0.0	0.0	0.0	0.0	0.0	1.2	0.2	0.2	0.7	0.7	0.0	0.0	0.0	0.0	0.2	0.0	0.2	100.0	100.0	100.0	100.0	100.0	CP090870.1 France-1 seq
	20	0.0	0.0	0.0	0.0	0.0	1.2	0.2	0.2	0.7	0.7	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	100.0	100.0	100.0	100.0	CP011528.1 Switzerland-1 seq
	21	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	CP047841.1 Germany-1RC.seq
	22	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	AP020324.1 Japan-1 seq
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	

Discussion

Meat is a major source of protein globally, as it delivers necessary nutrients such as amino acids, iron, phosphorus, and B-complex vitamins, among many others, required for human health (30). *Staphylococcus aureus* is often identified organisms from meat processing facilities, contact and noncontact surfaces, raw materials, and various types of products (31). The presence of *S. aureus* in food poses a substantial risk for foodborne illnesses, as it can produce a variety of enterotoxins (32). The findings revealed a great occurrence of *S. aureus*, accounting for 43.4% (152/350) of the samples. Furthermore, the incidence of *S. aureus* in meat collected from restaurants was 44.3% (31/70). Several investigations in the literature have documented foodborne outbreaks caused by *S. aureus*. However, these studies report highly variable findings regarding the occurrence of *S. aureus* in foods. The prevalence of *S. aureus* isolated from food in Spain was reported to be 48.4% (31/64) (33), which closely corresponds to the results of the current study. Additionally, the findings of this study were lower than those reported in previous studies, including 85% in the USA (34), 74.8% in Korea (35), and 66.67% in India (36). Furthermore, the findings of this study were greater than those reported in previous studies, such as 5% in Turkey (37), 6% in Portugal (28), and 7.14% in India (38). The variation in the prevalence of *S. aureus* may be attributed to several factors, including



contamination during the transport of animals from farms to slaughterhouses, prolonged contact between healthy and infected animals in abattoirs, evisceration caused by intestinal contents and the water used for washing and rinsing carcasses, as well as poor hygiene practices during the handling, transportation, and storage of carcasses for subsequent use (39). In restaurants, workers play a significant role in cross-contaminating hands and meat during handling. Practices such as not wearing gloves, infrequent handwashing, and wearing unclean clothing can significantly contribute to increased bacterial contamination (40). However, reducing the presence of *S. aureus* and other harmful bacteria in restaurants can be accomplished by properly washing and sanitizing equipment and tools both before and after they are used for handling meat (41, 15). Additionally, variations in study results may be attributed to differences in sampling strategies, isolation methods, carcass sampling locations, types of meat cuts analyzed, contamination during and after slaughter, and meat storage and processing practices (42). In this investigation, a PCR assay was employed to detect various genes, including the *nuc* gene, which was specifically used to identify *S. aureus* isolates. PCR assay is recognized as a highly sensitive and reliable method for distinguishing coagulase-positive *S. aureus* from other coagulase-negative *Staphylococcus* species (43, 44). Additionally, the present study demonstrated that *S. aureus* possesses the *nuc* gene, aligning with previous research findings that confirmed all coagulase-positive *S. aureus* strains carry the *nuc* gene (45). Furthermore, this study found that the *coa* gene was present in 100% of the *S. aureus* isolates, suggesting its crucial role in the bacterium's pathogenicity. The findings of this study align with previous research that reported all *S. aureus* isolates harbor the *coa* gene, albeit with varying molecular weights, including 514 bp, 595 bp, 757 bp, and 802 bp (46). A different investigation, however, found that the *coa* gene, which consistently had a molecular weight of 580 bp, was present in every *S. aureus* isolate (47). Numerous studies have identified food-borne staphylococcal enterotoxins as significant contributors to staphylococcal food poisoning. The presence of enterotoxigenic *S. aureus* in food represents a potential risk for the occurrence of staphylococcal food poisoning. In addition, the prevalence rates of the *sea*, *seb*, *sec*, and *tst* genes in *S. aureus* isolates were 82.5%, 17.5%, 75%, and 32.5%,

respectively. Conversely, none of the *S. aureus* isolates contained the *sed* or *see* genes. When *S. aureus* isolates are genotypically characterized, their genetic composition is examined to pinpoint particular genes linked to their virulence, such as several enterotoxin genes (*sea*, *seb*, *sec*, etc.) that are linked to food poisoning. Numerous previous studies have indicated that the prevalence rates of staphylococcal enterotoxin genes in *S. aureus* vary among isolates. For instance, in China, *S. aureus* isolated from retail meat exhibited the following prevalence rates: 8.7% for the *sea* gene, 52.2% for *seb*, 4.3% for *sec*, and 43.5% for *sed* (48). Meanwhile, *S. aureus* isolated from chicken meat showed the following prevalence rates: 9% for the *sea* gene, 6.9% for *seb*, 6.9% for *sec*, 4.8% for *sed*, and 2.4% for *see* (49). In addition, the highest prevalence rates of staphylococcal enterotoxin (SE) genes detected in *S. aureus* isolates from retail chicken meat in India were *seb* (80.95%), *sec* (14.29%), and *sed* (9.53%) (36). Furthermore, the highest prevalence rates of SE genes detected in *S. aureus* isolates from beef meat were *sea* (72.22%), *seb* (16.67%), and *tst* (5.56%), while those from mutton meat were *sea* (70%), *seb* (15%), and *tst* (10%) (50). Furthermore, the highest prevalence rates of SE genes detected in *S. aureus* isolates from food in France were *sea* (53.6%) and *seb* (37.5%) (6). Variations in genotypic profiles—such as differences in molecular weights, gene prevalence, or genetic mutations—offer information about the diversity of *S. aureus* strains, their potential for pathogenicity in accordance with their geographic (51, 52).

Conclusion

The study was extensive and covered diverse geographic regions across Erbil City, Iraq. *Staphylococcus aureus* was isolated from meat in restaurants, suggesting that contamination occurred during slaughter, handling, transport, or storage under unhygienic conditions. In addition, *S. aureus* was isolated from utensils used in restaurants, indicating that contaminated utensils contributed to the spread of *S. aureus* and the contamination of meat with pathogenic bacteria. In addition, many restaurants may use unpackaged meat or store it at non-refrigerated temperatures, creating conditions that promote bacterial growth and multiplication, leading to potential food poisoning for consumers. Furthermore, the presence of *S. aureus* on utensils



used in restaurants indicates that the utensils were not washed, cleaned, or sterilized frequently enough, leading to the contamination of meat with *S. aureus*, which poses a significant health risk to people. The *S. aureus* isolates carried various types of genes encoding virulence factors, indicating differences in the sequence types of *S. aureus* isolated in this study.

Acknowledgment

We sincerely extend our gratitude to the College of Veterinary Medicine at the University of Mosul for providing the essential resources that made this research possible.

Conflict of interest

The author of this manuscript declares no conflicts of interest regarding the preparation or analysis of this study.

Funding source

This research had no specific fund; however, it was self-funded by the authors.

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