



Phenotypic and genetic study of *Staphylococcus aureus* isolated from of different animal meats in Mosul abattoir, Iraq

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Abstract

Staphylococcus aureus (*S. aureus*) is one of the most important foodborne pathogens. The study aimed to isolate the bacterium from the meat of different animals' species in Mosul abattoir and to study the phenotypic and genetic characteristics and identify *S. aureus* during the period from August 26 to November 20, 2024. The results of the characteristics showed that 131/270 bacteria were isolated, with a total isolation rate of 48.5% of all studied meat samples. Polymerase chain reaction (PCR) was used to detect the enterotoxin genes of these bacteria. This technique was applied to 46 random isolates to identify some virulence factors of *S. aureus* (*Sea*, *Seb*, *Sec*, *Sed*, *See*, and *Tsst* genes). The results yielded positive results for the *Sea*, *Seb*, *Sec*, and *Tsst* genes with molecular weights of 219, 478, 257, and 559 base pairs, respectively, except for the *Sed* and *See* genes, which were not detected in this study. In addition, some isolates carried one or more enterotoxin genes. Molecular analysis (PCR) results confirmed the virulence genes of the *S. aureus* isolates which are registered later in GenBank. Evolutionary analysis of the genetic similarity between the isolates showed complete similarity to global isolates recorded in the GenBank, indicating that the origin of the isolates represents a diversity of meat sources, possibly coming from different global sources. The results reveal that meat contamination in slaughterhouses results from insufficient knowledge of basic hygiene practices, as well as unsanitary operations in the slaughterhouse, which expose consumers to meat-borne infections and food poisoning.

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Introduction

Consumption of meat and their products are often linked to key dimensions of sustainability and this is the primary reason for focusing on these edible foods, Furthermore, meat promotes sustainable development as a source of income and employment for millions of people around the world, most of whom live in third world countries (1,2). Since meat is consider as a source of many essential elements for human health and nutrition, containing amino acids, minerals, and vitamins, the presence of these important elements in meat helps reduce deficiencies of these elements among

consumers and maintain health (3,4). Converting inedible grasses and crop waste by animals to produce meat and meat products which are usable for human food, contributing to food security (5,6). Given the importance of meat and its fundamental role in public health, many countries are keen to ensure food safety, especially meat. This importance is heightened when animals are slaughtered and their meat is produced, processed, and handled in highly polluted and unsanitary environments (7). This requires the implementation of strict procedures and standards to ensure good quality of animal products, including meat (8). *Staphylococcus aureus* is commonly found on the skin and

nasal cavities as commensals in both humans and animals. It can migrate from these natural habitats to other parts of the human or animal body, causing a range of clinical diseases and infections, or be transmitted through food, causing food poisoning (9,10). This is due to its ability to produce numerous extracellular and enterotoxins (11). These toxins are heat-resistant which causes a serious public health problems in humans (12) and a major food poisoning worldwide (13,14) and ranks third among the most common foodborne pathogens worldwide (15,16). Raw meat is exposed to numerous microbial contaminants, leading to spoilage or deterioration, rendering it unfit for human consumption. This meat can also be a vector for these pathogens or their toxins, which can be transmitted to humans via food and this is considered a major cause of foodborne illness (17,18). Toxic shock syndrome (TSS) is a serious and potentially fatal illness caused by the TSST protein, which is closely related to staphylococcal enterotoxins (SEs). Studies have shown a strong association between virulence genes and the development of specific disease symptoms associated with these toxins, confirming their pivotal role in disease development (19,20). Meat contamination can initially occur on farms during the presence of these animals and their direct contact with contaminated surfaces and tools used in the field (21). Contamination can also occur in abattoirs, as a secondary stage of meat contamination during slaughter and evisceration, or during meat storage. Finally, contamination can occur during transportation, processing in abattoir and butcher's shops (7). There are various methods that can be used to isolate and identify *S. aureus*, including conventional methods and molecular biological techniques. Conventional methods such as bacterial cultures on selective media, then study characteristics of *S. aureus* colonies on culture media, and biochemical tests, but molecular techniques are more accurate, rapid and providing the results of *S. aureus* within three to five hours (9). Previous studies have indicated that meat is contaminated with various microorganisms during animal slaughter or transportation of carcasses to markets and butcher shops (22). *S. aureus* has been identified and detected from a variety of foods, including animals' meat, fish meat, and milk and milk products (18).

Therefore, the aim of the current study was to determine the phenotypic and genetic characteristics of isolates of *S. aureus* and their enterotoxins from carcasses of different animals' species as well as knowing their sequences that related in other countries by using polymerase chain reaction technique.

Materials and methods

Ethical approval

This project was approved by the College of Veterinary Medicine/Animal Welfare Committee and was carried out at

the Veterinary Public Health Laboratories, College of Veterinary Medicine, University of Mosul, Iraq. It contained the approved identifier UM.VET 2024.038.

Sample collection and isolation of bacteria:

In a previous study (part of this study), a total of 131/270 (48.5%) isolates of *Saureus* were isolated from different parts of different carcasses (cattle, sheep, and goats) in Mosul slaughterhouses during the period from August 26 to November 20, 2024 (23). These isolates were diagnosed as *S. aureus* according to the conventional microbiological methods (24) which were cultivated on selective media, biochemical tests for bacterial identification (25,26).

DNA extraction and amplification

According to the manufacturer's procedure for the KPG Karmania pars DNA isolation kit for Gram-positive microorganisms (CN: KPG-GPB), the DNA was extracted from 46 isolates of *S. aureus*. The designed primers such as *nuc* gene F: 5'-CCT GAA GCA AGT GCA TTT ACG A-3' and R: 5'-CTT TA GCC AAG CCT TGA CGA ACT-3' (27), for virulent genes of staphylococcal enterotoxin, *Sea* gene F: 5'-AAA GTC CCG ATC AAT TTA TGG CTA-3', R: 5'-GTA ATT AAC CGA AGG TTC TGT AGA-3' (28) and for *Seb* gene F: 5'-TCG CAT CAA ACT GAC AAA CG-3', R: 5'-GCA GGT ACT CTA TAA GTG CC-3' (29) and for *Sec* gene F: 5'-GAC ATA AAA GCT AGG AAT TT-3', R: 5'-AAA TCG GAT TAA CAT TAT CC-3' (29) and for *Sed* gene F: 5'-CTA GTT TGG TAA TAT CTC CT-3', R: 5'-TAA TGC TAT ATC TTA TAG GG-3' (36) and for *See* gene F: 5'-TAC CAA TTA ACT TGT GGA TAG AC-3', R: 5'-CTC TTT GCA CCT TAC CGC-3' (30) and for *Tsst* gene F: 5'-GCT TGC GAC AAC TGC TAC AG-3', R: 5'-TGG ATC CGT CAT TCA TTG TTA T-3' (31), all primers were prepared at a concentration of 10 pmol of reverse and forward primers in each reaction, the total of amplification reaction mixture (25 µL) was prepared by using a 12.5 mL master mix reaction prepared from the Add Bio reaction kit., 1 µL of each primer was then added and mixed with 6.5 µL of DNase/RNase-free water, and finally 4 µL of DNA was added for PCR technique. The polymerase chain reaction was performed (32) using a GeneAmp Applied Biosystem. The PCR program used is an initial denaturation at 94°C for 5 minutes, then by 35 cycles (94°C for 1 minute, 52-55°C (depending to the annealing temperature of the primer) for 1 minute, 72°C for 5 minutes), followed by one cycle at 72°C for 10 minutes.

DNA Sequencing

The polymerase chain reaction (PCR) amplicons for four *S. aureus* isolates which were obtained in this study were sent to Macrogenal, South Korea, for purification and sequencing. The *Seb* and *Tsst* genes were selected as target genes from the current study for sequencing. The obtained sequences of these virulence genes were then compared with previously

sequences of *S. aureus* available in GenBank using the NCBI BLASTn program. Phylogenetic trees were constructed by using the Neighbor-Joining method with the CLUSTALW program. PCR, DNA sequencing, and bioinformatics analysis clarified the genetic relationships among *S. aureus* isolates and helped to understand the phylogenetic context of the isolates in this study.

Results

Conventional microbiological diagnosis of *S. aureus* in meat of slaughtered animals in Mosul abattoir (Table 1) revealed the isolation of 131/270 meat samples from the studied animals (cows, sheep and goats), with a total isolation rate of 48.52% of all meat samples. Our study showed that the percentages of *S. aureus* isolate from cow, sheep and goats' carcasses in Mosul slaughterhouse when examining 90 samples for each the neck, thoracic and flank

region were 44.4 %, 47.8 % and 53.3% respectively. The study also showed that there were no significant differences between the percentages of all types. The studied also showed no significant differences between cows, sheep and goats for both the neck and chest area (Table 2).

For further analysis of the enterotoxin genes of isolates which previously confirmed as *S. aureus*, PCR technique was applied to 46 random isolates to identify the enterotoxins (*Sea*, *Seb*, *Sec*, *Sed*, *See*, and *Tsst* genes) which probably to be present in these selected samples. In this study, we found that all isolates showed positive PCR amplicons for the *Sea*, *Seb*, *Sec* and *Tsst* genes with molecular weights of 219, 478, 257, and 559 base pairs, respectively, with the exception of the *Sed* and *See* genes, which were not detected in this study (Table 3). The highest rate 80.4% of enterotoxins for this bacterium in meat samples was *Sea*, while the lowest rate 34.8% was associated with the *Sec* type.

Table 1: Number of examined animals and number of positive & negative isolates of *S. aureus*, and the percentage of their isolation in Mosul abattoirs

Animal	Examined (n)	Positive isolate (n)	Positive isolate (%)	Negative isolate (n)	Negative isolate (%)
Cow	90	50	55.6	40	44.4
Sheep	90	36	40	54	60
Goats	90	45	50	45	50
Total	270	131	48.52	139	51.48

Table 2: Shows the number of *S. aureus* isolates and their percentage from the sampling area of animal carcasses in the Mosul slaughterhouse

Animal	Neck region			Thoracic region			Flank region		
	Carcasses (n)	Isolates (n)	%	Carcasses (n)	Isolates (n)	%	Carcasses (n)	Isolates (n)	%
Cow	30	16	53.3	30	15	50	30	19	63.3
Sheep	30	10	33.3	30	11	36.7	30	15	50
Goats	30	14	46.7	30	17	56.7	30	14	46.7
Total	90	40	44.4	90	43	47.8	90	48	53.3

Table 3: Shows the number and percentage of *S. aureus* enterotoxins genes in the carcasses of studied animals

Animal	No. of samples	<i>Nuc gene</i>		<i>Sea gene</i>		<i>Seb gene</i>		<i>Sec gene</i>		<i>Sed gene</i>		<i>See gene</i>		<i>Tsst gene</i>	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cow	16	16	100	14	87.5	4	25	4	25	0	0	0	0	9	56.3
Sheep	15	15	100	11	73.3	8	53.3	5	33.3	0	0	0	0	7	46.7
Goat	15	15	100	12	80	7	46.7	7	46.7	0	0	0	0	6	40
Total	46	46	100	37	80.4	19	41.3	16	34.8	0	0	0	0	22	47.8

In this study, the results for *S. aureus* were also arranged into four different gene profile according to the presence of different genes in each isolate (Figure1). Furthermore, some isolates carried one or more enterotoxin genes. The most common frequency genotype of *S. aureus* isolates was group I (*Sea*, *Seb*, *Sec*, and *Tsst*) with 29/46 (63%), group II (*Sea*, *Seb* and *Tsst*) with 10/46 (21.7%), group III (*Sea*, *Seb*), with

5/46 (10.9%) while the lowest frequency genotype was IV (*SeA*) with 2/46 (4.4%).

After confirming the presence of enterotoxins by using PCR in isolates of *S. aureus*, the amplicon of two enterotoxins was sent to GenBank for registration. The *Seb* gene registration under GenBank accession no. PV 544161, as well as isolates carrying the *Tsst* gene registered under

accession no. PV541276, PV541277, and PV541278. The percentage of similarity between the *S. aureus* isolates registered in GenBank and other global isolates for both the *Seb* and *Tsst* genes was found 100%. The *Seb* gene matched 100% with some isolates from Germany, Japan, Belgium, and the United States while the *Tsst* gene matched 100% with isolates from Italy, France, and the United Kingdom, but it was found to be lower in Germany and India. The phylogenetic tree analysis showed that the isolates were similar in that they were *S. aureus* isolates carrying the *Seb* and *Tsst* genes (Figures 2 and 3).

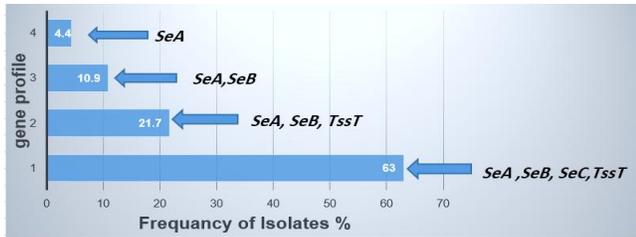


Figure 1: Show the frequency of staphylococcal enterotoxin genes from different isolates in meat (n=46).

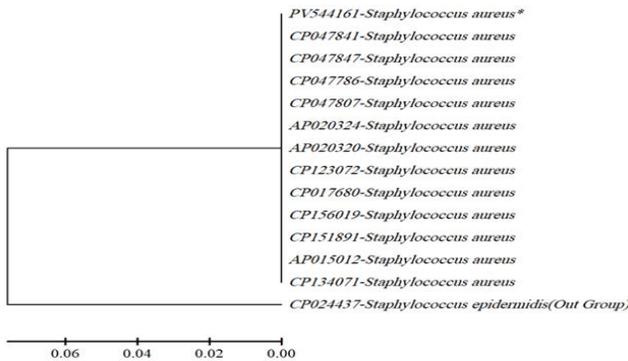


Figure 2: Phylogenetic tree of recorded local isolates of *Seb* gene *S. aureus* compared with other global isolates in GenBank using MEGA11 software.

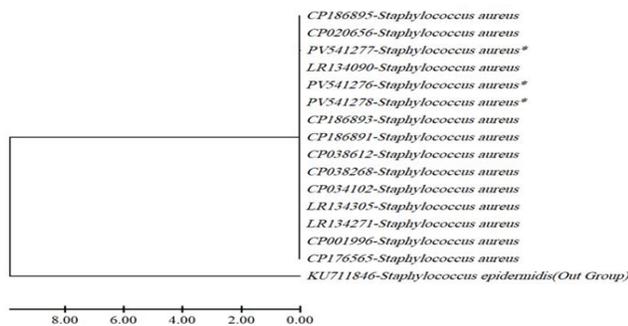


Figure 3: Phylogenetic tree of recorded local isolates of the *Tsst* gene *S. aureus* compared with other global isolates in GenBank using MEGA11 software.

Discussion

Ensuring food safety and quality is a fundamental requirement for consumers. Therefore, providing clear, honest, and straightforward food information is a vital step in building trust and ensuring food safety. Therefore, food safety is one of the most important areas of public health worldwide (33). Meat and meat products are major sources of foodborne illness and food poisoning. Among the pathogens is *S. aureus*, an opportunistic human pathogen and the third most common pathogen causing foodborne illness worldwide (34). In this study, the isolation rate of these bacteria was 48.52%, which is not a small percentage compared to 9.3% (35) in slaughterhouses in Addis Ababa, Ethiopia, while the percentage was higher than what we obtained in our study in a study conducted in Egypt. Ahmed and coworkers (36) recorded 55% in animals slaughtered in slaughterhouses, and it was 65.6% in a study conducted in United State of America (37), while the researchers (7) found in a local study conducted in Mansoura Governorate / Egypt that the isolation rate of staphylococci bacteria from meat was 43.4% (152/350), which is close to the percentage we obtained in our study.

Studying the virulence factors of bacteria is important for understanding their causes and targeting them for treatment or vaccine development (38). In current study, the results of molecular analysis confirmed the virulence genes of *S. aureus* isolates which are registration in Gen bank. Phylogenetic analysis of the genetic similarity between the isolates was 100% identical to global isolates registered in GenBank, indicating that the origin of the isolates represents a diversity of meat sources that may have come from various global sources. *S. aureus* possesses an array of virulence factors, enabling the organism to thrive as a pathogen causing a wide range of human and animal infections. Staphylococcal enterotoxins are responsible for more than 93% of staphylococcal food poisoning cases, due to their tolerance to acidic conditions (39). Because food poisoning cases caused by these bacteria are not reported, accurate estimates of their incidence are difficult to obtain (40).

Several recently published studies have identified other virulence factors of these bacteria, other than those discussed in our study (*Seg*, *She*, *Sei*, *Ser*, *Ses*, and *Set*), as potential food poisoning factors. Furthermore, studies have identified the virulence factor toxic shock syndrome (TSS), which is clinically characterized by fever, skin reddening and peeling, and hypotension. In severe cases, organ failure and death occur (41). Virulence factors enable it to adhere to host cells, subvert host immune defenses, invade tissues, cause sepsis, and induce toxin-associated syndromes (37). This underlies the persistence of *S. aureus* infections without a robust host immune response, making them more difficult to eradicate. Based on their mechanism of action and their role in pathogenesis (42,43).

Therefore, insufficient knowledge of basic hygiene practices and the presence of unsanitary operations in the slaughterhouse, in addition to weak compliance with the application of good handling standards during the slaughter of animals, all of these practices may lead to contamination of meat with pathogenic microorganisms and the consumer being exposed to diseases and food poisoning (44).

Conclusion

The presence of *S. aureus* in foods, such as meat, is a serious concern. In this study the results indicate that the main sources of Staphylococcal contamination in abattoir are the materials used, such as knives, tables, and workers' hands. The potential contamination of these materials is attributed to the unsanitary conditions of slaughterhouses and, consequently, to cross-contamination between these facilities. It is recommended that government and public health authorities monitor and regulate slaughterhouse activities to ensure compliance with approved and accepted standards to reduce meat contamination with *S. aureus*. These bacteria may possess different types of genes encoding virulence factors, which poses a significant problem for consumers.

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Conflict of interest

The author confirms no conflicts of interest in the preparation or application of the manuscript.

Editorial board note

R.A. Alsanjary is member of the editorial board of the Iraqi Journal of Veterinary Sciences, he did not participate in any stage of the decision-making process for this article.

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دراسة مظهرية وجينية لبكتيريا المكورات العنقودية الذهبية المعزولة من لحوم حيوانات مختلفة في مجازر الموصل، العراق

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الخلاصة

تعد بكتيريا المكورات العنقودية الذهبية من أهم مسببات الأمراض المنقولة عبر الغذاء. هدفت الدراسة إلى عزل هذه البكتيريا من لحوم الحيوانات المختلفة في مجازر الموصل ودراسة خصائصها الظاهرية والوراثية خلال الفترة من ٢٦ آب إلى ٢٠ تشرين الثاني ٢٠٢٤. أظهرت نتائج الخصائص الظاهرية للبكتيريا عزل ٢٧٠/١٣١ بكتيريا، بنسبة عزل إجمالية بلغت ٤٨,٥% من جميع عينات اللحوم المدروسة. استُخدمت تقنية تفاعل البلمرة المتسلسل للكشف عن جينات السموم المعوية للبكتيريا. طبقت هذه التقنية على ٤٦ عينة عشوائية لتحديد بعض عوامل الضراوة لبكتيريا المكورات العنقودية الذهبية (جينات *Sea*، *Sec*، *Sed*، *See*، و *Tsst*) أسفرت النتائج عن نتائج إيجابية لجينات *Sea* و *Sec* و *Tsst* بأوزان جزيئية ٢١٩ و ٤٧٨ و ٢٥٧ و ٥٥٩ زوجًا قاعديًا على التوالي، باستثناء جيني *Sed* و *See*، اللذين لم يتم اكتشافهما في هذه الدراسة فضلًا عن ذلك، حملت بعض العزلات جينًا واحدًا أو أكثر من جينات السموم المعوية. كما أكدت نتائج التحليل الجزيئي جينات الضراوة لعزلات المكورات العنقودية المسجلة في بنك الجينات. أظهر التحليل التطوري للتشابه الجيني بين العزلات تشابهًا تامًا مع العزلات المسجلة عالميًا، مما يشير إلى أن أصل العزلات يمثل تنوعًا في مصادر اللحوم، وربما يأتي من مصادر عالمية مختلفة. كشفت النتائج أن تلوث اللحوم في المجازر ناتج عن عدم كفاية المعرفة بممارسات النظافة الأساسية، بالإضافة إلى العمليات غير الصحية في المسالخ، مما يعرض المستهلكين للعدوى المنقولة باللحوم والتسمم الغذائي.