Iraqi Journal of Veterinary Sciences

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Molecular detection of some Staphylococcal enterotoxins in the meat of slaughtered animals in abattoirs, Mosul- Iraq

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Article information

Article history:
Received 28 April 2025
Accepted 04 June 2025
Published 21 June 2025

Keywords:
Meat
Staphylococcal aureus
Enterotoxin genes
PCR
Abattoir

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Abstract

Foodborne pathogens are an international health problem, especially *Staphylococcus (S.) aureus*, which is considered one of the most important foodborne pathogens. The study aimed to isolate and identify the *S. aureus* bacteria from the meat of slaughtered animals in Mosul abattoirs during the period from August 26 to November 20, 2024. The conventional microbiology diagnosis for *S. aureus* in the meat of slaughtered animals in Mosul abattoir reveals isolation of 131/270, with a total isolation rate reaching 48.5% from all meat samples. For the purpose of further analysis to detect the enterotoxin genes, the isolates that were previously confirmed as *S. aureus* by using specific *Nuc* gene, PCR was applied to 46 random isolates to identify S. enterotoxins (*sea*, *seb*, *sec*, *sed*, *sef*, and *Tsst* genes) which gave a positive result for *Sea*, *seb*, *sec*, and *Tsst* genes with molecular weight 219, 478, 257 and 559 bp, respectively, except the *seD* and *seE* gene are not detected in this study, moreover some isolates carrying either one or more than one of enterotoxins genes. From the results of this study, we believe that meat contamination in the abattoirs may come from poor sanitation practices in slaughterhouses that could expose the consumers to meat-borne infections and food poisoning. It could be recommended that activities in the abattoir should be monitored and regulated by the government and sanitation agencies to ensure that approved and acceptable standards are adhered to reduce contamination of meat that gets to final consumers.

DOI: 10.33899/ijvs.2025.159635.4258,

Introduction

Meats and meat products are a major part of the ideal diet in many parts of the world. Because they are linked to health, culture, and economic reasons (1). They are a major source of animal protein needed by the individual (2); however, it may be a source of danger to human health, as it is a suitable environment for the growth of many different types of microorganisms that are transmitted from meat to humans, causing health problems, including diarrhea (3,4). At present, meat consumption is considered one of the most prominent indicators of economic status and a measure of the progress of people (5). Many studies indicate that carcasses are exposed to contamination with various types of microorganisms in slaughterhouses during the slaughtering of animals or their evisceration before transporting the carcasses to markets and butcher shops (6,7). According to much earlier research, *S. aureus* was determined from a variety of foods, including meat (8), camel milk (9), cow's milk (10), and fish (11). Historically and from an evolutionary perspective, meat is considered a resource of important value to human societies as a food with essential elements against the backdrop of biological and social needs that have continued for 3 million years (12). *S. aureus* is an opportunistic and commensal bacteria in the body that can cause a wide range of diseases, from simple skin

inflammation to severe invasive diseases that are potentially fatal (13,14). Approximately 10 to 50% of healthy populations in the world harbor these bacteria as a part of the normal microflora in the skin, nasal passage, and throat (15). *S. aureus* colonizes and infects many humans as well as animals such as cattle, buffalo, goats, and sheep for meat production (16). *S. aureus* usually causes staphylococcal food poisoning, which is poisoning resulting from eating food or drinks containing intestinal toxins (17). In Human beings, the species of these bacteria are a common cause of Staphylococcal food poisoning (SFP), but in animals, the infection occurs with *S. aureus* or excrete toxins in animal products, especially raw foods and processed meat products, are also considered an important source of *S. aureus* contamination (18,19). Staphylococcal food poisoning is suddenly characterized by nausea, vomiting, and symptoms of cramps, pain in the abdomen as well as diarrhea in period 2 to 6 hours of ingestion of the toxin and generally lasts 12 hours, and the symptoms may be 4-10 days (20). These toxins occasionally require prolonged boiling or steam sterilization to reduce their virulence because they are thermally stable (21,22). Despite this, staphylococcal food poisoning remains a reportable condition, and controlling this disease is of great economic importance, especially in the food industry worldwide (23,24). These enterotoxins can be detected by using different techniques like immunoassays, immunodiffusion, or latex aggregation (25). However, these diagnostic kits, which are available commercially now, do not cover the detection of all enterotoxins (26). More currently, tests have been used to detect the enterotoxin genes of *S. aureus*, polymerase chain reaction (PCR) either directly from food samples or from bacterial isolates in culture media to increase their concentration (27), Loop-mediated amplification (LAMP) tests based on PCR can also be used (28,29).

Therefore, the current study aimed to identify various S. enterotoxins-contaminated animal carcasses and determine their percentages using the polymerase chain reaction technique.

Materials and methods

Ethical approval

The study project was approved by the Animal Welfare Committee and conducted at the College of Veterinary Medicine, University of Mosul, Iraq. It included an authorized ID of UM.VET 2024.038.

Sample collection and isolation of bacteria

Two hundred and seventy samples were collected from meats of different animals (cow, sheep, and goat) slaughtered in the Mosul abattoirs for the period from August 26 to 20 November 2024. The swabs were taken from the different parts of carcasses and placed in sterile tubes containing sterile peptone water to activate the bacteria. Then, by means of a cooling box, the samples were transferred for conventional microbiological diagnosis of *S. aureus* in the Laboratory of Veterinary Public Health department, College of Veterinary Medicine/University of Mosul. The samples were treated according to the bacteriological rules followed for diagnosis and detection of bacteria (30). After culture on mannitol salt agar as a selective media, the isolates of *S. aureus* were tested by biochemical tests like catalase test and coagulation test (31,32).

DNA extraction and amplification

DNA was extracted from *S. aureus* isolates according to the extraction method mentioned in the manufacturer's instructions for the extraction kit, the KPG Karmania pars gene DNA Extraction Kit from Gram-Positive Bacteria (CN: KPG-GPB). All primers listed in table 1 are prepared from Eurofins Genomics Germany Stock solution and are stored in the freezer at -20°C to prepare 10 pmol of reverse and forward primers; prepare the reaction mixture for the purpose of amplification using the reaction kit provided by the Korean company Add Bio. The mixing process is carried out according to the company's instructions for the mixing kit. 12.5 master mix, then withdrawn 1 microliter for each of both primers and mixed with 6.5 microliters of DNase/RNase-free water, finally added 4 microliters of DNA for the purpose of performing the polymerase chain reaction (PCR).

Results

Conventional microbiological diagnosis of *S. aureus* in meat slaughtered in Mosul slaughterhouses revealed the isolation of 131/270 samples, with an overall isolation rate of 48.5% of all meat samples (Table 2). The percentage of isolation on the right side of Mosul city was 39.3%, while the isolation on the left side was 55.6%, and this result showed a significant level difference at $P<0.01$. All *S. aureus* isolates showed positive results in biochemical tests, and these isolates were then confirmed using the *Nuc* gene, which was 100% specific, yielding 166 base pairs of amplification specific to the *S. aureus* genus (Figure 1).

For the purpose of further analysis to detect the enterotoxin genes of isolates that were previously confirmed as *S. aureus*, PCR was applied to 46 random isolates to identify enterotoxins (*seA, seB, seC, seD, seE, and TssT* genes) which were probably present in these selected samples. In this study, we found that all isolates showed positive PCR amplicons for *seA, seB, seC, and TssT* genes (219, 478,257 and 559 bp), respectively, except the *seD and seE* genes are not detected in this study moreover, some isolates carrying either one or more than one of enterotoxins genes. The highest rate, 80.4%, of enterotoxins of this bacteria in meat samples is *seA*, while the lowest rate, 34.8%, is related to *seC* type (Figures 2-5).

Table 1: The primers and PCR amplification programs of various toxin genes of *S. aureus*

Gene	Primer	Sequence (5-3)	Amplicon size [bp]	Programme*	Reference
nuc	nuc-1 nuc-2	5-CCTGAAGCAAGTGCATTTACGA-3 5-CTTTAGCCAAGCCTTGACGAACT-3	166	I	33
sea	SEA-1 SEA-2	5-AAAGTCCCGATCAATTTATGGCTA-3 5-GTAATTAACCGAAGGTTCTGTAGA-3	219	I	34
seb	SEB-1 SEB-2	5-TCGCATCAAACTGACAAACG-3 5-GCAGGTACTCTATAAGTGCC-3	478	I	27
sec	SEC-1 SEC-2	5-GACATAAAAGCTAGGAATTT-3 5-AAATCGGATTAACATTATCC-3	257	II	27
sed	SED-1 SED-2	5-CTAGTTTGGTAATATCTCCT-3 5-TAATGCTATATCTTATAGGG-3	317	II	27
see	SEE-1 SEE-2	5-TACCAATTAACTTGTGGATAGAC-3 5-CTCTTTGCACCTTACCGC-3	171	I	35
tst	TSST-1 TSST-2	5-GCTTGCGACAACTGCTACAG-3 5-TGGATCCGTCATTCATTGTTAT-3	559	I	36

*I: 35 cycles (94°C – 30s, 54°C – 30s, 72°C – 30s); II:: 35 cycles (94°C – 30s, 52°C – 30s, 72°C – 30s).

Table 2: Number of examined carcasses with the number and percentage of the positive isolates for *Staphylococcal aureus* on both sides of Mosul city

The city	Examined (n)	Positive (n)	Percentage	Negative isolates (n)	Percentage
Right side	117	46 a	39.3	71	60.7
Left side	153	85 b	55.6	68	44.4
Total	270	131	48.5	139	51.5

Lowercase letters are the significant differences between vertical rows at P<0.01.

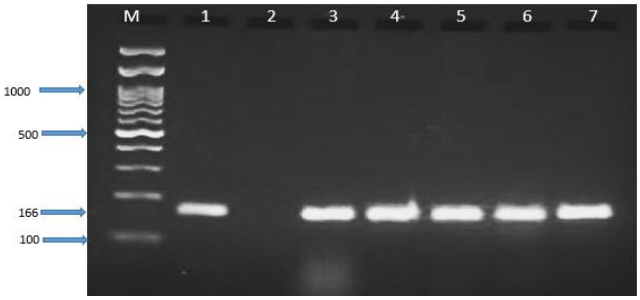

Figure 1: 3-7 Amplification of *Nuc* gene 166 bp, M: marker 100bp,1: control positive, 2: control negative

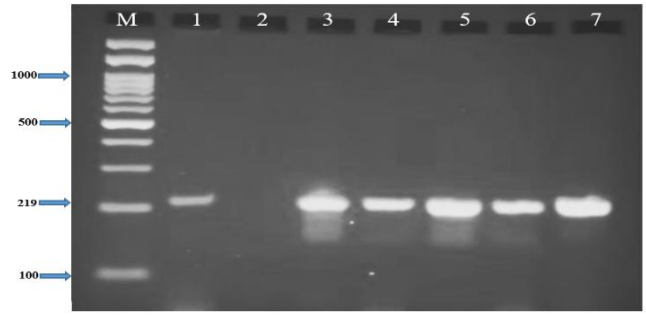

Figure 2: 3-7 Amplification of *SeA* gene 219 bp, M: marker 100bp,1: control positive, 2: control negative.

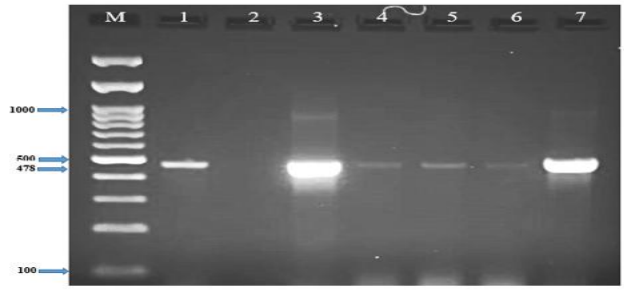

Figure 3: 3-7 Amplification of *SeB* gene 478 bp, M: marker 100bp,1: control positive, 2: control negative.

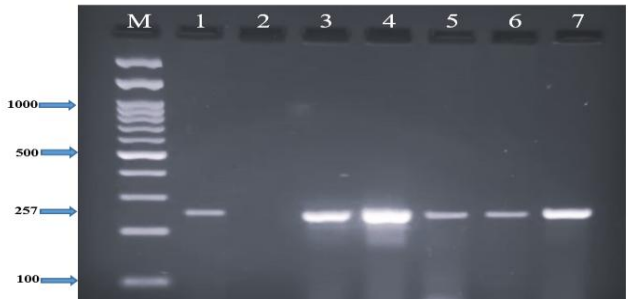

Figure 4: 3-7 Amplification of *SeC* gene 257 bp, M: marker 100bp,1: control positive, 2: control negative.

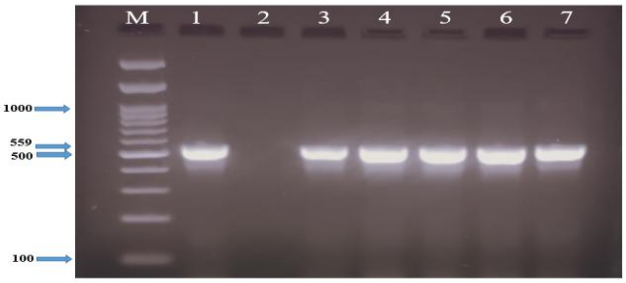

Figure 5: 3-7 Amplification of *TssT* gene 559 bp, M: marker 100bp,1: control positive, 2: control negative.

Also, in this study, the results arranged *S. aureus* into four different gene frequencies according to the existence of different genes in each isolate (Table 3). The most frequent gene profile of the *S. aureus* isolates in group I (*seA, seB, seC, and TssT*) was 29/46 (63%), the gene profile II (*seA,seB, and TssT*) was 10/46 (21.7%), the gene profile III (*seA and seB*) was 5/46 (10.9%), and the genes profile IV (*seA*) was 2 (4.4 %).

Table 3: Frequency rates of enterotoxin genes from meats

Gene	Enterotoxin genes	Isolates n(%)
I	*seA,seB, seC, TssT*	29 (63.0)
II	*seA, seB, TssT*	10 (21.7)
III	*seA, seB*	5 (10.9)
IV	*seA*	2 (4.4)

Discussion

A slaughterhouse is a facility where animals are slaughtered for food consumption. These slaughterhouses provide healthy, clean meat and meat products for human consumption, but they can also pose a public health risk due to poor sanitation and hygienic handling procedures (37). This justifies the current findings in our study, which showed a significant increase in the presence of *S. aureus* as a zoonotic contaminant in a Mosul abattoir (38), with a prevalence of 48.5%. Comparing the results of the study with the results of other studies, we found that they were higher than the 9.3% reported by Adugna and coworkers (39) in a slaughterhouse in Addis Ababa, Ethiopia, and the 20.3% reported by Thomas *et al.* (40) in a slaughterhouse in Woleta Sodo. The current finding in our study was similar to the results of a study in northeastern Ethiopia, where the prevalence was 54.45% (41) and 55% in Egypt (42).

The difference in *S. aureus* prevalence in this study and other studies may be due to the study area, sampling strategy, isolation techniques used, carcass sampling site, pre-and post-slaughter contamination, meat processing, and operator awareness and skill (38,43,44). Coagulase-positive *S. aureus (S. aureus*) is one of the most common causes of foodborne illness due to the high mortality rate associated with its resistance to multiple antibiotics (45). They are commensal organisms in the human nasopharynx, mouth, and skin. Their presence is also attributed to poor meat handling by butchers and the surrounding environment (46).

S. aureus can be transmitted to meat in slaughterhouses through talking, coughing, touching, sneezing, or laughing (47). Based on their antigenicity, twenty-three types of staphylococcal toxins were identified. The staphylococcal enterotoxins are well characterized, have similar molecular weights, and are approximately 230 amino acids long, and there are available commercial kits for detecting them. Food poisoning caused by *S. aureus* is typically associated with improper handling or storage of meat and meat products, which leads to bacterial growth in meat and toxin production (48,49).

The common enterotoxins of these bacteria are responsible for more than 90% of cases of staphylococcal sepsis (SFP) due to their stability in water activity and acidity conditions (50). The remaining 10% are responsible for outbreaks with the recently described staphylococcal enterotoxins SE and TSST-1. Accurate estimates of *S. aureus* food poisoning rates are difficult to obtain because most cases go unreported. Recently, research has been published on additional food poisoning factors *seG, seH, seI, seR, seS,* and *seT* as potential food poisoning agents, in addition to toxic shock syndrome (TSS), which is clinically characterized by high fever, hypotension, skin reddening, and peeling, and in severe cases, multiple organ failure and death. The enterotoxin type TSS-1 is associated with food poisoning, whether in humans, animals, or food, but its prevalence is not as well-known as that of food poisoning (51).

In general, the presence of unhygienic practices in abattoirs can be linked to a lack of inadequate knowledge of basic hygiene practices, poor compliance with the application of good food handling standards (52), as well as a lack of infrastructure or facilities, and weak government oversight systems by food regulatory workers, which may contribute to the continuation of these unsanitary practices that lead to an increased risk of human infection (38).

Conclusion

The presence of S. species in food such as meat is of great concern. From this study, it can be concluded that the major sources of contamination of meat by S. species in abattoirs are poor sanitation practices in slaughterhouses that could expose the consumers to meat-borne infections and food poisoning. It could be recommended that activities in the abattoir should be monitored and regulated by the government and sanitation agencies to ensure that approved and acceptable standards are adhered to reduce contamination of meat that gets to final consumers.

Acknowledgments

The researchers would like to extend their sincere thanks to the deanship of the College of Veterinary Medicine, University of Mosul, for providing all the resources and facilities that made this research possible.

Conflict of interest

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

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الكشف الجزيئي عن بعض السموم المعوية لجراثيم المكورات العنقودية الذهبية في لحوم الحيوانات المذبوحة في مجازر الموصل – العراق

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الخلاصة

تُعد مسببات الأمراض المنقولة بالغذاء مشكلة صحية عالمية وخاصةً بكتيريا المكورات العنقودية الذهبية، التي تُعتبر من أهم مسببات الأمراض المنقولة بالغذاء. هدفت الدراسة الحالية إلى عزل وتحديد بكتيريا المكورات العنقودية الذهبية من لحوم الحيوانات المذبوحة في مجازر الموصل خلال الفترة من ٢٦ أب إلى ٢٠ تشرين الثاني / ٢٠٢٤. كشف التشخيص الميكروبيولوجي التقليدي للمكورات العنقودية الذهبية في لحوم الحيوانات المذبوحة في مجازر الموصل عزل ١٣١ عينة من أصل ٢٧٠ عينة، بنسبة عزل إجمالية بلغت ٤٨,٥% من جميع عينات اللحوم. لغرض إجراء الكشف عن جينات السموم المعوية للعزلات التي تم تأكيدها سابقًا على أنها المكورات العنقودية الذهبية باستخدام جين *Nuc* ، تم تطبيق تفاعل البلمرة المتسلسل على ٤٦ عزلة عشوائية لتحديد السموم المعوية للمكورات العنقودية الذهبية باستخدام بادئات لجينات *sea* و *seb* و *sec* و *sed* و *see* و *Tsst* والتي أعطت نتائج إيجابية لجينات *sea* و *seb* و *sec* و *Tsst* بأطوال جزيئية ٢١٩ و ٤٧٨ و ٢٥٧ و ٥٥٩ زوجًا أساسيًا على التوالي، باستثناء جين *sed* و *see* اللذين لم يتم الكشف عنهما في هذه الدراسة، علاوة على ذلك، فإن بعض العزلات كانت تحمل واحدًا أو أكثر من جينات السموم المعوية. تُظهر النتائج أن تلوث اللحوم في المسلخ قد يكون ناتجًا عن ممارسات صحية سيئة في المجازر مما قد يُعرّض المستهلكين للعدوى المنقولة باللحوم والتسمم الغذائي. يُوصى بمراقبة الأنشطة في المجازر وتنظيمها من قِبل الحكومة وهيئات الصحة العامة لضمان الالتزام بالمعايير المعتمدة والمقبولة للحد من تلوث اللحوم التي تصل في النهاية إلى المستهلك.