Iraqi Journal of Veterinary Sciences

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Molecular detection of the genes encoding virulence factors of coagulase-positive *Staphylococcus aureus* isolated from restaurants

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Article information

Article history:
Received: 03 December 2024
Accepted: 13 March 2025
Published: 15 March 2025

Keywords:
S. aureus
Restaurants
Virulence factors
Gene profiles

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Abstract

Foodborne illness outbreaks have been associated with animal foods, and foodborne disease (FBD) is an international health issue. *Staphylococcus* (*S.*) *aureus* is considered one of the most important foodborne pathogens. The objectives of the present study were to isolate and identify *S. aureus* isolated from Meat, hand, and instrument of restaurants in Erbil city and to genes encoding virulence factors based on classical and molecular biology methods. Three hundred fifty samples of all kinds were collected from various areas in Erbil, Iraq (30, 40, 60, and 100-meter streets) from August 2024 to November 2024. The results of the current study found that the prevalence rate of *S. aureus* was 43.4% (152/350). The prevalence rate of *S. aureus* was different according to the area; in the 30-meter street was 38.8% (31/80); in the 40-meter street was 50% (40/80); in the 60-meter street was 36.7% (33/90), and in the 100-meter street was 48% (48/100). Additionally, all *S. aureus* possessed the *nuc* and *coa* genes at 100% (40/40), while *S. aureus* possessed the *clfA* gene at 95% (38/40), and the *clfB* gene was 92.5% (37/40). The gene profiles of *S. aureus* isolates are divided into four primary groups. The percentage of *S. aureus* isolates possessed the first gene profile was 85% (34/40), the second gene profile was 5% (2/40), the third gene profile was 7.5% (3/40), and the fourth gene profile was 2.5% (1/40). *Staphylococcus aureus* is isolated from meat and tools used in restaurants, significantly spreading the *S. aureus* in restaurants. All genes detected most of the S. aureus isolates based on the PCR assay.

DOI: 10.33899/ijvs.2025.155576.4042,

Introduction

Staphylococci are significant pathogens transmitted through food that can cause important public health problems (1). They have been classified as the third most common cause of diseases internationally among foodborne diseases that have been determined (2), and their pathogenicity is based on several components of the surface of the bacteria (3). *Staphylococcus* (*S.*) *aureus* is a serious foodborne illness. It is a complex pathogen that affects sentient beings, causing many diseases, such as septicemia and pneumonia (4). *Staphylococcus aureus* is one of the most significant microorganisms that can contaminate or recontaminate the food prepared by workers' hands, tools, or scraps (5). *Staphylococcus aureus* is considered an important foodborne pathogen globally due to its ability to generate several extracellular toxins, which usually induce an abrupt onset of nausea, vomiting, and abdominal cramping (6). The capacity of certain strains of *S. aureus* to produce heat-stable enterotoxins that result in staphylococcal food poisoning (SFP), one of the most prevalent causes of gastroenteritis in the world, is especially pertinent for the food processing sector (7). Additionally, staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-like (SEls) are the main cause of food-related diseases (8). These five traditional enterotoxins are said to be responsible for 95% of SFP cases, with an outstanding 5% of illnesses caused by newly recognized SEs (9). According to many earlier research, *S.*

aureus was determined from a variety of foods, including meat (10), fish (11), cow's milk (12), and camel milk (13). Foods such as meat and its products, particularly ham, are linked to the emergence of staphylococcal occurrences poisoning (14). In recent decades, *S. aureus* has been found in domesticated animals raised for food, such as pigs, cattle, chickens, and other types of animals (15). Food containing a high protein content should be handled by hand, with care, usually in conjunction with improper heating and/or storage (16). In addition, meat is highly susceptible to spoiling and is commonly associated with the transmission of foodborne illnesses. Raw meat contamination is a major cause of foodborne diseases (17). Meat can be contaminated from the first stage on the farm during direct contact of animals with contaminated surfaces or in the slaughterhouse, which is considered a second stage of contamination of meat during slaughtering, evisceration, and storage; finally, meat will contaminate during transmission, processing, and cooking. Various methods were used to determine the S. aureus isolates, including traditional and molecular biology. The Conventional methods are based on biochemical testing and the structural features of *S. aureus* colonies. However, molecular techniques are quicker and more accurate, providing results for identifying *S. aureus* in three to five hours (18).

The objectives of our study are to identify the pathogen *S. aureus* in the different types of samples taken from restaurants in various parts of Erbil city and to identify the genes in *S. aureus* isolates that encode virulence factors by the PCR assay.

Materials and methods

Ethical approval

All samples were collected with the owners' agreement and utilized based on the ethical criteria issued by the Institutional Animal Care and Use Committee at Mosul University's College of Veterinary Medicine, using the approved ID of UM. Vet. 2024.047.

Samples Collection

Three hundred and fifty samples (knife, meat, table, hand, and machine) were collected from various areas in Erbil, Iraq (30-meter streets, 40-meter streets, 60-meter streets, and 100-meter streets). Studying began in August 2024 and ended in November 2024. All types of samples (knife, meat, table, hand, and machine) were collected in sterile swabs in sanitary containers before being sent immediately to the College of Veterinary Medicine's Health Laboratory at Mosul University. All peptone water containers were placed in an incubator and underwent a pre-enrichment process for 18 to 24 hours at 37°C. After streaking each sample on Mannitol salt agar, they were incubated at 37°C for a full day.

S. aureus Isolation and Characterization

Phenotypic examination, coagulase and catalase activity tests, and gram staining were employed to investigate and determine phenotypic characterizations of the S. aureus colonies in conformity with the conventional procedures used for isolating and identifying *S. aureus* colonies (19).

Extraction of DNA

The genomic DNA from *S. aureus* was analyzed, and the positive isolates were analyzed and cultured in a mannitol salt medium for over eight hours at 37°C. The Genomic DNA Extraction Kit (Addbio, Korea) utilized to isolate the DNA was processed following the manufacturer's guidelines for Gram-positive bacteria. Following this, the concentration of DNA was measured using Nanodrop (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*, *clfA*, *clfB*, and *coa* genes of *S. aureus* were discovered by the PCR technique. The molecular weight of the *nuc* gene was 166 bp (20), *clfA* was 288 bp (21), *clfB* was 203 bp (21), and *coa* was 674 bp (22). The PCR reaction required 25 µl in total volume, and the mixture was made in a 200 µl tube (Addbio, Korea). The resulting amplicons underwent a gel electrophoresis evaluation on a 2% agarose gel (Peqlab, Erlangen, Germany), utilizing a reference of a 100 bp ladder. The reaction mixture consisted of 6.5 µl of DNeasy-free water from Promega Corporation (USA), 4 µl of the *S. aureus* DNA template, 12.5 µl of the GoTaq Mix Master (2×) from addbio company (Korea), 1 µl of each primer 1 and 2 (Table 1).

Statistical analysis

All analyses were performed using JMP® 16.1 software (23). The Chi-square test was used to determine if there was a significant difference between the percentages of *S. aureus* isolated from different streets in Erbil city. The results were substantial, with a $P<0.05$.

Results

The *S. aureus* colonies that provided positive data appeared on Mannitol salt agar with a golden-yellowish color. Additionally, specific biochemical tests that verified the existence of *S. aureus*, such as the coagulase and catalase assays, produced positive results. The concentration of extracted DNA from *S. aureus* in this study was 18.5 mg/µl to 30 mg/µl. The PCR assay confirmed that all *S. aureus* isolates were positive (Figures 1-7).

Table 1: Various primers utilized in the PCR programs to detect the genes of *S. aureus*

Gene	Primer	Sequence (5- 3)	Amplicon size [bp]	Program*	Reference
nuc	nuc-1 nuc-2	5-CCTGAAGCAAGTGCATTTACGA-3 5-CTTTAGCCAA GCCTTGACGAACT-3	166	I	(20)
cliff	clfA-1 clfA-2	5-ATTGGCGTGGCTTCAGTGCT-3 5-CGTTTCTTCCGTAGTTGCATTTG-3	288	I	(21)
clfB	clfB-1 clfB-2	5-ACATCAGTAATAGTAGGGGCAAC-3 5-TTCGCACTGTTTGTGTTTGCAC-3	203	II	(21)
coa	coa-1 coa-2	5-ATAGAGATGCTGGTACAGG-3 5-GCTTCCGATTGTTCGATGC-3	674	I	(22)

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).

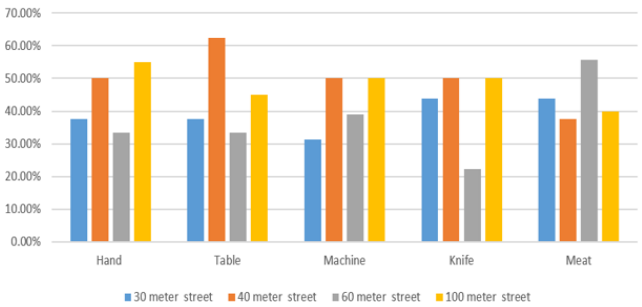

Figure 1: Comparative prevalence of *S. aureus* isolated from restaurants in various areas of Erbil city.

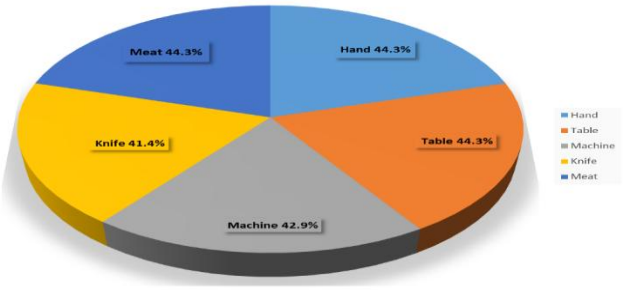

Figure 2: Prevalence of *S. aureus* isolated from different samples

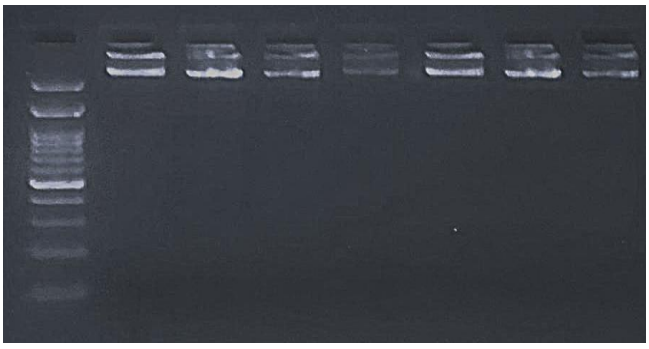

Figure 3: Visualization and comparative concentration of *S. aureus* whole genome DNA using agarose gel electrophoresis.

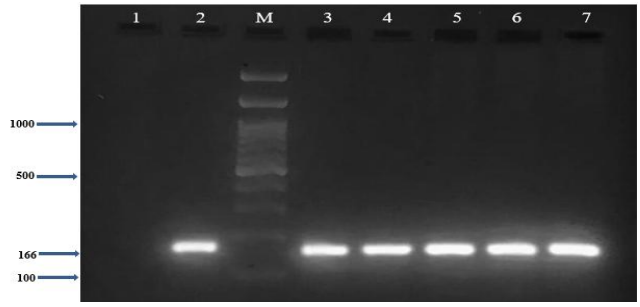

Figure 4: A 2% agarose gel electrophoresis illustrating the typical amplicon of the *nuc* gene product of *S. aureus* isolates.

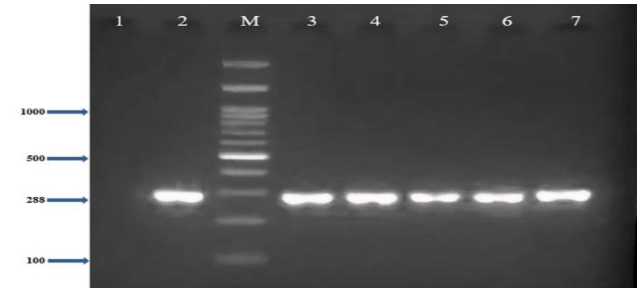

Figure 5: A 2% agarose gel electrophoresis illustrating the typical amplicon of the *clfA* gene product of *S. aureus* isolates.

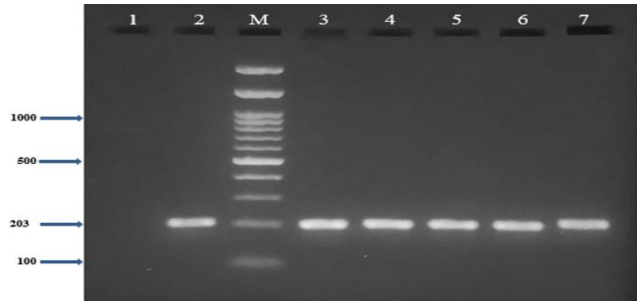

Figure 6: A 2% agarose gel electrophoresis illustrating the typical amplicon of the *clfB* gene product of *S. aureus* isolates.

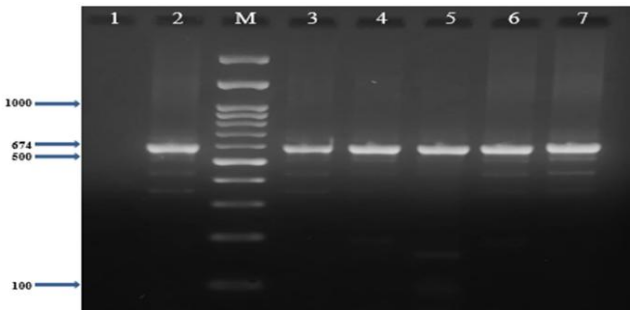

Figure 7: A 2% agarose gel electrophoresis illustrating the typical amplicon of the *coa* gene product of *S. aureus* isolates.

According to the present study, 43.4% (152/350) of *S. aureus* were determined to be prevalent in Erbil city. The high percentage isolated of *S. aureus* was 50% (40/80) on a 40-meter street. Subsequently, the prevalence rate of *S. aureus* in a 60-meter street was 48% (48/100). Although the lowest isolated *S. aureus* ratio observed in a 30-meter street and a 100-meter street was 38.8% (31/80) and 36.7% (33/90), respectively (Table 2). Statistical analysis revealed that the percentage of *S. aureus* isolated from various streets in Erbil city did not differ significantly (P<0.05).

Table 2: Number and percentage of *S. aureus* isolated from different streets in Erbil city

Street	Samples (n)	Positive n (%)
30 meters	80	31 (38.8)
40 meters	80	40 (50)
60 meters	100	48 (48)
100 meters	90	33 (36.7)
Total	350	152 (43.4)

Furthermore, the present investigation appeared to indicate various incidence rates of *S. aureus* in different regions. In a 30-meter street, the incidence rate of *S. aureus* was 38.8% (31/80). The high incidence rate of *S. aureus* isolated in a 30-meter street from knives and meat was 43.8% (7/16). Subsequently, the incidence rate of *S. aureus* isolated from the hand and table was 37.5% (6/16). The low incidence rate of *S. aureus* isolated from machines was 31.3% (5/16) (Table 3).

Additionally, the present study declared that the rate of occurrence of *S. aureus* isolated on a 40-meter street was 50% (40/80). The high occurrence of *S. aureus* strains obtained from the table was 62.5% (10/16). The low occurrence rate of *S. aureus* detected in meat samples was 37.5% (6/16). Meanwhile, the occurrence of *S. aureus* isolated from the hand, machine, and knife was 50% (8/16) (Table 4).

The incidence rate of *S. aureus* in 60-Meter Street was 36.7% (33/90). The high incidence rate of *S. aureus* from meat was 55.6% (10/18). The low incidence rate of *S. aureus* detected from a knife was 22.2% (4/18). Subsequently, the incidence of *S. aureus* isolated from the hand, table, and machine was 33.3% (6/18), 33.3% (6/18), and 38.9% (7/18) (Table 5).

Table 3: Number and percentage of *S. aureus* isolated from different samples in 30-Meter Street

Sample type	Samples (n)	Positive n (%)
Hand	16	6 (37.5)
Table	16	6 (37.5)
Machine	16	5 (31.3)
Knife	16	7 (43.8)
Meat	16	7 (43.8)
Total	80	31 (38.8)

Table 4: Number and percentage of *S. aureus* isolated from different samples on 40-Meter Street

Sample type	Samples (n)	Positive n (%)
Hand	16	8 (50)
Table	16	10 (62.5)
Machine	16	8 (50)
Knife	16	8 (50)
Meat	16	6 (37.5)
Total	16	8 (50)

Table 5: Number and percentage of *S. aureus* isolated from different samples on 60-Meter Street

Sample type	Samples (n)	Positive n (%)
Hand	18	6 (33.3)
Table	18	6 (33.3)
Machine	18	7 (38.9)
Knife	18	4 (22.2)
Meat	18	10 (55.6)
Total	90	33 (36.7)

Finally, the occurrence rate of *S. aureus* isolated in a 100-meter street was 48% (48/100). The high occurrence rate of *S. aureus* isolated from the hand was 55% (11/20). The low occurrence rate of *S. aureus* in meat was 40% (8/20). Subsequently, the occurrence rate of *S. aureus* isolated from the table, machine, and the table was 45% (9/20), 50% (10/20), and 50% (10/20), respectively (Table 6).

Furthermore, the high prevalence of *S. aureus* detected in restaurants from hand, table, and meat was 44.3% (31/70). The low prevalence of *S. aureus* obtained in restaurants from knives was 41.4% (29/70), and machines 42.9% (30/70) (Table 7). The statistical analysis showed no significant difference between the types of samples in 30, 40, and 100 meters of the street (P<0.05). Still, there was a substantial difference between knives and meat (P<0.05) in 60 meters of the street.

Based on Table 8, the results of the PCR assay supported the conclusions attained from traditional methods, confirming that the *nuc* and *coa* genes were present 100% (40/40) of *S. aureus* (Figures 4 and 7). Furthermore, our study showed that 95% (38/40) of *S. aureus* isolates possess the *clfA*, and 92.5% (37/40) possess the *clfB* genes (37/40) (Figures 5 and 6).

Moreover, the results of the current study showed that *S. aureus* was split into two different gene profiles according to the presence of various genes in each isolate (Table 9). The *S. aureus* isolates showed that the most frequent gene profile I (*nuc* + *mecA* + *clfA* + *clfB* + *coa*) was 34/40 (85%), the gene profile II (*nuc* + *clfA* + *coa*) was 2 (5%), the gene profile III (*nuc* + *clfB* + *coa*) was 3 (7.5%), and the genes profile IV (*nuc* + *coa*) was 1 (2.5%).

Table 6: Number and percentage of *S. aureus* isolated from different samples on 100-Meter Street

Sample type	Samples (n)	Positive n (%)
Hand	20	11 (55)
Table	20	9 (45)
Machine	20	10 (50)
Knife	20	10 (50)
Meat	20	8 (40)
Total	100	48 (48)

Table 7: Comparative of the percentage of *S. aureus* isolated from different streets in Erbil city

Sample type	30 meter	40 meter	60 meter	100 meter	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)

Table 8: The number and percentage of genes found in *S. aureus* isolates

Gene	Number (n)	Percentage (%)
nuc	40	100% (40/40)
clfA	40	95% (38/40)
clfB	40	92.5% (37/40)
coa	40	100% (40/40)

Table 9: Types of gene profiles of *S. aureus* isolates (n = 40)

Gene profile	Staphylococcus genes	Isolate n(%)
I	*nuc* + *clfA* + *clfB* + *coa*	34 (85)
II	*nuc* + *clfA* + *coa*	2 (5)
III	*nuc* + *clfB* + *coa*	3 (7.5)
IV	*nuc* + *coa*	1 (2.5)

Discussion

It is a well-known truth that the primary way that harmful bacteria spread is through foodborne contamination. It is one of the leading causes of death and health complications, as well as enteric diseases in less economically developed countries (24). Meat is regarded as the most significant food source in the world since it gives customers the necessary amino acids, iron, phosphorus, and B complex vitamins, among numerous other nutrients (25). *S. aureus* is one of the most frequently identified organisms in meat processing plants, including contact and non-contact surfaces, raw materials, and various types of products (26). frequently, the incidence of *S. aureus* in the current study was high at 43.4% (152/350), as well as the incidence rate of *S. aureus* isolated from various areas in Erbil city was different, and this could be attributed to a variety of factors such as contamination the during transport of animals from farms to slaughterhouses, prolonged contact between healthy animals and infected animals in the abattoir, evisceration caused by both intestinal contents and the water applied to wash and rinse carcasses, and inadequate hygiene during the handling, transportation, and storage of carcasses for later use (27). In restaurants, the workers play an important role in causing cross-contamination between hands and meat while handling contaminated meat; for example, not wearing gloves, not washing their hands frequently, and wearing unclean clothes can lead to elevated bacterial contamination (28,29). However, minimizing the amount of *S. aureus* and other harmful bacteria in restaurants can be achieved by thoroughly washing and sanitizing equipment and tools before and after using them with meat (30,31).

Additionally, the incidence of *S. aureus* isolated from meat was 44.3% (31/70). The results of the analysis of this research were higher than in other studies, which appeared to show that the frequency of *S. aureus* in meat was 16.4% in the USA (32) and in African countries 33.1% (33). While earlier studies found that the prevalence of *S. aureus* in beef meat was higher than the results of the present study, in China, it was 50% (34), and in the USA, it was 65.6% (35). There are reasons why the prevalence of *S. aureus* varied between this study and other studies, including the sampling strategy, isolation techniques, location of carcass sampling, various cuts of meat, contamination before and after slaughter, meat storage, and meat processing (36). While the prevalence of *S. aureus* isolated from hands and tables was 44.3% (31/70), machines were 42.9% (30/70), and knives

were 41.4% (29/70). In addition to comparison with studies outside Iraq, in Thailand, the prevalence of *S. aureus* contamination in restaurants in the hands of food preparation workers was 78%, on tables was 26%, on plates was 23%, and on knives was 16% (37). The symbiotic bacterium *S. aureus* has been detected in the gastrointestinal tract, nose, and human skin. According to a prior study on nasal carriers operating in the production of food contexts, the *Staphylococcus* carrier can cause food contamination when in contact with respiratory secretions, which results in food poisoning due to *Staphylococcus* bacteria (38). *Staphylococcus aureus* can produce heat-stable toxins that are not destroyed by cooking (39).

In this investigation, a PCR assay was applied to identify the various types of genes, such as the *nuc* gene, which was used to identify *S. aureus* isolates. Based on numerous research studies, the PCR assay is a very sensitive and reliable method for identifying coagulase-positive *S. aureus* in contrast to coagulase-negative *Staphylococcus* species (40,41). In addition, the current study appeared to show that *S. aureus* possesses the *nuc* gene, and the results of this study agree with the results of the previous studies that declared that all coagulase-positive *S. aureus* have the *nuc* gene (31,42). Furthermore, this investigation revealed that the *coa* gene was 100% in all *S. aureus* isolates, indicating that this gene is essential pathogenicity of the bacterium. The results of this study were consistent with other studies that found that all *S. aureus* isolates possessed the *coa* gene with different molecular weight ranges of 514 bp to 802 bp (43). However, a different study found that the *coa* gene was present in every *S. aureus* isolate with the same molecular weight of 580 bp (44). In addition, adhesion-encoding genes, such as the *clfA* and *clfB* genes, were highly detected in *S. aureus* isolates. Early studies showed that *clfA* and *clfB* were detected in *S. aureus* isolates at 76% and 76.67%, respectively (45). According to studies, the *clfA* gene was found in 19-100% of *S. aureus* isolates, whereas the *clfB* gene was found in 91.8-92.9% of isolates (46-50).

Conclusions

It was a long study conducted in various geographic areas of Erbil City, Iraq. *Staphylococcus aureus* was isolated from meat in restaurants, which indicated that meat had been contaminated from slaughterhouses by handling, transport, and storage under unsanitary conditions. In addition, *S. aureus* was isolated from utensils used in restaurants that infected all utensils to help spread *S. aureus* and contaminated meat with pathogenic bacteria. Furthermore, all restaurants may use unpackaged meat or store it in non-refrigerated temperatures that help to provide suitable conditions for the growth and amplification of bacteria and cause food poisoning for consumers. Furthermore, *S. aureus* exceeds the utensils used in restaurants, which means that they do not wash, clean, and sterilize the utensils frequently, leading to meat contamination through *S. aureus*, which causes a huge problem for people. *S. aureus* possessed various types of gene encoding virulence factors, meaning a difference in sequence types of *S. aureus* was isolated in this study.

Acknowledgments

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

Conflict of interest

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

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الكشف الجزيئي عن الجينات التي تشفر عوامل الضراوة لجراثيم المكورات العنقودية الذهبية الإيجابية للأنزيم المُخَثِّر المعزولة من المطاعم

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الخلاصة

ارتبطت حالات انتشار الأمراض المنقولة بالغذاء مع الأغذية ذات المصدر الحيواني والتي تعد من اهم المشاكل الصحية الدولية. تعد جراثيم المكورات العنقودية الذهبية من أكثر الجراثيم التي تسبب الأمراض والتي تنتقل بالأغذية. كانت أهداف الدراسة الحالية هي عزل وتحديد جراثيم المكورات العنقودية الذهبية المعزولة من اللحوم والأدوات والأيادي في المطاعم في مدينة أربيل والتعرف على الجينات التي تشفر عوامل الضراوة بناء على طرق البيولوجيا الكلاسيكية والجزيئية. تم جمع ثلاثمائة وخمسين عينة مختلفة من مناطق مختلفة في أربيل، إقليم كردستان العراق (شوارع ٣٠ و٤٠ و٦٠ و١٠٠ متر) من أغسطس ٢٠٢٤ إلى نوفمبر ٢٠٢٤. أظهرت نتائج الدراسة الحالية أن معدل انتشار المكورات العنقودية الذهبية كان ٤٣,٤٪ (١٥٢/٣٥٠). كان معدل انتشار المكورات العنقودية الذهبية متفاوتا وفقا للمنطقة، في شارع ٣٠ مترا كان ٣٨,٨٪ (٣١/٨٠)، وفي شارع ٤٠ مترا كان ٥٠٪ (٤٠/٨٠)، وفي شارع ٦٠ مترا كان ٣٦,٧٪ (٣٣/٩٠)، وفي شارع ١٠٠ مترا كان ٤٨٪ (٤٨/١٠٠). بالإضافة إلى ذلك، تمتلك جميع المكورات العنقودية الذهبية جينات *nuc* و *coa* بنسبة ١٠٠٪ (٤٠/٤٠) بينما تمتلك المكورات العنقودية الذهبية جين *clfA* بنسبة ٩٥٪ (٣٨/٤٠) وجين *clfB* بنسبة ٩٢,٥٪ (٣٧/٤٠). تنقسم ملفات تعريف الجينات لعزلات المكورات العنقودية الذهبية إلى أربع أنماط جينية أساسية. كانت نسبة عزلات المكورات العنقودية الذهبية التي تمتلك النمط الجيني الأول ٨٥% (٣٤/٤٠)، والنمط الجيني الثاني ٥% (٢/٤٠)، والنمط الجيني الثالث ٧,٥% (٣/٤٠)، والنمط الجيني الرابع ٢,٥% (١/٤٠). تم عزل المكورات العنقودية الذهبية من اللحوم والأدوات المستخدمة في المطاعم والتي تلعب دورا هاما في نشر المكورات العنقودية الذهبية في المطاعم. امتلكت معظم عزلات المكورات العنقودية الذهبية جميع الجينات بناء على اختبار تفاعل البوليميرات المتسلسل.

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