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Detection the *spa* type of methicillin-resistant *Staphylococcus aureus* isolated from local Basturma in Mosul city, Iraq

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered the primary foodborne pathogen because of its diverse virulence factors, including staphylococcal enterotoxins that can lead to food poisoning in humans that are eating contaminated and undercooked food, such as raw milk and undercooked ground beef. Iraqi traditional meat products like basturma are made to preserve meat. Forty-five samples of locally made basturma were gathered from various local shops located throughout the city of Mosul. The study's original April 2023 deadline was extended to June 2023. This project depended on the classical methods and PCR method for detecting the *spa, nuc*, and *mecA* genes in *S. aureus*. The results of our investigation demonstrated that *S. aureus* could be identified in local basturma samples using conventional microbiological techniques and PCR methods at a rate of 3 out of 45 (6.7%). The *mecA* gene (100%), with a molecular weight of 147 bp, was discovered to be present in all *S. aureus* isolates that referred to all isolates as MRSA. The spa type of all *S. aureus* isolated was t213. Three novel *S. aureus* sequences are registered in the NCBI Genbank and have the accession numbers PP388962.1, PP388963.1, and PP388964.1. Extremely comparable *S. aureus* isolates from this investigation. Additionally, the *S. aureus* sample revealed a tight association with another *S. aureus* isolates from throughout the globe.

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Introduction

Spa typing is one of the most popular MRSA typing techniques. It is used to characterize the distribution and evolution of MRSA clones and to detect MRSA transmission, which depends on the sequencing of the *spa* gene (1,2). In addition to ensuring consistent and updated nomenclature, the 2003 installation of the Ridom StaphTyper software made it possible for researchers, groups of disease control, and doctors to exchange and liken information (3). Spa sequence typing was recently used for *S. aureus* molecular typing (4). The polymorphism in the X region of the *Staphylococcus* Protein A (*spa*) gene is determined by sequencing (5). *S. aureus* is a pathogen responsible for many infectious disorders, including skin and soft tissue infections, toxin-mediated illnesses, including pneumonia and bacteremia, are caused by the bacteria *S. aureus* up to 50% of people on the planet may also have *S. aureus* in their natural flora (6). Penicillin-binding protein 2a (PBP2a), translated by the *mecA* gene, is the mechanism through which *S. aureus* develops methicillin resistance (7,8). Quickly later, methicillin was prescribed as the medicament used for treating *S. aureus*, which is resistant to penicillin; MRSA was first identified in 1961 (9). Since then, MRSA cases have been documented in hospitals and community settings throughout the world, beginning in the early 1990s. When testing for staphylococci, oxacillin and cefoxitin were chosen over methicillin in the early 1990s, although the acronym MRSA is still used for historical reasons (10). It's critical to identify MRSA carriers to prevent and monitor

these infections. There is rising concern about MRSA infections contracted outside of hospitals as well as those obtained there. (11). Foodborne infections are a significant public health issue worldwide. As per the World Health Organization, foodborne disease (FBD) can be described as an infectious or toxic circumstance brought on by food or water consumption or has been suggested to be caused by it (12,13). A prominent problem in global public health programs is foodborne illness initiated by *S. aureus*, one of the most common types of FBD. It's among the most often cited reasons for FBD in American reports (14). *S. aureus* generates a diverse range of toxins. There are nine main serological types of heat-resistant enterotoxins in the group of Staphylococcal enterotoxins (SEs) (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ) (15). Foods frequently related to SFD included food-producing animals and food such as meat, egg products, milk and its products, salads, and cakes (16). Iraq produces a lot of basturma, a meat product, throughout the nation, but primarily in Mosul City (Nineveh governorate). They should only be consumed after heat processing (frying or grilling) and may be partially dried or smoked (17). meat and its byproducts food products are frequently contaminated during manufacture, storage, or sale, either directly from unsanitary conditions or indirectly via contaminated food animals (18).

The present investigation aimed to isolate and characterize MRSA from basturma, identify the risks linked to raw cured fermented meats and other primary product categories, and identify the spa gene using PCR technique to determine the correlation between the MRSA isolated in this study and MRSA isolated globally.

Materials and methods

Ethical approval

All samples were gathered with owner consent and used according to the ethical guidelines provided by the Institutional Animal Care and Use Committee at Mosul University's College of Veterinary Medicine, with an approved ID of UM. Vet. 2023.057.

Samples collection

Between April 2023 and June 2023, 45 samples of regional sausages were collected from various shops across several areas, including 8 samples of basturma taken from each of the following areas: Al-Noor, First Qadsia, Ras Al-Jada, Bab Al-Tob, and Al-Zahra, and 5 samples from Al-Tahrer in Mosul city/Iraq. The basturma from the area were gathered in sterile containers and put in containers with peptone water, and all samples were delivered to the Research Centre and Laboratories at Mosul University in an icebox. All samples were incubated for 18 to 24 hours at 37°C for pre-enrichment. Afterward, these samples were streaked over 7.5% Mannitol salt media and Blood media plates, which were incubated for a full day at 37°C.

S. aureus isolation and characterization

Gram staining, conventional biochemical methods, such as catalase and coagulase tests, and morphological evaluation were used to examine the distinctive *S. aureus* colonies (19).

Isolation of DNA

The positive isolates were cultivated on a mannitol-salt medium at 37°C for 24 h, allowing the genomic DNA extraction of *S. aureus*. The DNeasy Blood and Tissue Kit (Qiagen, Germany) was utilized for DNA isolation, following the constructor's Gram-positive bacteria-specific methodology. After being measured with Nanodrop (Biodrop, UK), the extracted DNA was kept at -20°C.

Reaction of PCR

Three genes were identified using the PCR assay: the *nuc* gene, the *mecA* gene, and the *spa* gene of MRSA. The *nuc* gene has a molecular weight of 166 bp (20), the *mecA* gene is 147 bp (21), and the *spa* gene's molecular weight varies (22). The mixture was comprised of This process was carried out in a 200 µl tube (Biozym, Oldenhorf, Germany). A total of 25 µl was used for the PCR reaction. The reaction mixture was composed of 12.5 µl of 2× GoTaq Green Mix-Master from Promega Corporation (USA), 1 µl of primer 1, 1 µl of primer 2, 6.5 µl of DNeasy-free water from Promega Corporation (USA), and 4 µl of the *S. aureus* DNA template and MRSA DNA template. Gel electrophoresis on a 2 % agarose gel (Peqlab, Germany) with a 100 bp ladder as a reference was used to analyze the resulting amplicons.

Protein A (spa) typing

Spa typing was carried out with specific primers; Table 1 provides further information. After amplifying the specific repeat area of Staphylococcal Protein A, PCR was used to sequence the DNA (22). The MegAlign software was used to assign all spa repeats and kinds. The Ridom Spa Server website (www.spaserver.ridom.de) was rendered, and numerical codes for spa typing and spa types were ascertained.

DNA sequencing

The samples were sent to Macrogen, a South Korean commercial sequencing business, to purify and sequence three PCR amplicons obtained from basturma isolates, which had all been previously determined to be *S. aureus*-positive by traditional PCR. The spa gene was the intended target for sequencing. The resulting *spa* gene sequences were compared using the NCBI BLASTn tool, edited at http://www.ncbi.nlm.nih.gov, against previously published *S. aureus* sequences available on GenBank. Using CLUSTALW from GenomeNet, an online multiple sequence alignment tool, the alignment and comparability of these sequences were further investigated. The Neighbor-Joining (NJ) program and the identical GenomeNet tool

CLUSTALW were utilized to create phylogenetic trees. To improve robustness, five hundred replicates of the *S. aureus* spa gene sequences were used as an outgroup while building the phylogenetic tree. This all-encompassing strategy aimed to better comprehend the phylogenetic context of the *S. aureus* isolates from basturma by utilizing purification, sequencing, and subsequent bioinformatics analysis to clarify the genetic relationships among the isolates.

Table 1: Sequences of Primers and PCR programs to detect *nuc*, *mecA,* and *spa* of *S. aureus* and MRSA

Gene	Primer	Sequence (5- 3)	Amplicon Size [bp]	Programme*	Reference
Nuc	nuc-1 nuc-2	5-CCTGAAGCAAGTGCATTTACGA-3 5-CTTTAGCCAA GCCTTGACGAACT-3	166	I	20
mecA	MEC A-1 MEC A-2	5-GTGAAGATATACCAAGTGATT-3 5-ATGCGCTATAGATTGAAAGGAT-3	147	II	21
Spa	SPA-1 SPA-2	5-AGACGATCCTTCGGTGAGC-3 5-GCTTTTGCAATGTCATTTACTG-3	Variable	I	22

PCR program: I: 35 times (94°C – 30s, 55°C – 30s, 72°C – 30s), II: 35 times (94°C – 30s, 54°C – 30s, 72°C – 30s).

Results

Based on the appearance of colonies on Mannitol salt agar, the colonies of S. aureus that yielded positive results exhibited a golden-yellowish color. Furthermore, affirmative outcomes were achieved for specific biochemical examinations like the coagulase and catalase tests, which substantiated the presence of positive S. aureus isolates. Our investigation demonstrated that among 45 isolates of S. aureus, 3 (6.7%) were identified as S. aureus, as depicted in Table 2 and Figure 1. Additionally, the results of the PCR assay disclosed the occurrence of the mecA gene in S. aureus. This confirmed that all the isolated S. aureus strains were MRSA, 12.5% (1/8) found in First Qadsia and 25% (2/8) found in Ras Al-Jada (Table 2 and Figure 2). As seen in Figure 3, the protein's X region found a gene spa in each isolate, and no discernible variations in the sizes of the amplified products were seen. Table 3 shows that one spa type was t213 (n = 3), which was created from the S. aureus isolates. (Table 3, Figure 3).

Table 2: The number of samples and percentage of positive *S. aureus* and MRSA isolates

Areas	Samples (No.)	No. of positive *S. aureus* (%)	No. of positive MRSA (%)
Al-Tahrir	5	0	0
First Qadsia	8	1 (12.5)	1 (12.5)
Al-Noor	8	0	0
Al-Zahra	8	0	0
Ras Al-Jada	8	2 (25%)	2 (25%)
Bab Al-Tob	8	0	0
Total	45	3 (6.7%)	3 (6.7%)

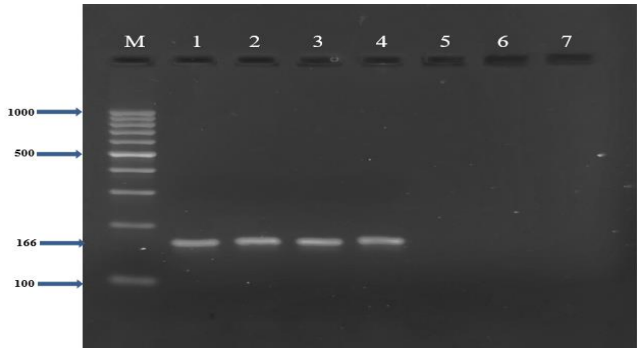


Figure 1 displays a 2% agarose gel electrophoresis image demonstrating the characteristic amplified product of the *nuc* gene. The amplification of DNA appears as a ladder-like pattern. Lane 1 is the positive control, and Lanes 2 – 4 represent positive isolates. Lanes 5 and 6 represent negative isolates. Lane 7 is the negative control, and Lane M is the DNA Marker 100 bp ladder (Biozym Diagnostic).

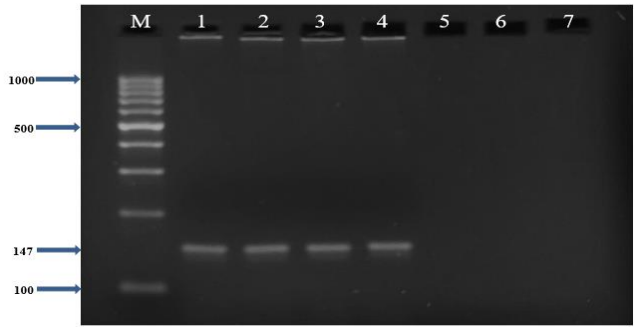


Figure 2: Illustrates a 2% agarose gel electrophoresis depiction presenting the characteristic amplified product of the *mecA* gene. The amplification of DNA appears as a ladder-like pattern. Lane 1 is the positive control, Lanes 2 – 4 represent positive isolates, Lanes 5 and 6 represent negative isolates, Lane 7 is the negative control, and Lane M is the DNA Marker 100 bp ladder (Biozym Diagnostic).

Table 3: Spa types' frequency of *S. aureus* isolates examined

Area	*Spa* type	Frequency	*Spa* repeat
First Qadsia	t213	1	07-23-12-21-24-33-22-17
Ras Al-Jada	t213	2	07-23-12-21-24-33-22-17

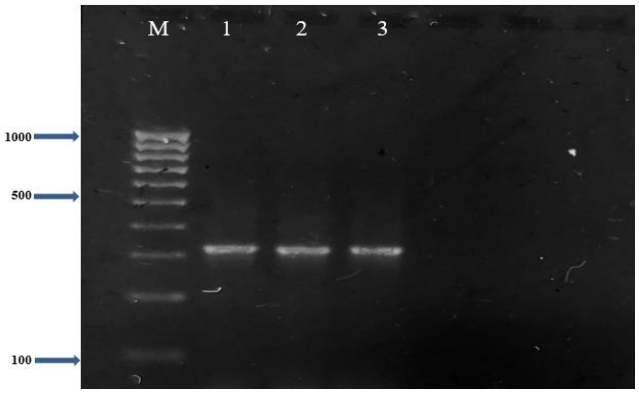


Figure 3: Illustrates a 2% agarose gel electrophoresis depiction presenting the characteristic amplified product of the protein A gene *spa* product. The amplification of DNA appears as a ladder-like pattern. Lanes 1 – 3 represent positive isolates, 4 - 6 represent negative isolates, Lane 7 is the negative control, and Lane M is the DNA Marker 100 bp ladder (Biozym Diagnostic).

In addition, three unique spa-type gene sequences were found from basturma. Individual sequencing analysis (BLASTn) was used to analyze the sequencing data. As indicated in Table 4, the S. aureus sequences in the NCBI Genbank are indexed underneath the previous accession numbers: PP388962.1, PP388963.1, and PP388964.1. Additionally, local spa-type gene sequences indicated a similarity between the S. aureus isolates in this study and those available in the GenBank database that were previously reported, according to a phylogenetic tree analysis performed in MegAlign software using the maximum likelihood technique. The sequence types obtained in this study (PP388962.1, PP388963.1, and PP388964.1) and the sequence types AP027135.1 Japan and EF203503.1 Australia had a 96.2% close relationship (Figure 4) (Table 5).

Table 4: The NCBI GenBank accession numbers for the *S. aureus* sequences in basturma

uidA gene	Bacteria	Types
PP388962.1	*S. aureus*	Bastruma
PP388963.1	*S. aureus*	Bastruma
PP388964.1	*S. aureus*	Bastruma

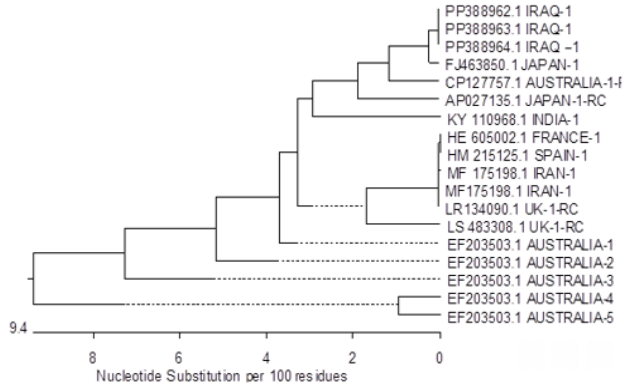
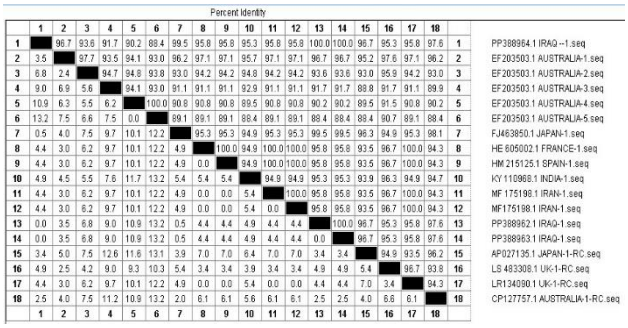


Figure 4 shows the phylogenetic tree for the partial spa gene sequence of the *S. aureus* local sequences available in the NCBI GenBank. Bootstrap supports (500 replications) are indicated by the numbers at the branches.

Table 5: DNASTAR-calculated the *spa* gene sequence similarity and divergence of each pair for *S. aureus.*



Sequence pair comparisons with the tree produced by MegAlign DNASTAR are used to calculate a percentage divergence. By utilizing % similarity, sequences are directly compared without considering evolutionary ties.

Discussion

The World Health Organization (WHO) defines foodborne diseases (FBDs) as infectious or toxic illnesses brought on by or supposed to be taken on by consuming food or water. Many different disease-causing microorganisms that can contaminate food can cause more than 250 FBD, including food poisoning in humans (23). *S. aureus* is a cause of foodborne infections in the majority of countries worldwide (24). Traditional methods in several countries involve dry-curing, rinsing, pressing, and drying whole-muscle beef chunks before coating them with a spice paste. This product is known as basturma. Unfortunately, *S. aureus* occurred in basturma due to several factors, including contaminated water used for washing and rinsing carcasses and intestinal contents during evisceration or poor hygiene during transportation and slaughter (25). Increased cross-contamination between workers and meat can occur from

improper personal hygiene practices during meat processing. For example, forgetting to wear gloves, wearing dirty clothes, and not washing your hands can all lead to increased levels of *S. aureus* contamination (26). Nonetheless, *S. aureus* and other dangerous germs can be removed from butcher shops by following proper sanitation procedures and cleaning procedures for tools and equipment before and after handling meat (27).

This investigation used PCR against the *nuc* gene to identify *S. aureus* isolates and the *mecA* gene to detect MRSA. PCR is a highly sensitive and accurate method for differentiating *S. aureus* from other Staphylococci species, as demonstrated by numerous research (28,29). For the quick identification of MRSA in food samples, the *nuc* and *mecA* gene PCR amplification strategy is very successful and regarded as the gold standard method for MRSA identification (30). In this study, 3 (6.7%) of the 45 basturma samples tested positive for *S. aureus* isolates with the *nuc* and *mecA* genes. The occurrence of *S. aureus* in this investigation was lower than that of prior research, which reported 30% in Saudi Arabia (31), 50% in Morocco (32), and 42.3% in the United States of America (33). The results of this study indicated that the occurrence of *S. aureus* was almost in agreement with studies that found 5.6% in Iraq (34) and 6.9% in Iowa (35). Differences between the current study's results and those from the previously stated research may be attributed to variations in the food's source, additional components, preparation methods, and laboratory techniques.

MRSA poses a severe risk to human and animal public health (31). In recent years, the death rate from MRSA infections has remained high. Numerous earlier investigations have documented the isolation of MRSA from animals (36). Based on the geographic distribution, MRSA can be isolated and identified differently from meat and its products in different parts of the same nation and between different countries. Furthermore, out of 45 basturma samples, 3 (6.7%) had MRSA identified. The previous studies showed that 2.9% of samples tested positive for MRSA during a one-year survey conducted among 3520 retail meats in the USA (37). Meanwhile, an Italian study discovered that MRSA was present in 6 out of 160 (3.75%) foods derived from animals (38). However, 3.6% (15/421) of retail meat in Korea included MRSA (39).

In the protein, the X region in each isolate, a gene spa was detected; the size of the amplified products detected was spa type t213. The previous study in Bangladesh between January 2021 and December 2022 showed that all *S. aureus* isolated from the inpatients or outpatients at the Hospital showed clinical signs; the t213 was discovered in all *S. aureus* isolates (40). Furthermore, between December 2008 and December 2009, t213 was the spa type of MRSA that was recovered from nasal swabs of patients in the Thames Valley Primary Care Research Partnership (41). Moreover, 212 rodents were found near a house in Guangzhou, Southern China, on the lawn and in the trash can. The spa type of MRSA isolated from them was t213 (42-46).

Moreover, the phylogenetic tree of the *spa* gene sequences in *S. aureus* isolated from Mosul city revealed a tight link between *S. aureus* obtained for this study. Sequence *S. aureus*, PP388962.1, PP388963.1, and PP388964.1, as well as AP027135.1 Japan and EF203503.1 Australia, were my sequence kinds, exhibited a 96.2% significant connection. These molecular targets are effective genotyping techniques for the spa genus of the *S. aureus* isolates. The previous studies identified *S. aureus* species based on the *spa* gene, further supported by the strong similarity results with this study.

Conclusion

The principal foodborne pathogen responsible for producing several types of staphylococcal enterotoxins, resulting in food poisoning in humans, was discussed in the study. Butcher shops can use knives, hooks, tables, machinery, and staff hands to contaminate meat products with *S. aureus*. These items are important in the contamination and spread of *S. aureus* to foods. One meat product that could be contaminated with *S. aureus* or other foodborne pathogens is basturma. Additionally, the PCR method is a useful molecular diagnostic tool for detecting the characterization and identification of *S. aureus* isolates from basturma, opening up new avenues for research and shedding light on the potential implications of *S. aureus* in foods of animal origin.

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Conflict of interest

The manuscript's author has confirmed that no conflicts of interest arose during the writing or data analysis phases.

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الكشف عن نوع *spa* في جراثيم المكورات العنقودية المقاومة للميثيسيلين المعزولة من الباسطرمة المحلية في مدينة الموصل، العراق

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الخلاصة

تعتبر جراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين العامل الممرض الرئيسي الذي ينتقل عن طريق الأغذية بسبب عوامل الفوعة المتنوعة، بما في ذلك السموم المعوية للمكورات العنقودية التي يمكن أن تؤدي إلى التسمم الغذائي لدى البشر نتيجة تناوله الأطعمة الملوثة وغير المطبوخة جيدا، مثل الحليب الخام ولحم البقر المفروم غير المطبوخ جيدا. يتم تصنيع منتجات اللحوم التقليدية العراقية مثل البسطرمة بهدف الحفاظ على اللحوم. تم جمع ٤٥ عينة من البسطرمة المصنوعة محلياً من مختلف المتاجر المحلية المنتشرة في جميع أنحاء مدينة الموصل. كانت فترة الدراسة ما بين شهر نيسان ٢٠٢٣ إلى شهر حزيران ٢٠٢٣. تم استخدام الطرائق التقليدية للتشخيص وطريقة تفاعل البلمرة المتسلس في تحديد جينات *spa* و *nuc* و *mecA*. وأظهرت نتائج الدراسة تم التعرف على المكورات العنقودية الذهبية في عينات الباستورما المحلية باستخدام كل من التقنيات الميكروبيولوجية التقليدية وطريقة تفاعل البلمرة المتسلسل، حيث كان العزل بمعدل ٣ من ٤٥ (٦,٧٪). تم تحديد وجود جين *mecA* ١٠٠% بوزن جزيئي ١٤٧ زوج أساس، في جميع عزلات المكورات العنقودية الذهبية. كان نوع السبا لجميع المكورات العنقودية الذهبية المعزولة هو t213. ثلاث تسلسلات جديدة لعزلات المكورات العنقودية الذهبية مسجلة في المركز الوطني لمعلومات التكنولوجيا الحيوية؛ جزء من المعاهد الصحة الوطنية في الولايات المتحدة) ولديها أرقام الانضمام: PP388962.1، PP388963.1، و.PP388964.1. اضافة الى ذلك أشارت الدراسة إلى أن جميع العزلات جراثيم المكورات العنقودية الذهبية هي جراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين. بالإضافة إلى ذلك، كشفت عينة المكورات العنقودية الذهبية عن وجود ارتباط وثيق مع عزلات أخرى من المكورات العنقودية الذهبية من جميع أنحاء العالم.

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