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Detection of positive mecA *Staphylococcus aureus* isolated from meat and butchers' shops by using PCR technique in Mosul city

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Abstract

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) can harm to public health as they can cause widespread food poisoning and resistance to multiple antibiotics. Therefore, we aimed to investigate the percentage of *S. aureus* and MRSA among meat and butcher shop tools, utensils, and workers' hands by detecting the presence of the *nuc* and *mecA* genes in *S. aureus* and MRSA isolates, respectively using PCR technique in Mosul city. In this study, we randomly selected 300 samples from meat and various butcher shop surfaces in Mosul city between September 2021 and January 2022. The findings confirmed that *S. aureus* carried the *nuc* gene in meat 64% and other samples, ranging from 36% in machines to 82% in knives. Additionally, out of 178 positive *S. aureus* isolates in this study, 94 samples showed a positive *mecA* gene for *S. aureus*. Significant differences were observed among the meat and various utensils of butcher shops. The meat had a higher prevalence of methicillin-resistant *S. aureus* isolates reach 75% more than the other tools and utensils (machines 38.9%, tables 36.7%, worker hands 38.5%). Further research is necessary to evaluate the existence of enterotoxin genes in *Staphylococcus aureus* of different meat products.

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Introduction

Food safety practices aim to prevent harmful microbiological and chemical agents from entering, growing, or surviving in the food supply chain (1,2). Worldwide, microbial toxins are ranked as the third most crucial factor in food-borne illnesses. In 2015, *Staphylococcus aureus* enterotoxins accounted for more than half of all food-borne outbreaks linked to bacterial toxins (3). According to the Centers for Disease Control (CDC), Staphylococcal food poisoning causes 240,000 cases annually, resulting in one thousand hospitalizations, and six deaths in the United States (4). Food contamination often occurs as a direct result of pathogens present in animals raised for food and/or due to inadequate hygienic practices during the manufacturing, storage, or sale of food products (5,6). Humans and animals are considered major reservoirs for the transmission of *S. aureus* (7). The severity of the disease is influenced by the number of toxins consumed and the general health of the consumer. Patients experience symptoms like nausea and vomiting soon after consuming the toxins. These symptoms are often accompanied by fever and watery diarrhea (8). *S. aureus* can develop resistance to a variety of widely used antimicrobials (9). Antibiotic misuse or overuse in animal production has been linked to the growth and spread of pathogens that are resistant to antibiotics (10). These microorganisms carry genes linked to antibiotic resistance that can spread to humans through food

handlers and animals that produce food (11). This poses a threat to the effective treatment of infectious diseases (12). Two years after the discovery of penicillin, initial resistant *S. aureus* strains were discovered (13). Methicillin was initially used as an antibiotic in 1959, and the first clinically recognized strain of methicillin-resistant *S. aureus* (MRSA) was discovered in 1961 (9). Penicillin, cephalosporins, and the majority of beta-lactam antibiotics are ineffective against Staphylococcal strains (14,15). Healthcare-associated MRSA (HA-MRSA) outbreaks in hospitals have occurred frequently throughout the world in recent decades (16). The penicillin-binding protein (Enzyme-PBP2a), encoded by the *mecA* gene, is the primary pathway responsible for methicillin resistance in *S. aureus*. Due to this protein's decreased affinity for beta-lactam antibiotics, peptidoglycan strand cross-linking occurs during microbial cell wall synthesis (17-19).

The *nuc* gene is frequently utilized as a specific target for the detection of *S. aureus* when using the molecular method. Given the importance of this bacterium in terms of food safety and its ability to resist antibiotics, we aimed to use PCR to confirm the presence of *nuc* and *mecA* genes in *S. aureus* and MRSA, respectively, among meat and butcher shops (tools, utensils, and workers' hands) in Mosul city.

Materials and methods

Ethical approval

The research design for this work was approved by the scientific committee of the Department of Veterinary Public Health.

Sampling

Three hundred samples were randomly selected in this study from veal meats and various butcher shops on the left side of Mosul city, with 50 samples collected from each of the following: knives, hooks, tables, machines, worker's hands, and veal meat. Additionally, meat samples 250±25 g consisting of lean meat from either chuck or round were purchased from 50 butcher shops during shop visits conducted between September 2021 and January 2022. Meat samples were collected aseptically in sterile containers, while other samples were taken using sterile swabs that were placed in sterile containers and transported to the laboratory (College of Veterinary Medicine-University of Mosul) for analysis.

Isolation and identification of *S. aureus*

All samples were initially cultivated on blood agar. Thereafter, two selective media, Mannitol Salt Agar and Vogel Johnson Agar (Lab M.L T. House, UK) were used for the selection of *S. aureus* and MRSA colonies. Each plate was incubated under aerobic conditions at 37°C for 24 hours at 37°C. The typical *S. aureus* colonies were examined by gram staining for bacterial morphology, and conventional biochemical techniques were used to confirm the isolates by coagulase and catalase tests (20).

DNA extraction of *S. aureus*

All the positive isolates of *S. aureus* were grown on mannitol salt agar for 24 hours at 37°C. Genomic DNA for *S. aureus* was extracted based on the manufacturer's protocol for the Gram-positive bacteria kit (Qiagen, H., Germany). The amount of extracted DNA was measured using a nanophotometer (Biodrop, UK), and the DNA was used as the template for PCR and stored at −20 °C for subsequent procedures.

PCR reaction

The PCR assay was used to identify the *mecA* gene of MRSA and the specific-species *nuc* gene of *S. aureus*. A sterile 200 µl Eppendorf tube (Biozym, Oldenhorf, Germany) was used for PCR reaction. The mixture had a total volume of 25 µl, which contained 1 µl of each F- and R-primer (each ten pmol/ µl). The thermos-nuclease (*nuc*) gene (166 bp) primers *nuc*-F: CCTGAAGCAAGTGCATTTACGA and nuc-R: CTTTAGCCAA GCCTTGACGAACT were used for confirming *S. aureus* species (21). Meanwhile, the *mecA* gene (147 bp) primers (*mecA*-F: GTGAAGATATACCAAGTGATT and *mecA*-R: ATGCGCTATAGATTGAAAGGAT) used for confirming the MRSA isolates (22). Eight µl of double-distilled water and 12.5 µl Go Taq Green Master Mix (Promega/USA) were added to the reaction. Then, a 2.5 µl DNA template of *S. aureus* or MRSA was added to the Eppendorf tube.

The thermocycler protocol for the *nuc* gene included a 5-minute initial denaturation step at 95°C, followed by thirty-five cycles of 95°C (denaturation), 54°C (annealing), 72°C (extension) for 30 seconds each. After that, the final extension was carried out for 5 min at 72°C. The program for the *mecA* gene was carried out as follows: Initial denaturation was performed at 94 °C for 5 min, then there were 10 cycles of 94°C (denaturation) for 45s, 65°C (annealing) for 45s, and 72°C (extension) for 1.5 min. Twenty-five more cycles of 94°C (denaturation) for 45s, 55°C (annealing) for 45s, and 72°C (extension) for 1.5 min were performed. The process was then completed with a final extension of 72°C for 10 min and held at 4°C. Amplicons were determined using a hundred-bp DNA ladder and gel electrophoresis.

Data analysis

JMP Pro 16.1 software 2021 from SAS Institute Inc., North Carolina, USA, was used to conduct descriptive and inferential statistics (23). The Chi-square test was used to compare the frequency of *S. aureus* or MRSA in meat, butcher shop tools and utensils to determine if there were any statistically significant difference. Findings with $P<0.05$, were considered significant.

Results

Although traditional microbiological procedures take some time, they are still considered the gold standard for detecting and verifying the presence of *S. aureus* in samples. Based on *S. aureus's* morphological characteristics, the positive isolates were identified as Gram-positive (spherical shape in clusters), catalase-positive, and coagulase-positive. They also displayed β-hemolysis on blood agar and formed golden-yellow colonies on mannitol salt agar. Molecular detection of the *S. aureus nuc* gene in various samples showed different levels of contamination ranging from 18 isolates in machines to 41 isolates in knives (Table 1). Of the 300 samples included in this study, 178 samples showed a positive *nuc* gene for *S. aureus* (Figure 1). Significant differences (P<0.01) were observed among the meat and various utensils in butcher shops. The Machines 36% had a lower proportion of *S. aureus* isolates than the other tools and utensils (knives 82%; meat 64%; hooks 62%; tables 60%) (Table 1). On the other hand, MRSA was also isolated from meats, tools, and various utensils from butcher shops. Out of the 178 positive *S. aureus* isolates in this study, 94 samples showed a positive *mecA* gene for MARS (Figure 2).

Table 1: The incidence of *S. aureus* in meat, tools, and various utensils of butcher shops

Sample items	No. of Samples	Positive samples	No. *S. aureus* isolates (n)	Percentages
Meat	50	32	32^{ab}	64%
Knives	50	41	41^{a}	82%
Hooks	50	31	31^{b}	62%
Tables	50	30	30^{b}	60%
Machines	50	18	18^{c}	36%
Worker Hands	50	26	26^{bc}	52%
Total	300	178	178	59.3%

Within the same column, frequencies with different letters are significantly different (P<0.01).

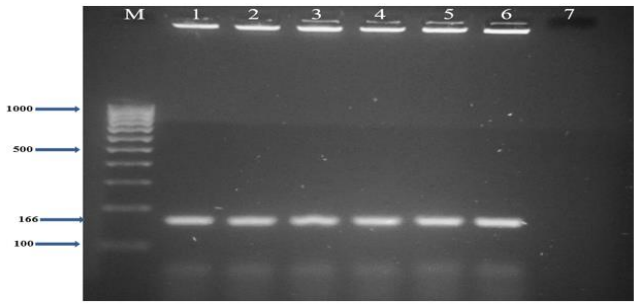

Figure 1: Positive samples are in lanes two through six. Positive control of the *nuc* gene (166 bp) is located in lane one, whereas negative controls are in lane seven. Lanes M: 100 bp DNA ladder (Biozym Diagnostic).

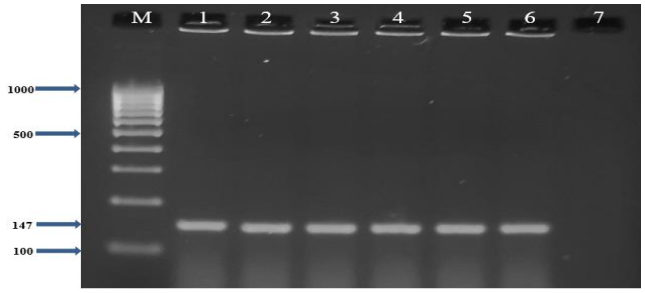

Figure 2: Positive samples are in lanes two through six. Positive control of the *mecA* gene (147 bp) is located in lane one, whereas negative controls are in lane seven. Lanes M: 100 bp DNA ladder (Biozym Diagnostic).

Significant differences (P<0.01) among the meat and various utensils of butcher shops were observed. The meat samples 75% had a higher prevalence of Methicillin-resistant *S. aureus* (MRSA) isolates compared to the other tools and utensils such as machines 38.9%, tables 36.7%, and worker hands 38.5% (Table 2).

Table 2: The incidence of MRSA in meats, tools, and various utensils of butcher shops

Sample items	No. of positive S. aureus	No. of MRSA	Percentages
Meat	32	24^{a}	75%
Knives	41	26^{ab}	63.4%
Hooks	31	16^{abc}	51.6%
Tables	30	11^{c}	36.7%
Machines	18	7^{bc}	38.9%
Worker Hands	26	10^{c}	38.5%
Total	178	94	50.7%

Within the same column, frequencies with different letters are significantly different (P<0.01).

Discussion

Meat is considered the most important food source worldwide, as it provides vital amino acids, B complex vitamins, iron, and phosphorous, among other nutrients, to consumers (24). However, meat is also recognized as a significant source of foodborne illnesses including food poisoning in humans (25). In most nations around the world,

the common cause of foodborne illnesses is *S. aureus* (26). Unfortunately, the prevalence of *S. aureus* in the current study was high, and this could be attributed to several factors such as contamination during evisceration caused by intestinal contents and water used for washing and rinsing carcasses, or poor hygiene during transportation and slaughter (27). Failure to maintain proper personal hygiene while handling meat can result in increased cross-contamination between meat and workers. For instance, not washing hands, wearing unclean clothes, and failing to wear gloves can all contribute to higher levels of bacterial contamination (28,29). However, proper sanitation and cleaning of utensils and equipment before and after handling meat in butcher shops can help eliminating *S. aureus* and other harmful microbes (30).

In this study, *S. aureus* isolates were identified using PCR targeting the *nuc* gene. Numerous studies have shown that PCR is an accurate and highly sensitive technique for distinguishing *Staphylococcus aureus* from other Staphylococci species (31, 32). The *nuc* gene PCR amplification approach for *S. aureus* identification is considered as the gold standard method and is highly effective for the rapid diagnosis of *S. aureus* in food samples (32). In our study, out of 50 meat samples, 32 (64%) were positive for *S. aureus* isolates that were positive for the *nuc* gene. Khalil (33), Hanson *et al.* (34), Ezzat *et al.* (35), Goja *et al.* (36), and Saleh *et al.* (37) conducted multiple studies that showed a lower prevalence of *S. aureus* 5.6, 6.9, 10, 12, and 22%, respectively). However, several studies consistently showed a high incidence, like our findings, with a prevalence 49.1, 50, and 50.4% (38-40), respectively. Another study reported a higher prevalence of *S. aureus* than our study, where the prevalence of *S. aureus* in retail meat was 76.47% (41) and 76.67% (42).

Contamination of utensils and tools in butcher shops such as knives 82%, hooks 62%, and tables 60% with *S. aureus* may explain the main cause of increased meat contamination. However, another study revealed lower prevalence of *S. aureus* than our study, where the prevalence of *S. aureus* was in butcher shop tools such as cutting tables 15%, hooks 15%, and knives 22%5 (43). A study conducted in the city of Mosul showed that more than 60% of red meat is displayed without refrigeration when sold in meat markets (44), which may facilitate the multiplication of microorganisms in meat at room temperature. In addition, failure to use proper packaging for meat after cutting may increase the cross-contamination between meat and butcher shop surfaces.

Lastly, more than 50% of the *S. aureus* isolates in our investigation have the same phenotypic characteristics as MRSA because they carried the *mecA* gene. There is evidence that livestock may act as a reservoir for *S. aureus* that can spread widely, especially from cattle to humans, which first appeared in animals and then jumped to human 40 years ago (45,46). Several studies from various countries have documented the isolation of MRSA from animal-derived foods, primarily from meat. For instance, in the Netherlands 11.9%, Brazil 23.3%, and Pakistan 63% (47-49) where samples of raw beef from the retail trade tested positive for MRSA (presence of the *mecA* gene). On the other hand, our findings showed that samples of workers' hands and tools had a higher prevalence of MRSA contamination (knives 63.4%; hooks 51.6%; workers' hands 38.5%) compared to the study in Pakistan (knives 45%; hooks 18%; Workers' hands 18%) (49). An important risk factor for increasing the incidence of MRSA, enhancing the evolutionary process of resistance, and accelerating the emergence and spread of MRSA is the inappropriate use of antimicrobial drugs in farm animals (50,51).

Conclusions

Although it was a short study conducted in a small geographical location, it shows that MRSA and *S. aureus* are prevalent in butcher shops. This finding indicates that *S. aureus* contamination of retail meat was widespread in Mosul city due to the lack of implementation of food safety practices from farms to butcher shops. The best evidence for this is the high percentage of Staphylococcus contamination on utensils in our samples. In addition, the non-use of meat packaging before it is sold and the absence of refrigeration when meat is displayed for sale in butcher shops are considered the main cause of increased Staphylococcal contamination. Moreover, the high incidence of MRSA may indicate that the indiscriminate administration of antibacterial drugs in the treatment of illnesses can lead to resistance in *S. aureus*. Further investigation is necessary to evaluate the hazards of MRSA colonization in animals and anyone who handles raw meat.

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Conflict of interest

The authors of the manuscript state that neither the writing nor the data analysis had any conflicts of interest.

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تحديد جين ميك أ في جراثيم المكورات العنقودية الذهبية المعزولة من اللحوم ومحلات القصابة باستخدام تقنية تفاعل البلمرة المتسلسل في مدينة الموصل

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الخلاصة

المكورات العنقودية الذهبية المقاومة للميثيسيلين يمكن أن تكون ضارة بالصحة العامة لأنها تؤدي إلى التسمم غذائي على نطاق واسع. لذلك، حاولنا تحديد مدى انتشار بكتيريا المكورات العنقودية والمرسا بين اللحوم والأدوات والأواني وأيدي العمال في محلات القصابة من خلال الكشف عن وجود جين نوس في عزلات المكورات العنقودية وجين ميك أ في عزلات المرسا باستخدام تقنية تفاعل البوليميراز المتسلسل في مدينة الموصل. في هذه الدراسة، تم اختيار ٣٠٠ عينة عشوائياً من اللحوم وأسطح محلات القصابة المختلفة في مدينة الموصل بين أيلول ٢٠٢١ وكانون الثاني ٢٠٢٢. وأكدت النتائج أن المكورات العنقودية الذهبية تحمل جين نوس في اللحوم وبنسبة ٦٤٪ وعينات أخرى تراوحت النسب من ٣٦٪ في المكائن إلى ٨٢٪ في السكاكين. بالإضافة إلى ذلك، من بين ١٧٨ من المكورات العنقودية الذهبية المعزولة في هذه الدراسة، أظهرت ٩٤ عينة موجبة لجين ميك أ للمكورات العنقودية الذهبية. كما كان هناك فروق معنوية بين اللحوم والأواني المختلفة لمحلات القصابة، حيث أن ٧٥٪ من عينات اللحوم كانت تحتوي على المكورات العنقودية الذهبية المقاومة للميثيسيلين وهي أعلى من باقي الأدوات والأواني (المكائن ٣٨,٩٪، المناضد ٣٦,٧٪، أيدي العمال ٣٨,٥٪). مزيد من البحوث ضرورية لتقييم وجود الجينات المعوية في بكتريا المكورة العنقودية الذهبية لمنتجات اللحوم المختلفة.