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Molecular detection of methicillin resistant *Staphylococcus aureus* isolated from local fish in Mosul city

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Abstract

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) can harm public health as they can cause widespread food poisoning and resistance to multiple antibiotics. Hence, our objective was to examine the prevalence of *S. aureus* and MRSA among fish shops, utensils, and workers' hands in Mosul City. We were able to find the *nuc* and *mecA* genes in *S. aureus* and MRSA isolates, respectively, via the PCR approach. In March and April of 2023, one hundred samples randomly selected from fish and various other store surfaces in Mosul were taken for this inquiry. *S. aureus* isolates the *nuc* gene, which can be identified in 19% of the samples. The worker hand samples had eight of the top isolates, in contrast to the fish's three isolates out of twenty. Additionally, because the *mecA* gene existed in 12 of the 19 *S. aureus* isolates (63.2%), methicillin resistance was shown. The hand samples of the workers had a higher percentage of methicillin-resistant *S. aureus* isolates 75% (6/8) than other samples. According to the study, a significant amount of *S. aureus* was showing in fish and associated utensils, revealing that the fishing industry was not suitably subsequent food safety laws. Based on this study, a significant amount of *S. aureus* was discovered in fish and associated utensils, revealing that the fishing industry was not appropriately following food safety laws.

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Introduction

Seafood, which includes fish, shrimp and oysters, is a high-protein food that is low in calories and saturated fat (1). Fish consumption per person has increased globally over the last few decades (2). Fish, rich in vitamins and minerals, has been demonstrated to have several positive health effects. It delivers vital nutrients throughout prenatal growth and development and lowers the risk of heart illness for people of all ages (1). Nonetheless, fish and fish products, especially those consumed raw or undercooked, are often associated with human illnesses (3). Direct contact with a contaminated aquatic environment and ingested contaminated fish are two possible causes of the presence of various species of bacteria, including harmful pathogens for humans. Therefore, pathogens found in fish reflect the overall health and safety of aquatic ecosystems (4). Seafood-borne illnesses constitute a severe risk to public health both locally and globally. Microorganisms like *Escherichia coli*, *Vibrio* spp., *Salmonella* spp., *Listeria* spp., *Shigella* spp., *and Staphylococcus* spp. are the most frequently implicated in seafood poisoning (5). Twenty-five percent of healthy individuals and animals have asymptomatic *Staphylococcus aureus* (*S. aureus*) in their noses and skin, and it has been linked to fish and other seafood products (6). Among the *Staphylococcaceae* family members, *S. aureus* seems to be the most invasive species, causing various diseases that frequently result in mortality (7). Indeed, *S. aureus* is capable of producing staphylococcal enterotoxins (SEs), which positions it as one of the potential foodborne pathogens (8)

Correct, the staphylococcal enterotoxins (SEs) produced by *S. aureus* can cause foodborne illnesses characterized by symptoms such as vomiting and diarrhea. It is essential to note that these toxins are heat-stable, meaning they can withstand cooking temperatures and do not degrade, making proper food handling and hygiene crucial in preventing foodborne illnesses caused by *S. aureus* (9). Most *S. aureus* strains (95%) are markedly resistant to penicillin and its derived compounds (10). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant human disease as well as a historically emerging zoonotic pathogen that is significant for both public health and veterinary care. Severe infectious illnesses such as food poisoning, suppurative pneumonia, Osteomyelitis, pyogenic endocarditis, and otitis media are all frequently caused by MRSA in humans (11,12). Clinically, the identification of methicillin resistance in *Staphylococcus aureus* is commonly done using PCR-based methods to detect the presence of the *mecA* gene and cefoxitin resistance. This antimicrobial resistance is mainly caused by the penicillin-binding protein, primarily encoded by the *mecA* gene (13,14). MRSA are hostile zoonotic biovars of *S. aureus* that meet the requirements for being methicillin- and cefoxitin-resistant. Several phenotypic and molecular characteristics can differentiate methicillin-susceptible *S. aureus* (MSSA) and MRSA. Non-penicillin antimicrobial classes such as macrolides, fluoroquinolones, aminoglycosides, tetracyclines, and lincosamides are among the many antibiotic classes for which MRSA consistently displays a multidrug resistance pattern (15-17). Consumers who consume products contaminated with MRSA present a severe risk to their health since they may spread resistance to others (18). Few reports about MRSA in fish and fisheries products are available (19).

Therefore, we aimed to find out the occurrence of MRSA in local fish, which may be a source of infection and a public health problem.

Materials and methods

Ethical approval

The research was approved by the Ethics Committee of the College of the Veterinary Medicine /University of Mosul. No. UM.VET.2023.017.

Samples collection

The study area was Nineveh Governorate, which included various regions (Al-Maidan district, Hay Al-Suqar, and Al-Nabi Younis district). From March to April 2023, samples of fish skin and local shops, including worker hands, knives, tables, and fish water, were collected. All samples were swabbed with sterilized swabs before being put in tubes with peptone water and pre-enrichment at 37°C for 18 to 24 hours. The samples were then brought in an icebox to the Research Center and Laboratories at Mosul University. After being streaked onto Blood media and Mannitol salt media 7.5% plates, the samples underwent a 24-hour incubation at 37°C.

S. aureus isolation and characterization

Gram staining, common biochemical techniques, including catalase and coagulase tests, and morphological appearance (20) were utilized to analyze the typical *S. aureus* colonies.

DNA Isolation

Positive isolates were cultured on mannitol salt medium for 24 hours at 37°C to isolate the DNA of *S. aureus*. DNA was recovered using the DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines for Gram-positive bacteria. Using Nanodrop (Jenway^{TM} Genova Nano, UK), the extracted DNA was weighed and kept at -20°C for additional testing.

PCR Reaction

Both the *mecA* gene and the particular species *nuc* gene of *S. aureus* have been identified using the PCR assay. In a 200 µL tube (Biozym, Oldenhorf, Germany) with a total volume of 25 µL, the reaction was carried out using 1 µL of each Forward and Reverse primers (each 10 pmol/L) (Eurofins Genomics, Ebersberg, Germany) (Table 1). The *nuc* gene has a molecular weight of 166 bp (21) compared to the *mecA* gene's 147 bp (22). Eight µL of double-distilled water (Promega) and 12.5 µL of 2Go Taq Green Master Mix made up the reaction. Finally, 2.5 l of *S. aureus* or MRSA DNA template were added to each reaction, and the amplicons were identified utilizing 2% agarose gel and a 100 bp ladder for gel electrophoresis (Peqlab, Erlangen, Germany).

Table 1: Primers and PCR programs for *S. aureus* (*nuc* gene) and MRSA (*mecA*) detection

Gene	Primer	Sequence (5- 3)	Fragment size [bp]	Program*	Reference
nuc	*nuc*-F	CCTGAAGCAAGTGCATTTACGA	166	I	(21)
	nuc-R	CTTTAGCCAA GCCTTGACGAACT			
mecA	*mecA*-F	GTGAAGATATACCAAGTGATT	147	II	(22)
	mecA-R	ATGCGCTATAGATTGAAAGGAT			

*PCR program: I: 35 cycles (94°C for 30s, 55°C for 30s, and 72°C for 30s); II: 35 cycles (94°C for 30s, 54°C for 30s, and 72°C for 30s).

Results

S. aureus colonies that tested positive had a Golden-yellowish colony, as shown on Mannitol salt agar. Additionally, positive results from specific biochemical assays, such as the coagulase and catalase tests, were used to confirm the presence of *S. aureus* isolates. According to our research, *S. aureus* was isolated in 19 out of 100 samples (19%). *S. aureus* was confirmed by the presence of the *nuc* gene in a high proportion of samples (8 out of 20) in the worker hand samples, compared to 2 out of 20 in the knife samples and 3 out of 20 in fish samples (Table 2 and Figure 1).

Table 2: Number of positive *S. aureus* in different sample items in fish shops

Sample type	Sample (n)	Positive n (%)
Fish	20	3 (15%)
Table	20	3 (15%)
Knives	20	2 (10%)
Worker hands	20	8 (40%)
Fish water	20	3 (15%)
Total	100	19 (19%)

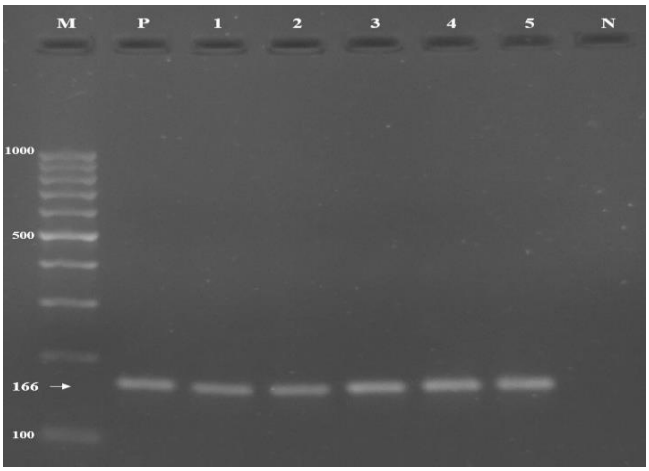

Figure 1: Fragment size of *nuc* gene (166 bp), lane M: ladder, lane P: +ve control (*S. aureus*), lane N: -ve control, and lanes 1-5: +ve samples.

Moreover, the results of the PCR assay showed that the *mecA* gene was present in most *S. aureus* isolates (MRSA), with a detection rate of 63.2% (12 out of 19). The worker hand samples showed a high percentage of MRSA isolates, with a detection rate of 75% (6 out of 8) that tested positive for the *mecA* gene. On the other hand, the table and fish water samples had a lower percentage of MRSA isolates at 33.3% (1 out of 3). The percentages of MRSA isolates that were detected in fish and knife samples were 66.7% (2 out of 3) and 100% (2 out of 2), respectively (Table 3 and Figure 2).

Table 3: Number of positive Methicillin-Resistant *S. aureus* in different sample items in fish shops

Sample type	Sample (n)	Positive MRSA No. (%)
Fish	3	2 (66.7%)
Table	3	1 (33.3%)
Knives	2	2 (100%)
Worker hands	8	6 (75%)
Fish water	3	1 (33.3%)
Total	19	12 (63.2%)

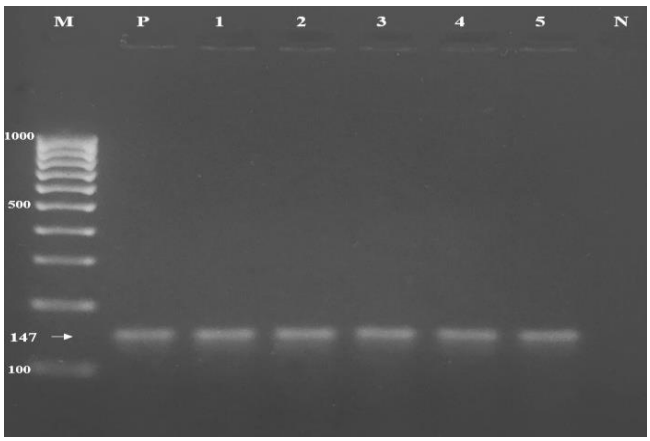

Figure 2: Fragment size of *mecA* gene (147 bp), lane M: ladder, lane P: +ve control *(S. aureus*), lane N: -ve control, and lanes 1-5: +ve samples.

Discussion

Although the focus of this study was mainly on the fish market environment, it is essential to address the results connected to fish since they reveal substantial implications for the practices of food safety in the fish markets. The findings showed a significant incidence of *S. aureus* and MRSA contamination in Mosul fish shops.

According to the current study, 19 of 100 (19%) samples were contaminated with *S. aureus*, and 12 19 (63.2%) were MRSA. Variations in *S. aureus* and MRSA percentages from very low to high were seen when comparing the global incidences of this pathogen in seafood items. Oh *et al.* (23) reported a similar percentage (19.8%) of *S. aureus* in raw fish and the environment in Korea. Furthermore, similar findings have been reported regarding raw fish in Brazil, where higher incidences of 22.2% MRSA were discovered (24). Sivaraman and his colleagues discovered a more significant number of MRSA isolates (50%) in the 174 fish market samples in India (25). However, the prevalence rates of MRSA reported by Hammad *et al.* (26) and Onmaz *et al.* (27) were lower than those found by our study at 6.6% and 9%, respectively, in retail fish samples.

A high percentage of *S. aureus* contamination on utensils and fish samples proves insufficient hygiene practices in the

fish shops. Moreover, it is determined that the lack of fish packaging before the sale and the absence of refrigeration when fish is on display for purchase in fish markets are the leading causes of elevated Staphylococcal contamination.

Due to the previously described reasons, it is thought that it is necessary to increase public awareness training for fish workers at every stage of production to reduce the prevalence of infections in fish that harm public health (28). Contamination could result from unclean activities, unsanitary utensils, or an infected handler (29). It is crucial to comprehend that most carriers are asymptomatic and are responsible for the ongoing spread of *S. aureus* in fish. The likelihood of *S. aureus* transferring from fish handlers to fish and vice versa is very high (30). According to some research on *S. aureus* and MRSA, food handlers' working habits, personal hygiene, and health conditions are generally unfavorable to sanitary standards, which increases cross-contamination in processed fish (26,31). The current research highlights the need for good hygiene practices during all processing stages, from shipping to retail stores, to reduce the hazard of *S. aureus* and MRSA transfer from fish to consumers (32-34).

MRSA is becoming more prevalent as a result of the widespread use of antibiotics in both humans and animals. Public health professionals, microbiologists, epidemiologists, and veterinary and medical practitioners must work together to defeat MRSA infections. MRSA infections are now resistant to most common commercial antibiotics (35,36). The examination and control of the issue of antibiotic resistance in both humans and animals requires screening resistant bacteria using the antibiotic sensitivity test. Additionally, it is essential to apply alternative-friendly treatment regimens, such as using symbiotic and herbal medicines (37).

Conclusions

These findings highlight the urgent need for improved food safety practices, including rigorous hygiene measures, proper packaging, and appropriate refrigeration, in fish shops and other retail food establishments. Implementing these practices is crucial to minimize bacterial contamination, including *S. aureus* and MRSA, and to prevent the emergence and spread of strains resistant to antibiotics, ensuring the safety of both consumers and those involved in the meat supply chain. Furthermore, it indicates that the efficiency of the related government agencies in the fish production sector is essential to control these pathogens.

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Conflict of interest

There were no conflicts of interest throughout the writing or data analysis.

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الكشف الجزيئي عن جراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين المعزولة من الإسماك المحلية في مدينة الموصل

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الخلاصة

يمكن لجراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين أن تضر بالصحة العامة لأنها تسبب تسممًا غذائيًا واسع النطاق ومقاومتها لعديد من المضادات الحيوية. لذلك، هدفت دراستنا إلى تقصي نسبة تلوث محلات الأسماك والأواني وأيدي العمال بجراثيم المكورات الذهبية العنقودية وب جراثيم المكورات الذهبية العنقودية المقاومة للميثيسيلين من خلال الكشف عن وجود جينات *nuc* و *mecA* في العزلات باستخدام تقنية تفاعل البلمرة المتسلسل في مدينة الموصل. تم جمع مائة عينة عشوائياً لهذه الدراسة من الأسماك وأسطح محلات الاسماك المختلفة في الموصل خلال شهري اذار ونيسان ٢٠٢٣. أظهرت نتائج الدراسة أن عزلات الجراثيم المكورات الذهبية العنقودية كانت تحمل جين *nuc* والذي كان موجوداً بنسبة ١٩٪ من العينات الكلية. حيث كانت أعلى العزلات (٨ من ٢٠) في عينات اليد العاملة مقارنة بعينات الأسماك (٣ من ٢٠). بالإضافة إلى ذلك، أظهرت ١٢ عزلة من أصل ١٩ من المكورات العنقودية الذهبية (٦٣,٢٪) وجود جين *mecA*، مما جعلها مقاومة للميثيسيلين. كما كانت عينات العمال تحوي نسبة أعلى من عزلات المكورات العنقودية الذهبية المقاومة للميثيسيلين ٧٥٪ (٦ من ٨ عينات)، مقارنة بالعينات الأخرى. نستنتج من هذه الدراسة بوجود نسبة غير قليلة من المكورات العنقودية الذهبية في الأسماك وأوانيها، مما يؤكد عدم اتباع ممارسات سلامة الغذاء في محلات الأسماك بشكل صحيح. نحتاج الى المزيد من البحوث الضرورية لتقييم وجود الجينات المعوية في المكورات العنقودية الذهبية لمنتجات اللحوم المختلفة لأهميتها في سلامة الغذاء.