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Prevalence, Virulence Determinants and Antimicrobial Resistance Genes of *Staphylococcus aureus* Strains Isolated from Retail Market Fish and Their Handlers in Egypt

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ABSTRACT

Staphylococcus aureus (*S. aureus*)is a significant foodborne bacterial pathogen resulting in food intoxication and other numerous human infections. The purpose of the present study was to ascertain the prevalence, virulence markers and antibiotic resistance genes of *S. aureus* isolates collected from some fish products and their handlers at retail markets in Cairo Governorate, Egypt. A total of 75 different fish products including sardine, herring and canned tuna (25 of each) and 100 hand swabs of fish handlers were screened for the presence of *S. aureus* utilizing *16S rRNA*, *nuc* and *clfA* primers. The *S. aureus* strains were subsequently assessed for the potential of virulence (*sea*, *seb*, *tst*, *pvl* and *lukM*) and antimicrobial resistance (*mecA*, *tetK* and *ermC*) using particular primers. The findings showed that sardine had the highest bacterial count (4.25± 2.9log10 cfu/ g), followed by herring (3.79± 2.4log10 cfu/ g) and canned tuna (3.8± 2.7log10 cfu/ g). In addition, it was found that 8 out of 75 fish samples (10.7%) and 12 out of 100 fish handlers' specimens (12%) possessed coagulase-positive *S. aureus*. For fish samples, the molecular analysis revealed that all the examined *S. aureus* strains harbored *pvl* and *tet K* genes, while *sea* and *tst* genes were detected in 12.5% (1/8) of the recovered isolates. On the other hand, other genes such as *seb*, *lukM* and *ermC* were not identified. Concerning human specimens, the findings revealed that all the obtained *S. aureus* strains carried *sea* and *tetK* genes, while *pvl*, *seb*, *ermC* and *tst* genes were identified in 83.3 (10/ 12), 33.3 (4/ 12), 33.3 (4/ 12) and 8.3% (1/ 12) of the examined isolates, respectively. Interestingly, all isolates obtained from the fish handlers were categorized as methicillin-resistant *S. aureus* (MRSA), whereas no MRSA isolates were detected from the fish. The current study supported the existence of virulent antimicrobial-resistant *S. aureus* in the tested fish products and their handlers, generating a serious risk to food safety. Accordingly, strict preventive and control strategies should be implemented to tackle this major food safety issue.

INTRODUCTION

All over the world, *Staphylococcus aureus* (*S. aureus*) is a foremost contributor to foodborne illnesses, especially foodborne intoxications **(Darwish *et al.*, 2022)**. *Staphylococcus aureus* is responsible for about 241,000 illnesses every year in the United States **(Pal *et al.*, 2022)**. The prevalence of staphylococci related foodborne infections in developing countries is thought to be significantly greater than that recorded due to the poor foodborne illness surveillance systems, with nuerous cases going unreported **(Bencardino *et al.*, 2021)**. Human *S. aureus*-enterotoxin poisoning is characterized by a sudden start of nausea, vomiting, stomach cramping and diarrhea. These symptoms usually appear within a few hours of consuming infected food **(Hu *et al.*, 2021)**. In more severe cases, toxic shock syndrome, dehydration, headache, muscle cramping, changes in blood pressure and pulse rate may occur. Toxic shock syndrome is a rapidly progressive disease with severe symptoms, including increased temperature, rash, hypotension and multi-organ failure **(Schmitz *et al.*, 2018)**.

Staphylococcus aureus is an opportunistic bacterial pathogen posing a zoonotic risk since it can spread among environments, humans and various animals, such as pets, livestock, fish and wildlife **(Soliman *et al.*, 2019)**. A number of studies have reported that *S. aureus* isolated from non-human primates had close similarity with human isolates, leading to the concern of amphixenoses or zoonotic diseases **(Pumipuntu *et al.*, 2023)**. Therefore, asymptomatic workers and food handlers may constitute a reservoir of virulent strains of *S. aureus* and may be vectors of their transmission to food via manual contact or through respiratory secretions, thus becoming the source of staphylococcal food poisoning **(Hassanain *et al.*, 2013; Elfadaly *et al*., 2018)**.

In Egypt, the production of traditional fish products by salting the fish are used as an alternative source of protein as well as being consumed on special occasions **(Farag *et al.*, 2022)**. These fish products (smoked and salted fish and canned tuna) are high not only in protein but also in amino acids, selenium, iodine, phosphorus, potassium, lipid and water-soluble vitamins, such as vitamin A, B and D, with a relatively lower price compared to other meat sources **(Morshdy *et al.*, 2022)**. However, bacteriological hazard in such products is one of the most important microbiological hazards, causing a large proportion of all foodborne illnesses **(Sadek *et al.*, 2023)**.

Staphylococcus aureus produces a lot of virulence factors, which allow it to invade the immune system such as enterotoxins (*sea*, *seb*), exfoliative toxins and toxic shock syndrome toxin, hemolysins and Panton–Valentine leukocidin (PVL) **(Rajkovic *et al.*, 2020; Pérez-Boto *et al.*, 2023)**. In addition to the enormous number of virulence factors, a high level of antimicrobial resistance genes like *ermC*, *tet*, *fexA* are also found in *S.aureus* strains **(Huang *et al.*, 2023)**. *Staphylococcus aureus* and staphylococcal enterotoxins (SEs) were isolated from salted mullet collected from Iranian markets **(Basti *et al.*, 2006)**, salted fermented fish product of the salted mullet, a traditional retailed in Jazan region, Saudi Arabia **(Gassem, 2019)** in addition to the salted fish, such as fesiekh, molouha and sardine retailed in Alexandria **(Elkassas & Mousa, 2021)** and in Cairo **(Abbas *et al*., 2022)**.

Therefore, the objective of this research was to characterize the isolated coagulase positive *S. aureus* from some fish products (salted sardine, smoked herring and canned tuna) and their handlers in correlation with the presence of virulence genes *(sea, seb, luk*

M, tst and pvl) and antimicrobial resistance genes (*mec A, tet K* and *erm C*) by using PCR technique.

MATERIALS AND METHODS

Ethical consideration

The research ethics review committee at the Faculty of Veterinary Medicine, Alexandria University accepted every aspect of the current study's design and procedures (approval No: Au 13 12 12 2023 057). Additionally, informed consent was received from each fish handler beforehand swab sampling.

Sample collection

The present study was conducted between August 2022 and October 2023 in Cairo Governorate, Egypt. A total of 75 different fish products were gathered including sardine, herring and canned tuna (25 of each). Samples were randomly collected, under complete aseptic condition, from various retail markets in Cairo province. Fish specimens were placed into sterile bags. In addition, a collection of 100 hand swabs was aseptically taken from fish handlers after obtaining their informed consent in advance. Swabs were collected in an aseptic manner and inserted back into its container filled with 3ml sterile nutrient broth and closed tightly **(Saad-Alla *et al*., 2022)**. All samples were packed into an icebox and sent as quickly as possible to the laboratory of Zoonotic Diseases Department, National Research Center, Giza, Egypt, for conventional bacteriological examination and assessment.

Total aerobic plate count

The level of microorganisms in fish products was evaluated; approximately, 5 gram of each fish sample (canned tuna, sardine and herring) was transferred into a stomacher bag containing 45ml buffered peptone water, and thoroughly mixed using a sterile blender for 1- 1.5 minutes. Afterward, ten-fold (decimal) serial dilutions were prepared from the first original dilution (1:10). Consequently, 1ml of each serial dilution was transferred into sterile petri dishes, and standard plate count agar (PCA) was poured on each plate and kept to be solidified. Then, all plates were incubated at 37°C for 48hrs. Following the incubation period, the separate colonies were counted for each plate **(Shell *et al.*, 2012)**.

Isolation and identification of *S. aureus*

Fish specimens were macerated, and then aseptically transported to the staphylococcal enrichment broth media (tryptic soy broth that includes 10% sodium chloride and 1% sodium pyruvate) and incubated at 37°C for 18hrs. Subsequently, one loopful (10µl) of the enriched culture was sub cultured on the surface of the selective Baird-parker agar (BPA) (Oxoid, CM0275) and incubated aerobically at 37^{o}C for 36hrs

to identify the presumptive *S. aureus* colonies (black colonies surrounded with a clear zone) **(Dallal *et al.*, 2015)**. Regarding fish handlers' swabs, every swab contained an enrichment of nutrient broth, incubated at 37°C for 6hrs and then plated on Baird-parker agar, and incubated for 36hrs at 37°C. For biochemical identification, catalase and tube coagulase tests were carried out, and only catalase coagulase positive isolates were selected and kept at -20^{o}C in brain heart infusion containing 30% glycerol to be used for further molecular verification at the species level **(Dhinesh *et al.*, 2021)**.

DNA extraction and molecular detection of virulence markers and antimicrobial resistance genes by PCR assay

The genotypic characterization of the isolated *S. aureus* strains was performed at the Department of Parasitology and Animal diseases, National Research Center, Giza. Total Genomic DNA of *S. aureus* isolates was obtained by using DNA gel extraction kit (NORGRN BIOTEK, Cat. No. 13100) after refreshing the presumptive *S. aureus* strains on nutrient agar plates. All *S. aureus* strains were derived from fish products, and human samples were screened by polymerase chain reaction (PCR) for eubacterial 16srRNA, thermonuclease encoding gene (*nuc*) and clumping factor A gene (*clfA*) (*S. aureus* specific determinants) **(Mahmoud *et al.*, 2019)**. Confirmation of *S. aureus* was achieved by a concurrent amplification of the three genes. *Staphylococcus aureus*-verified isolates were used as targets for the identification of five selected virulence genes including *sea*, *seb*, *tst*, *pvl* and *lukM* genes, encoding staphylococcal enterotoxins A, B, toxic shock syndrome-Toxin1, Panton-Valentine leukocidin and leukotoxin M, respectively. Besides, *mecA* (methicillin resistance), *ermC* (erythromycin resistance) and *tetK* (tetracycline resistance) genes were detected. PCR assay for the target genes was conducted using primers sets and cycling conditions listed in Table (1). The uniplex PCR reaction mixture, with a total of 25µl, consisted of 12.5µl of 2X COSMO PCR RED Master mix (Willowfort, Cat.No. WF10203001); 1µl of each primer of 10µM working concentration (Macrogen, South Korea); 5.5µl of nuclease-free water, and finally 5µl of DNA template. The reaction mixture was transferred to the thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products (5µL) were analyzed by electrophoresis in 1x TBE electrophoresis buffer and on 1.5% (w /v) agarose gel at 100V for 60min. A PCRRanger 100bp DNA ladder (NORGRN BIOTEK, Cat.No. 11300) was exploited to determine the sizes of DNA amplicons **(Hassanain *et al.*, 2011)**. The gel documentation system captured images of agarose gels following their staining with GoldView I Nuclear Staining Dye. (Solarbio, Cat.No. G8140).

Table 1. Oligonucleotide primer sequences and cycling conditions used in PCR assay

Target gene	Sequence (5′- 3′)	Initial den.	Amplification cycle			Final exten/	Product size (bp)
			Denat.	Anneal	Exten.		
16S	AACTGGAGGAAGGTGGGGAT	94°C/	94°C/	55°C/	72°C/	72°C/	371
rRNA	AGGAGGTGATCCAACCGCA	5 min	20 sec	20 sec	50sec	5 min	
nuc	GCGATTGATGGTGATACGGTT	94°C/	94°C/	55°C/	72°C/	72°C/	280
	AGCCAAGCCTTGACGAACTAAAGC	5 min	60 sec	30 sec	90 sec	3.5min	
clf A	GCAAAATCCAGCACAACAGGAAACGA	94°C/	94°C/	55°C/	72°C/	72°C/	638
	CTTGATCTCCAGCCATAATTGGTGG	5 min	1 min	1 min	1 min	10 min	
mecA	TCCAGATTACAACTTCACCAGG	94°C/	94°C/	55°C/	72°C/	72°C/	162
	CCACTTCATATCTTGTAACG	5 min	30 sec	30 sec	1 min	5 min	
Sea	GAAAAAAGTCTGAATTGCAGGGAACA	94°C/	94°C/	55°C/	72°C/	72°C/	560
	CAAATAAATCGTAATTAACCGAAGGTTC	5 min	30 sec	30 sec	45 sec	10 min	
Seb	TCGCATCAAACTGACAAACG	94°C/	94°C/	55°C/	72°C/	72°C/	478
	GCAGGTACTCTATAAGTGCC	4 min	2 min	2 min	1 min	5 min	
tet (K)	GTA GCG ACA ATA GGT AAT AGT	94°C/	94°C/	55°C/	72°C/	72°C/	360
	GTA GTG ACA ATA AAC CTC CTA	3 min	30 sec	30 sec	30 sec	4 min	
erm(C)	AAT CGT CAA TTC CTG CAT GT	94°C/	94°C/	55°C/	72°C/	72°C/	299
	TAA TCG TGG AAT ACG GGT TTG	3 min	30 sec	30 sec	30 sec	4 min	
tst	AAGCCCTTTGTTGCTTGCG	94°C/	94°C/	55°C/	72°C/	72°C/	445
	ATCGAACTTTGGCCCATACTTT	3 min	1 min	1 min	2 min	5 min	
PVL	ATCATTAGGTAAAATGTCTGGACATGATCCA	94°C/	94°C/	55°C/	72°C/	72°C/	433
	GCATCAAGTGTATTGGATAGCAAAAGC	5 min	30 sec	30 sec	1 min	5 min	
lukM	AAACGCGCAGTTAATAAAAG	95°C/	95°C/	55°C/	72°C/	72°C/ 5	975
	AGCATTAGGTCCTCTTGTCG	2 min	30 sec	35 sec	1 min	min	

RESULTS

Total bacterial count of the examined fish samples

Based on the quantitative evaluation data of the aerobic plate count, the canned tuna, herring and sardine fish samples were contaminated at mean levels of (3.8± 2.7) log_{10} (CFU/ g), (3.79± 2.4) log_{10} (CFU/ g) and (4.25± 2.9) log_{10} (CFU/ g), successively (Table 2).

Table 2. Aerobic plate count of the examined fish samples

Fish produc. (25 of each)	Minimum	Maximum	Mean	SE±
Tuna	2	4.77	3.8	2.7
Herring	2	4.38	3.79	2.4
Sardine	2	4.95	4.25	2.9

Prevalence of *S. aureus*

A total of eight isolates were presumptively identified as *S. aureus* out of seventy five tested fish products samples from Cairo, Egypt. The *S. aureus* contamination was 16% (4/25) among herring and sardine samples. However, there was no contamination of *S. aureus* among the canned tuna samples. Moreover, a total of twelve presumptive *S. aureus* isolates were obtained from hand swabs specimens of humans. Consequently, all presumptive *S. aureus* (20) isolates were confirmed by amplification of *S. aureus*-specific *16s rRNA*, *nuc* and *clfA* genes (encodes eubacterial 16s rRNA, thermnuclease and clumping factor A) using PCR assay. All isolates (20) were confirmed as *S. aureus* using specific primer sequences. Accordingly, the overall prevalence rate of *S. aureus* was 10.7 (8/75) and 12% (12/100) in fish samples and human specimens, respectively. Twenty *S. aureus* isolates were subsequently subjected to genotypic identification of virulence and antimicrobial resistance determinants (Table 3).

Table 3. Prevalence of *S. aureus* in the examined fish products and their human handlers

Sample source	Sample type	No. of examined samples	Positive rate	
			No.	%
Fish species	Tuna	25	0	0.0
	Herring	25	4	16.0
	Sardine	25	4	16.0
	Total	75	8	10.7
Fish handlers	Hand swabs	100	12	12.0

Assessment of virulence-associated genes and antimicrobial resistance determinants

Regarding fish samples, the molecular analysis revealed that all the examined *S. aureus* strains harbored *pvl* and *tet K* genes, whereas *sea* and *tst* genes were detected in 12.5% (1/ 8) of the recovered isolates. On the other hand, other genes *such as seb*, *lukM, mecA* and *ermC* were not identified (Table 4 & Figs. 1, 2).

Table 4. Screening of *S. aureus*-specific, virulence and antimicrobial resistance genes sourced from fish samples

	***S. aureus* specific gene**			**Virulence-associated gene**					**Antibiotic resistant gene**		
Sample ID	*16 S rRNA*	*nuc*	*clfA*	*sea*	*seb*	*luk M*	*tst*	*pvl*	*mecA*	*tetK*	*ermC*
1	+	+	+	-	-	-	-	+	-	+	-
2	+	+	+	-	-	-	-	+	-	+	-
3	+	+	+	-	-	-	-	+	-	+	-
4	+	+	+	-	-	-	-	+	-	+	-
5	+	+	+	-	-	-	-	+	-	+	-
6	+	+	+	+	-	-	+	+	-	+	-
7	+	+	+	-	-	-	-	+	-	+	-
8	+	+	+	-	-	-	-	+	-	+	-

Fig. 1. Characteristic colony growth and appearance of *S. aureus* isolates on selective paird-parker agar plates after incubation

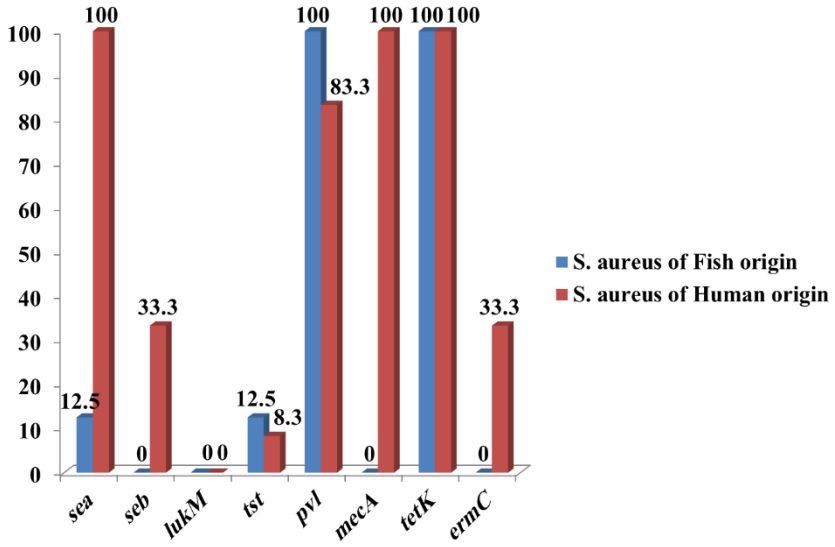

Fig. 2. Virulence markers and antimicrobial resistance genes identified in *S. aureus* isolates from different fish and human samples

Concerning human specimens, the results exhibited that all the obtained *S. aureus* strains carried *sea*, *mecA* and *tetK* genes while *pvl*, *seb*, *ermC* and *tst* genes were identified in 83.3 (10/ 12), 33.3 (4/ 12), 33.3 (4/ 12) and 8.3% (1/ 12) of the examined isolates, respectively. Meanwhile, *lukM* genetic determinant was not detected (Table 5 & Figs. 3, 4).

Table 5. Screening of *S. aureus* specific, virulence and antimicrobial resistance genes identified in human specimens

	S. aureus specific gene			Virulence-associated gene					Antibiotic resistant gene		
Sample no.	*16 S rRNA*	*nuc*	*clfA*	*sea*	*seb*	*lukM*	*tst*	*pvl*	*mecA*	*tetK*	*ermC*
1	+	+	+	+	-	-	-	+	+	+	+
2	+	+	+	+	-	-	-	+	+	+	+
3	+	+	+	+	-	-	-	+	+	+	-
4	+	+	+	+	-	-	-	+	+	+	-
5	+	+	+	+	-	-	+	+	+	+	-
6	+	+	+	+	-	-	-	+	+	+	-
7	+	+	+	+	-	-	-	-	+	+	-
8	+	+	+	+	+	-	-	+	+	+	-
9	+	+	+	+	+	-	-	+	+	+	+
10	+	+	+	+	-	-	-	+	+	+	-
11	+	+	+	+	+	-	-	-	+	+	-
12	+	+	+	+	+	-	-	+	+	+	+

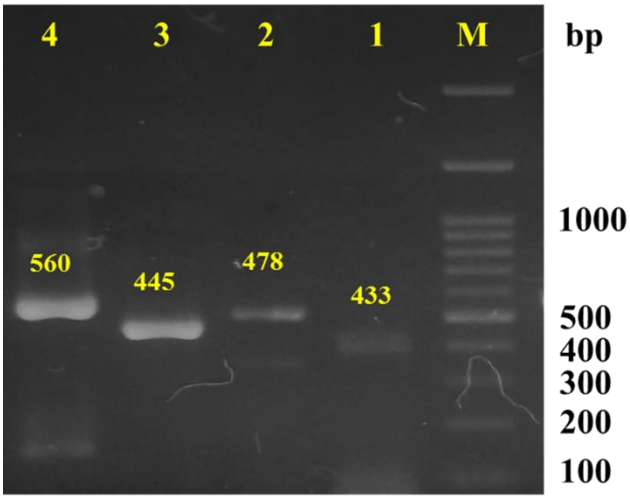

Fig. 3. Representative agarose gel electrophoresis of PCR product of four virulence genes. Lane M: 100 bp DNA marker, Lane 1: Amplified amplicon of *pvl* gene, Lane 2: Amplified product of *seb* gene, Lane 3: PCR product of *tst* gene, and Lane 4: PCR amplicon of *sea* gene

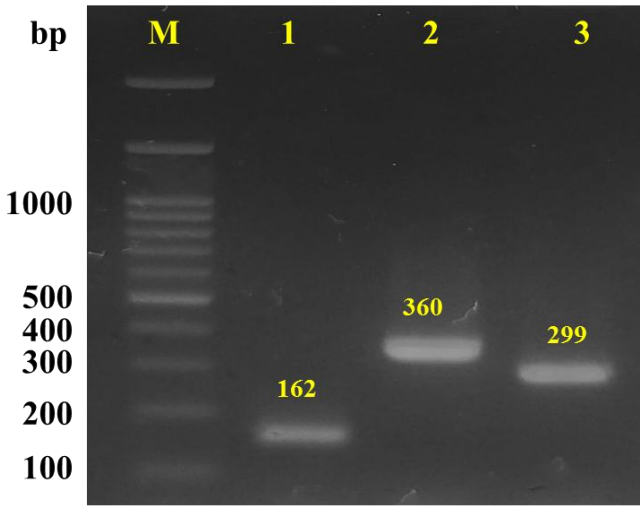

Fig. 4. Representative agarose gel electrophoresis of PCR product of three antimicrobial resistance genes. Lane M: 100 bp DNA marker, Lane 1: Amplified amplicon of *mecA* gene, Lane 2: Amplified product of *tetK* gene, and Lane 3: PCR product of *ermC* gene

DISCUSSION

The bacterial population, associated with fish, is typically linked to the characteristics of the aquatic environment, viz. the salinity and the amount of the bacteria present in the water **(Hassanain *et al.*, 2021)**. The microbiota of the retail fish can be impacted by the degree of hygiene standards, where in food handlers' involvement in fish

processing is essential in assessing the finished product's sanitary state, with improper handling raising the risk of contamination by human-associated microorganisms including enter toxigenic and/or multiple drug-resistant bacterial pathogens **(Rifat *et al.*, 2022)**. The total aerobic plate count in fish is often associated with food safety concerns, and it is sometimes used to evaluate quality, shelf life and contamination following heat processing **(Eltholth *et al.*, 2018)**. Accordingly, the results in this report (Table 2) presented that the highest aerobic plate counts (CFU/ g) in the inspected fish products were found in salted sardine (4.25± 2.9) $\log_{10}$ (CFU/ g), followed by smoked herring (3.79± 2.4) $\log_{10}$ (CFU/ g) and canned tuna samples (3.8± 2.7) $\log_{10}$ (CFU/ g). These findings agree with those of **Ghita *et al.* (2017)** who stated that, the total viable counts for salted, smoked and canned fish products should not exceed 10^5- 10^6 (CFU/ g). The greatest value of APC in salted sardine could be resulted from halophilic bacteria that was generated by the high content of NaCl. These outcomes concur with those recorded in a previous report conducted by **Gassem (2019)**, who reported that, the microbial load of salted fermented fish from Saudi Arabia ranged from 2.81- 4.72 $\log_{10}$ (CFU/ g).

Staphylococcus aureus is a prevalent foodborne bacterial pathogen that results in a variety of healthcare-associated infections globally, as well as a large number of foodborne intoxications triggered by the production of heat-stable enterotoxins, such as *Sea* and *Seb* **(Darwish *et al.*, 2022)**. Given that this bacterium grows efficiently in environments with 5- 15% salt concentration, salted food may be at a potential risk **(Akbar *et al.*, 2019)**. Data on the microbiological safety of *S. aureus* in fish products and their handlers in Egypt are limited. In this study, *S. aureus* was detected in 16% of retail smoked herring and salted sardine samples, demonstrating a similar occurrence to prior studies on salted fish products in India (17.0%) **(Simon & Sanjeev, 2007)**, Egypt (15%) **(Morshdy *et al.*, 2022)**, and Viet Nam (18%) **(Phan *et al.*, 2023)**. However, it was lower than the reported prevalence for raw salted fish from South India (24.4%) **(Bujjamma & Padmavathi, 2015)**, Egypt (26%) **(Mostafa *et al.*, 2016)**, Nigeria (31%) **(Mohammed *et al.*, 2020)**, and Pakistan (26%) **(Rashid *et al.*, 2021)**. Conversly, the prevalence in this investigation was greater than that monitored in the samples of fish products in Italy (2.3%) **(Normanno *et al.*, 2005)** and Turkey (9%) **(Ertas Onmaz *et al.*, 2015)**.

As illustrated in Table (3), the comparatively low (12%) *S. aureus* incidence in the hands of fish handlers was noticed. The achieved results are not consistent with earlier research studies, that showed the high incidence of *S. aureus* in 60% of food handlers' hands in Brazil **(Albuquerque *et al.*, 2007)**, 62% in India **(Simon & Sanjeev, 2007)**, and 74% in Malaysia **(Tan *et al.*, 2014)**. This widespread dissemination of *S. aureus* among fish handlers could be attributed to inadequate hand sanitation and irregular face masks used. Moreover, fish handlers may cross-contaminate fish with *S. aureus* by sneezing, coughing, or having skin sores. *Staphylococcus aureus* isolates' virulence was molecularly assessed by detection of *sea*, *seb*, *luk M*, *pvl*, *tst* genes. Enterotoxins genes *sea* and *seb* associated with *S. aureus* are the most common cause of staphylococcal food

borne intoxication. These toxins are of clinical significance since they can withstand heat and digestive enzymes **(Hassanain *et al.,* 2022)**. Our research study revealed 12.5% of *sea* and 0 % of *seb* presence in the coagulase positive *S. aureus* isolates from fish samples, reflecting a higher proportion of *sea* in comparison to *seb*. In a related research project by **Mohammed *et al.* (2020)**, it was found that salted fish in Khartoum, Sudan had higher concentrations of *sea* (38%) than those with *seb* (35.7%) genes. In a different study **(Arfatahery *et al.,* 2016)**, isolates of *S. aureus* from the Iranian fisheries products have 18.5% of *seb* gene and 45.2% of *sea* gene. The presence of enterotoxins genes containing *S. aureus* in salted fish products is a major threat facing the Egyptian food safety and represents a potential source for zoonotic food borne transmission of Staphylococcal enterotoxin intoxication. *S. aureus* is a pathogen that involves a complicated manner of adhesion to and entrance of various hosts, along with coordinated expression during numerous phases of infection in order to evade immune responses **(Cheung *et al.,* 2021)**. *pvl* virulence factor is a pore forming cytotoxin. In fact, roughly 90% of cases of necrotizing pneumonia caused by *S. aureus* has linked to the isolation of *S. aureus* strains harboring the *pvl* gene. Furthermore, it has been associated with infections of skin and soft tissues, such as abscesses, carbuncles or furuncles **(Bhatta *et al.,* 2016)**. A prior study discussed the existence of *pvl* in aquatic products, however it discovered that no samples of raw fish, that was ready to eat in Japan, tested positive for *pvl* gene **(Hammad *et al.,* 2012)**. On the other hand, our research revealed that *pvl* gene was present in all *S. aureus* isolates. Therefore, our findings suggest that fish handlers could be the potential source of *S. aureus* contamination in the tested fish specimens since *pvl* gene was originally and commonly associated with community-acquired MRSA (CA-MRSA) isolates and was infrequently identified in hospital-acquired MRSA (HA-MRSA) isolates **(Shohayeb *et al.,* 2023)**. Moreover, only one isolate contained *tst* gene which encodes TSST-1 protein, and this protein is a crucial virulence determinant of *S. aureus* leading to a multiple organ dysfunction **(Zheng *et al.,* 2020)**. This finding illustrated the elevated levels of toxicity associated with *S. aureus*, which could pose a risk to both fish handlers and general public consumers.

Staphylococcus aureus and MRSA are not typical fish microbiota members **(Vaiyapuri *et al.,* 2019)**. Fish contamination with *S. aureus* and MRSA may have arisen from the harvest location or from the incorrect handling of the fish by customers, processors or handlers before consumption. In the current study, not all isolates carried *mecA* gene, and this agrees with the findings of **Vázquez-Sánchez *et al.* (2012)** who reported that, there were no MRSA strains collected from fishery products in Galicia, Spain. On the contrary, other investigations recorded the presence of *mecA* genetic element in *S. aureus* isolates collected from fish products in South Africa (16.30%) and Nigeria (10.30%) **(Mohammed *et al.,* 2020)**. Humans frequently have enterotoxigenic *S. aureus* in their upper respiratory tract and skin lesions, and this bacteria is easily transmitted to food during handling. If an enterotoxins-carrying *S. aureus* strain is found

in the food and its multiplication is not suppressed, this could result in a widespread epidemic of food poisoning **(Bencardino *et al.*, 2021)**. The present report revealed a 100% of *sea* gene and 33.3% of *seb* gene presence in the coagulase positive *S. aureus* isolates from fish handlers' samples, indicating a higher prevalence of *sea* gene in comparison with *seb* gene, and this finding does not match that reported in the study of **Simon and Sanjeev (2007)** who elucidated that, the most prevalent toxin types detected in the handlers were *seb* (40%), followed by *sea* (20%) genes. Moreover, our investigation revealed that, all *S. aureus* isolates were MRSA due to harboring the *mecA* genetic determinant. This value is higher than those obtained by **Sezer *et al.* (2015)** (28.9%) and **Mohammed *et al.* (2020)** (6%). The documented antibiotic resistance of human isolates is predictable since antibiotic medications have been prescribed without any laboratory testing in most Egyptian governorates leading to an inappropriate use of antimicrobial agents and purchase of antibiotics like the over –the- counter medications **(Manyi-Loh *et al.*, 2018)**.

CONCLUSION

The findings of the existing study highlight the first occurrence of virulent and antimicrobial resistant *S. aureus* in salted fish and fish handlers at retail markets in Cairo Governorate, Egypt. Although the prevalence of *S. aureus* in fish and their handlers was low, it is still possible for these isolates to disseminate throughout the community. Therefore, enhancing processing procedures and executing suitable sanitation standards in fish processing handlers are recommended to safeguard the fisheries' products. It is advisable to handle retail fish with hygienic gloves to mitigate the risk of *S. aureus* contamination. In addition, handlers should be trained on the value of maintaining hygienic and sanitary conditions, as well as implementing good manufacturing practices (GMP) and hazard analysis critical control points (HACCP).

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