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Isolation of Highly Antibiotic-Resistant *Staph. aureus* Bacteria from Salted Fish Sold in Markets

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ABSTRACT

The present study was done to estimate the microbial quality of salted fish (moloha and fesikh) through detection of *Staph. aureus* with identified vancomycin and methicillin-resistant genes (VMRSA) from salted fish for their public health significance. A total of 50 samples of salted fish (25 moloha and 25 fesikh) were purchased from markets. Samples were examined microbiologically for detection of incidence and molecular characterization of *Staph. aurous* and assessment of the sanitary quality of salted fish. Total aerobic plate count showed mean values of 3.3×104 ± 1.3×10cfu/ml for moloha and 6.2×104 ± 3.6×10cfu/ml for fesikh, the coliform count showed a mean value of 2.1×102 ± 1.1×10cfu /ml and 3.5×102 ± 1.6 ×10 cfu/ml for moloha and fesikh respectively. *Staph. aureus* count showed a mean value of 1.9×102 ± 1.2×10 cfu/ml for moloha and 2.3×102 ± 1.2 ×10 cfu/ml for fesikh. Moloha samples exceed standard total aerobic bacterial count, Coliform's count, *Staph. aureus* count were14 (56%),7 (28%) , 19 (76%) respectively, and fesikh samples exceed standard total aerobic bacterial count, Coliforms count, *S. aureus* count were18 (72%), 10 (40%), 23 (92%) respectively. *Staph. aureus* isolates were more sensitive to Vancomycin (66.66%), and the most resistant antibiotic was Oxytetracycline (95.24%) then Erythromycin and Doxycycline (92.86%). Incidence of isolation of *Staph. aureus* from examined moloha (25 samples) and fesikh (25 samples) by 76% and 92%. Methicillin-resistant *Staph. aureus* (MRSA) was 32% and 48%. Methicillin and vancomycin-resistant *Staph. aureus* (MVRSA) was 20 % and 36% respectively. MecA gene was detected in 60% of examined *Staph. aureus* isolates. Vancomycin-resistant genes (VanA, vanB, vanX) of examined *Staph. aureus* isolates were positive for vanA (30%) but vanB and vanX genes were not detected. Results revealed that both products are considered as a source of MVRSA, an emerging public health problem.

1. INTRODUCTION

Staph. aureus is an opportunistic human pathogen and foodborne pathogens (Gündoğan *et al.*, 2005). It ranks third as the factor beyond food-borne diseases (Boerema *et al.*, 2006). Antibiotic-resistant *Staph. aureus* isolates considered a human challenge (Brouillette and Malouin, 2005). Exposure to an antibiotic can result in mutations in bacteria than antibiotic resistance (Beer and Wentzel, 2003). *S. aureus* shows multiple antibiotic resistance patterns (Enright, 2003). Methicillin-resistant staphylococcus has problems in antibiotic treatment (Pereira *et al.*, 2009). MRSA is cleared by the mecA gene (Normanno *et al*., 2005). The MRSA was hard in treatment and rapidly spread (Pereira *et al.*, 2009).

Fish is considered one of the most outbreaks of foodborne illness (Huss and Valdimarsson, 1990), and a vehicle for many bacteria (Hosseini *et al*., 2004). *S. aureus* poisoning is self-limiting and not reported to healthcare services, so it is higher than recorded (Lawrynowicz – Paciorek *et al*., 2007).

Fish was a good media for pathogenic bacteria (Hosseini *et al*., 2004).

S. aureus detected from fishery resulted in foodborne illness (EFSA, 2010). Human was a carrier of *S. aureus* and source contamination for food (Boynukara *et al*. 2008).

S. *aureus* prevalence in food affected with contamination and holding temperatures during distribution and selling (Wei *et al.*, 2006). *S. aureus* causes endocarditis, sepsis, infection of (skin, soft tissue, respiratory tract, urinary tract, intestinal tract, blood) (Rallapalli *et al*., 2008).

Vancomycin was effective in the therapy of most diseases caused by multidrug-resistant *S. aureus* (Weigel *et al.*, 2003). VanA-type resistance has vanA gene, characterized by high levels of resistance to glycopeptides, vancomycin, and is chromosomally or plasmid-located (Moubareck *et al.*, 2009). Vancomycin interferes with cell wall formation in bacteria with Gram-positive stain. (Solenberg et al., 1997). Poonam *et al.* (2010) recorded that MRSA resistant to methicillin and oxacillin. Cross-resistant between MRSA and β-lactam, vancomycin is considered as the effective treatment of MRSA and there is a gradual increase in the number of cases with VRSA.

Amitabh Talwar *et al* (2013) reported that MRSA is one of the animals and human bacteria responsible for a wide spectrum of infections, few ideal antibiotics are available for the treatment of such infections. MRSA infections overcome most antibiotics developed recently and increased use of vancomycin (Oliveira *et al* 2001*)*. MRSA strains carry mec A gene confers resistance to all β-lactam antibiotics (Chambers1997). Bad use of antibiotics resulted in antibiotic resistance (Beer and Wentzel, 2003). *S. aureus has* a record of multiple antibiotic resistance (Enright, 2003). Herrero *et al.* (1999) reported that *S. aureus* was not natural microflora of marine and freshwater fish.

Feldhusen (2000) and Ahmed *et al.*, (2012) isolated *S. aureus* from fish and it had public health importance. Jay (2000) considered *S. aureus* as frequently foodborne pathogens that cause gastroenteritis and was much higher than several other microbial food-borne disease outbreaks. Davies *et al.* (2001) reported that fish and seafood considered as a vehicle for many bacterial pathogens like *S. aureus*. NCCLS (2002) reported the method technique used to perform an antimicrobial susceptibility test of *S. aureus* for vancomycin and methicillin disk. Albuquerque *et al.* (2007) isolated 5 *S. aureus* strains from the hands of fish handlers and all isolates were resistant to Ampicillin. Tinover and Goering (2009) recorded that MRSA was resistant to all other beta-lactam antibiotics and have an extended host spectrum. Gardete and Tomasz (2014) resistance gene mainly located on mobile elements, such as plasmids or prophages, and transferable through gene transfer. Bala *et al.* (2016) said VRSA is now known as a major emerging public health problem.

Guido *et al.*, (2008) recorded that the vanA-type resistance is the common resistance determinant on plasmids spread either vertically by dissemination, or horizontally into different strains.

2. MATERIAL AND METHODS:

2.1. Samples collection:

The total number of samples of salted fish was 50 (moloha and fesikh) (25 for each), were randomly collected from Kafrelsheikh shops and transferred directly to the laboratory under a complete aseptic condition without delay and subjected to the following microbial count

2.2. TBC using standard plate count agar according to APHA (2001): Each count was occurred according to the specific medium and the determination technique of each microbial group. From serially diluted samples, enumeration of was done.

2.3. TCC was done according to APHA (2001): using the MPN technique.

2.4. *S. aureus* count (APHA, 2004):

Ten grams from each sample were homogenized with 90 ml 1/4 ringer's solution to make serial tenfold dilution up to 106 from the original dilution (1:10). Only 0.1 ml from each dilution was spread over double plates of Baird Parker using a sterile bent glass spreader and incubated 48hr at 37oC (opaque black shining convex colonies with narrow white margins surrounded by a clear zone) were counted and the average number per gram was calculated. Typical *S. aureus* colonies were picked up, purified, and stabbed into semisolid tubes for biochemical identification (catalase test, coagulase test, DNAse test, mannitol fermentation test, oxidation-fermentation test, and detection of hemolysin) **(MacFaddin, 2000) and (Iurlina and Fritz, 2004)**.

2.5. Isolation and identification of *Staph .aureus* according to the method recommended by Lancette and Benett (2001).

For isolation of *S. aureus*, we take (0.1ml) from the mixture of prepared samples of salted fish meat spread onto the surface of Baird Parker agar (BPA) supplemented with egg yolk tellurite emulsion incubated at 37oC for 18-24 hours. Black colonies surrounded by whit halo zone considered as presumptive *S. aureus* confirmed with Gram stain, coagulase test, catalase test, and another biochemical examination by Vitek-2 compact test and API STAPH identification test strips according to the manufacturer's instructions.

2.6. Disk diffusion method for *S. aureus* Antibiotic susceptibility (NCCLS, 2002).

An antibiotic sensitivity test was performed by the disc diffusion method. Different antimicrobials were used such as using the following discs: Methicillin 5 µg /disc**,** Vancomycin 30 µg /disc, Gentamycin 10 µg /disc, Enrofloxacin 5 µg /disc, Norfloxacin 5 µg /disc, Ciprofloxacin 5 µg /disc, Tylosin10 µg /disc, Oxytetracycline 30 µg /disc, Erythromycin10 µg /disc, Doxycycline 30 µg /disc, Ampicillin10 µg /disc, Amoxicillin 10 µg /disc, Cefotaxime 30 µg g/disc Chloramphenicol 30 µg /disc. The interpretation of the measured zone was done according to CLSI (2018).

2.7. PCR:

2.7.1. DNA extraction: DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Oligonucleotide Primers used were supplied from **Metabion (Germany)** are listed in the table (1).

2.7.2. PCR amplification:

2.7.2.1. MecA uniplex PCR. Primers were utilized in a 12.5- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan. The reaction was performed in an applied bio-system 2720 thermal cycler.

2.7.2.2. VanA, vanB and vanX multiplex PCR. Primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan. The reaction was performed in an applied bio-system 2720 thermal cycler.

2.7.3 - Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) a gel pilot 100 bp ladder (Qiagen, Gmbh, Germany) and general 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

3. RESULT AND DISCUSSION

Salted fish was commonly contaminated by *S. aureus* which plays an important role in food poisoning (Jay, 2000).

Humans are reported as symptomatic carriers of *Staph. aureus,* so food will be contaminated

with *Staph. aureus* during the manufacturing process from workers or bad handling (lack of hygiene in food production) (Jett *et al.*,2001).

Coagulase positive *S. aureus* strain was spherical and form in grape-like clusters, and on plates of Baird Parker appear opaque black shining convex colonies with narrow white margins and surrounded by a clear zone, with the typical colonies were yellow colonies surrounded by yellow halo zone on mannitol salt agar. Further phenotypic characteristics of *Staphylococcus aureus* were clear in Table (2) and agree with Organji *et al.* (2018) who reported that *Staphylococcus* spp. was isolated from food products, detecting Staphylococci on specific media, phenotypical character, biochemical character, and 16S rRNA sequencing, and with Sampimon *et al.*, 2009 whom use biochemical tests as API Staph System (includes 20 tests), for detection of gram positive *Staphylococococci.*

The reported results in table (3) and table (4) showed the statistic analytic results of examined moloha and fesikh samples (25 moloha and 25 fesikh). The total aerobic plate count showed mean values of 3.3×104 ± 1.3×10cfu/ml for moloha and 6.2×104 ± 3.6×10cfu/ml for fesikh, the coliform count showed a mean value of 2.1×10 2 ± 1.1×10cfu /ml and 3.5×102 ± 1.6 ×10 cfu/ml for moloha and fesikh respectively. *Staphylococcus aureus* count showed a mean value of 1.9×102 ± 1.2×10 cfu/ml for moloha and 2.3×102 ± 1.2 ×10 cfu/ml for fesikh. which means that fesikh is more contaminated than moloha, and the high bacterial number of *S. aureus* indicates the quality of fish and the level of the spoilage Ali (2014).

Nashwa Ezzelden *et al.* (2011) isolated 144 strains of coagulase-positive *S. aureus*, 55 strains from fesikh, and 42 from molouha. The antimicrobial sensitivity reveals that 84.7% of *S. aureus* isolates were sensitive to methicillin and they assume that isolation of *S. aureus* was due to the contamination during fishing, unhygienic handling, and processing.

Xiaojuan et al., (2016) stated that methicillin-resistant *S.aureus* (MRSA), has public health importance, *S. aureus* isolated from ready to eat foods tested for antibiotic sensitivity, 69 (12.5%) of examined samples were positive for *S. aureus* with the most probable number (MPN)/g rang 0.3–10, with five samples exceeding 10 MPN/g., seven were identified as MRSA from 69 *S. aureus* isolates, mecA-positive (6), negative mecC, 75.8% of the S. aureus MSSA isolates and all of the MRSA isolates were multi antibiotic-resistant.

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions

gene	sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
van A	CATGACGTATCGGTAAAATC	885	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Patel *et al.*, 1997**
	ACCGGGCAGRGTATTGAC							
van B	GTGACAAACCGGAGGCGAGGA	433						**Kariyama *et al.*, 2000**
	CCGCCATCCTCCTGCAAAAAA							
van X	ATGGAAATAGGATTTACTTT	609						**Saha *et al.*, 2008**
	TTATTTAACGGGGAAATC-							
mec A	GTA GAA ATG ACT GAA CGT CCG ATA A	310	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**McClure *et al.*, 2006**
	CCA ATT CCA CAT TGT TTC GGT CTA A							

Table (2): Phenotypic characteristics of *Staphylococcus aureus* strain

Basic Characteristics	**Properties (*Staphylococcus aureus*)**
Capsule	Non-Capsulated
Catalase	Positive (+ve)
Citrate	Positive (+ve)
Coagulase	Positive (+ve)
Gas	Negative (-ve)
Gelatin Hydrolysis	Positive (+ve)
Gram Staining	Positive (+ve) Gram positive cocci
H_2S	Negative (-ve)
Hemolysis	Positive (+ve)- Beta
Indole	Negative (-ve)
Motility	Negative (-ve)
MR (Methyl Red)	Positive (+ve)
Nitrate Reduction	Positive (+ve)
OF (Oxidative-Fermentative)	Fermentative
Oxidase	Negative (-ve)
Pigment	Mostly Positive (+ve)
Shape	Cocci
Spore	Non-Sporing
Urease	Positive (+ve)
VP (Voges Proskauer)	Positive (+ve)
Fermentation of	
Cellobiose	Negative (-ve)
Fructose	Positive (+ve)
Galactose	Positive (+ve)
Glucose	Positive (+ve)
Lactose	Positive (+ve)
Maltose	Positive (+ve)
Mannitol	Positive (+ve)
Mannose	Positive (+ve)
Raffinose	Negative (-ve)
Sucrose	Positive (+ve)
Xylose	Negative (-ve)
Enzymatic Reactions	
Acetone Production	Positive (+ve)
Alkaline Phosphatase	Positive (+ve)
Arginine Dehydrolase	Positive (+ve)
Hyaluronidase	Positive (+ve)
Lipase	Positive (+ve)
Ornithine Decarboxylase	Negative (-ve)

Table (3): Statistical analytical results of the examined moloha samples.

Counts	**Number of samples**	**Positive samples**		**Counts / ml**		
		No.	**%**	**Minimum**	**Maximum**	**Mean ± SEM**
TABC cfu/ml	**25**	**25**	**100**	**3.9×10^{3}**	**1.89×10^{5}**	**$3.3\times10^{4} \pm 1.3\times10$**
CC Mpn/ml	**25**	**25**	**100**	**0.3×10**	**3.9×10^{3}**	**$2.1\times10^{2} \pm 1.1\times10$**
SC cfu/ml	**25**	**19**	**76**	**1×10**	**3×10^{3}**	**$1.9\times10^{2} \pm 1.2\times10$**

Table (4): Statistical analytical results of the examined fesikh samples

Microbial parameter	standard	moloha samples **exceed** standard		fesikh samples **exceed** standard	
		No.	**%**	**No.**	**%**
TAB.C cfu/ml	**10^4 cfu/g***	**14**	**56**	**18**	**72**
C.C Mpn/ml	**10^2 cfu/g***	**7**	**28**	**10**	**40**
S.C cfu/ml	**negative**	**19**	**76**	**23**	**92**

TABC: total aerobic bacterial count. Cfu/ml: colony forming unit per ml. CC: Coliforms count. Mpn/ml: most probable number

Table (5): Moloha and fesikh examined samples (25 for each) exceed Egyptian standards.

Counts	Number of samples	Positive samples		Counts / ml		
		No.	**%**	**Minimum**	**Maximum**	**Mean ± SEM**
TAB.C cfu/ml	**25**	**25**	**100**	**7.24×10**	**1.15×10^6**	**$6.2\times10^4 \pm 3.6\times10$**
C.C Mpn/ml	**25**	**25**	**100**	**1.5×10**	**4.6×10^3**	**$3.5\times10^2 \pm 1.6\times10$**
S.C cfu/ml	**25**	**23**	**92**	**2.6×10**	**1.65×10^4**	**$2.3\times10^2 \pm 1.2\times10$**

SC: *Staphylococcus aureus* Count

*** Egyptian Organization for Standardization and Quality (E.O.S.Q.C.) (2007).**

Table (6): Susceptibility of isolated *Staph. aureus* to antibiotics (42 isolates).

Antibiotic	42 isolates			
	Sensitive		Resistant	
	No.	%	No.	%
Methicillin 5 µg /disc	16	38.09	26	61.90
Vancomycin 30 µg /disc	28	66.66	14	33.33
Gentamycin 10 µg /disc	14	33.33	28	66.67
Enrofloxacin 5 µg /disc	17	40.47	25	59.53
Norfloxacin 5 µg /disc	15	35.71	27	64.29
Ciprofloxacin 5 µg /disc	16	38.09	26	61.91
Tylosin10 µg /disc	6	14.28	36	85.72
Erythromycin10 µg /disc	3	7.14	39	92.86
Oxytetracycline 30 µg /disc	2	4.76	40	95.24
Doxycycline 30 µg /disc	3	7.14	39	92.86
Ampicillin10 µg /disc	9	21.43	33	78.57
Amoxicillin 10 µg /disc	10	23.81	32	76.19
Cefotaxim 30 µg /disc	12	28.57	30	71.43
Chloramphenicol 30 µg /disc	8	19.04	34	80.96

Table (7): Incidence of isolation of *Staph. aureus*, methicillin-resistant *Staph. aureus* (MRSA) and methicillin and vancomycin resistant *Staph. aureus* (MVRSA) from examined moloha (25 samples) and fesikh (25) samples

type	*Staph.aureus*				MRSA				MVRSA			
	moloha		fesikh		moloha		fesikh		moloha		fesikh	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
	19	76	23	92	8	32	12	48	5	20	9	36
Total (50)	42 (84%)				26 (52%)				14 (28%)			

Table (8): Result of PCR for mecA gene (310bp) and multiplex PCR for vancomycin resistant genes VanA (885bp), vanB (433bp), vanX (609bp) of *Staph. aureus*

Sample	**mecA**	**VanA**	**vanB**	**vanX**
1	+	+	-	-
2	+	-	-	-
3	+	-	-	-
4	-	-	-	-
5	+	+	-	-
6	+	+	-	-
7	+	-	-	-
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-

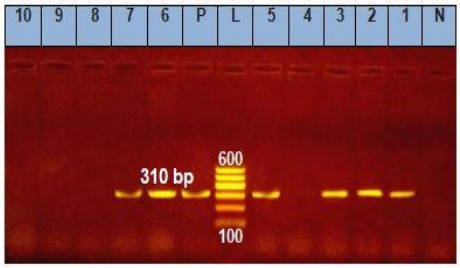

Photo (1): Agarose gel electrophoresis of PCR for mecA gene (310bp) of *Staph. Aureus*. Photo details. Lane L: 100 bp ladder as molecular size DNA marker. Lane P: Control positive genes. Lane N: Control negative.Lanes 1,2,3,5, 6, and 7: Positive for mecA gene.

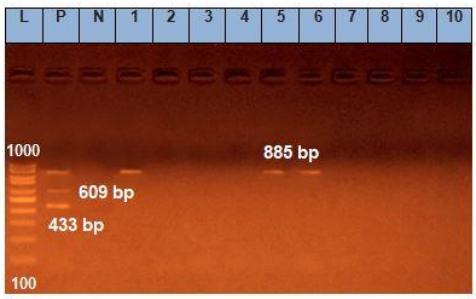

Photo (2): Agarose gel electrophoresis of multiplex PCR for vancomycin resistant genes VanA (885bp), vanB (433bp), vanX (609bp) of *Staph. aureus* Lane L: 100 bp ladder as molecular size DNA marker. Lane P: Control positive genes. Lane N: Control negative. Lanes 1, 5, and 6: Positive only for vanA gene.

Table (5) illustrated that Moloha and fesikh examined samples (25 for each) exceed Egyptian standards in which Moloha samples exceed standard total aerobic bacterial count, Coliform's count, *Staph. aureus* count were14 (56%),7 (28%) , 19 (76%) respectively, and fesikh samples exceed standard total aerobic bacterial count, Coliforms count, *S. aureus* count were18 (72%), 10 (40%), 23 (92%) respectively. A high percentage of samples exceed the standard of *Staph. aureus* count in both Moloha and fesikh may be due to Human contamination in which humanity was a carrier of *S. aureus* and source contamination for food (Boynukara *et al*. 2008).

Herrero *et al.* (1999) reported that *S. aureus* was not natural microflora of marine and freshwater fish, and Albuquerque *et al.* (2007) isolated 5 *S. aureus* strains from the hands of fish handlers and all isolates were antibiotic resistant to Ampicillin. *S. aureus* is one of the frequently occurring foodborne bacteria (Jay, 2000). *S. aureus* considered an important cause of infection, bacteremia (Kools and Bannerman,1994), infective endocarditis(Wood, 1992), cerebrospinal fluid shunt infection (Grasmick *et al.*,1983), subdural empyema(Hall *et al*.,1987), vertebral osteomyelitis (Buttery et al., 1997) and urinary tract infection(Kirchhoff *et al*., 1985).

Increase deaths worldwide due to multidrug resistance bacteria strains (Laxminarayan et al., 2016), especially MRSA which is isolated from different species (Paterson *et al.*, 2014). Different studies report the isolation of *S. aureus* from aquatic products (Hammad *et al.*, 2012; Cho *et al*., 2014), which has a risk to consumers.

Table (6) showed that Susceptibility of isolated *S. aureus* to antibiotics and it was more sensitive to vancomycin (66.66%), and the most resistant antibiotic was oxytetracycline (95.24%) then erythromycin and doxycycline (92.86%)

S. aureus has in general have virulence factors and acquire multidrug resistance and MRSA showed high resistance mainly to ampicillin, gentamycin, tetracycline, erythromycin and ciprofloxacin, chloramphenicol, and lower rates of resistance to vancomycin, (Skovgaard, 2002)

Brouillette and Malouin, (2005) and Elbargisy *et al.*, (2016) isolated *S. aureus* and antibiotic resistance was 88.5, 52.8, 40, and 25.7% for ampicillin, tetracycline, cefoxitin, and oxacillin, respectively, and 100% sensitive to gentamicin, 40% of isolates were MRSA, and antibiotic resistance was mostly associated with MRSA.

High rate of *S. aureus* resistance to one or more antibiotics (Aydin et al., 2011). The increased prevalence of MRSA all over the world (Jones *et al.*, 2003).

Dongli *et al.*, (2017) reported that *s. aureus* is one of the food-borne bacteria, they isolate *S. aureus* (37.2%) from food with high resistance to macrolides, β-lactams, and tetracyclines, and susceptible to vancomycin, negative mecC, positive mecA (9) with multidrug-resistant, aquatic products were a carrier for multidrug-resistant *S. aureus*, with public health importance, and it can be transmitted from handlers to the food chain (Sospedro *et al.*, 2012). MRSA infection causes morbidity and mortality (Klevens *et al.*, 2007; Centers for Disease Control and Prevention, 2013), *S. aureus* was detected in fish products in Northwest Spain (43.0%) (Vazquez-Sanchez *et al*., 2012) and Korea (40.7%) (Cho *et al*., 2014), from raw fish in Japan (87.0%) (Hammad *et al*., 2012)., freshwater fish samples (52.1%) higher rates of *S. aureus* detection and bacterial loads than saltwater fish samples (23.9%), and five freshwater fish samples exceeded 110 MPN/g., about 80.0% of MRSA isolates were obtained from freshwater fish, high threat of *S. aureus* in freshwater fish due to the higher antibiotic resistance of MRSA isolates, and that due to

contamination with sewage in freshwater in comparative with saltwater (Gomez *et al*., 2016; Yang *et al*., 2016; Boopathy, 2017).

Enright *et al.*, (2000) isolated *S. aureus* with resistant to ampicillin and 90.6% of isolates were resistant to three antibiotics, and 30.3% of isolates displayed resistance to six agents, among which 5.9% of isolates were resistant to 9 agents, 9 isolates were mecA positive, detected by PCR.

MRSA resists β-lactam antibiotics, 90.6% of isolates showed 3 or more antibiotic-resistant, higher than ready foods in China (Yang et al., 2016*)*. High antibiotic-resistant MRSA are detected in China (Zhang et al., 2016), these demanded emerging protocol for antimicrobial-resistant strains.

Dan *et al*., (2010) reported that resistance of MRSA isolates was high, 9 MRSA isolates were mecA gene positive. Yang *et al*. (2016) shown that two MRSA isolates were detected in aquatic products in China. Antibiotic resistance among *Staph. aureus* strains is important (Ito *et al.,* 2003). Level of exposure to antibiotic play role in the development of resistance to a particular antibiotic (Sisak *et al.,* 2006). *S. aureus* reported as one of the major bacteria of foodborne diseases by fishery products in the Europ and Staphylococci in fish indicates contamination, poor hygiene, or diseased fish (EFSA, 2010). Sergelidis *et al.,* (2014) examined salted fish products and isolate Staphylococcus aureus (7%) and (2%) MRSA isolates which carried the mecA gene and discuss risk assessment of salted fish products. Chambers (1997) isolate MRSA from seafood, becomes resistant by a gain of mecA gene, with higher resistance rates were observed against ampicillin (85·7%), erythromycin (71·4%), gentamicin (57·1%), and oxacillin (28·5%).

Hare *et al.* (2006) recorded that vancomycin was important for the treatment of infections caused by MRSA, the incidence of vancomycin-resistant *S. aureus* increase all over the world.

Suat *et al.,* (2016) showed that *S. aureus* was identified in 26% of ready-to-eat food samples and antibiotic resistance studies showed 34.6% of the *S. aureus* isolates show resistance antibiotic, tetracycline was (28.8%), and variable resistance between 1.9% and 7.7% for gentamicin, chloramphenicol, vancomycin, ciprofloxacin, and erythromycin while multi-drug resistance was 3.8%.

Data in Table (7) showed the incidence of isolation of *S. aureus,* MRSA, and MVRSA from examined moloha (25 samples) and fesikh (25 samples). Incidence of isolation of *S. aureus* from examined moloha (25 samples) and fesikh (25 samples) was 76% and 92%, methicillin-resistant *S. aureus* (MRSA) was 32% and 48% and methicillin and vancomycin-resistant *S. aureus* (MVRSA) was 20 % and 36% respectively. A reported relation between methicillin-resistant and β-lactam antibiotics resistance (Otalu *et al*., 2011). The mecA gene is carried on *S. aureus* chromosomes cassette mec and reported as a primary mechanism of methicillin resistance (Archer and Niemeyer, 1994). Kwon *et al*. (2006) isolated MRSA which carried the mecA gene and this mecA gene present on Staphylococcal cassette chromosome mec (SCC mec).

Tiemersma *et al.* (2004) recorded that in the Netherlands, 0.6% of all isolated *S. aureus* between 1999 and 2003 were resistant to methicillin, and these results were below than Europe: 23.7%.

Table (8) illustrates the result of PCR for mecA gene (310bp) and multiplex PCR for vancomycin-resistant genes VanA (885bp), vanB (433bp), vanX (609bp) of *S. aureus*, Agarose gel electrophoresis. In photo (1): of PCR for mecA gene (310bp) of *Staph. aureus,* and In photo (2): multiplex PCR for vancomycin-resistant genes VanA (885bp), vanB (433bp), vanX (609bp) of *S. aureus*. The result of PCR for the methicillin-resistant gene (mecA gene) was detected in 60% of examined *S. aureus* isolates and multiplex PCR for vancomycin-resistant genes

(VanA, vanB, vanX) of examined *S. aureus* isolates was positive for vanA (30%) but vanB and vanX gene not detected. Al-Amery *et al.,* (2019) reported that all isolates of VRSA carried vanA and vanB genes and classified as MRSA with the zoonotic transmission. McGuinness *et al.,* (2017) reported that MRSA has increased resistance to vancomycin, effective for the treatment of severe MRSA affections, resistance in VRSA is conferred by the vanA gene, vancomycin-resistant genes were A, B, C1, and C2/3, X, H, R, and S. Moellering (2012) recommends that antibiotics should not be used (prescribed) except if it is highly indicated and only after susceptibility tests. Obaidat *et al.,* (2015) isolate 156 *S. aureus* from 330 imported fresh fish samples, isolates were tested for mecA gene, antibiotic resistance, about 88.5% of the *S. aureus* show resistance to antimicrobial, high resistance to ampicillin; low resistance to tetracycline, erythromycin, and very low resistance to cefotaxime, gentamicin, and ciprofloxacin, some antibiotic resistance shows a strong correlation ($P \leq 0.01$) with enterotoxigenic in *S. aureus.* The glycopeptide antibiotic vancomycin has proven effective in treating severe MRSA infections (Sorrell *et al.,* 1982).

Yanguang *et al.,* (2020) reported that the infection MRSA has global public health importance, bactericidal action of vancomycin remains one of the important antibiotics for the treatment of MRSA affections, but its effect gradually decreased. VRSA is mediated by a vanA gene, Methicillin resistance is conferred by mecA (Walsh *et al.,* 1996).

Ghoniem *et al.,* (2014) isolate 145 *S. aureus* strains, 58.64% of them were vancomycin-sensitive and 20.68% were VRSA, vanA gene was detected in 51.9% of isolates.

CONCLUSION

Results obtained in this study confirmed the presence of *S. aureus* in salted fish samples, the occurrence of resistant strains to methicillin and vancomycin antibiotics was a potential risk.

The microbial quality of moloha salted fish is better than the fesikh salted fish and both products are considered as a source of MVRSA, an emerging public health problem.

Recommendations

Improving hygienic conditions with good handling of salted fish should be followed to limit the spread of such bacteria to humans, antibiotic-resistant organisms can be transmitted to humans through eating food contaminated with bacteria. Not use antibiotics in animals as growth promoters.

Antibiotic susceptibility tests should be done, proper use of antibiotics. Genetic antibiotic determinants should also be investigated using molecular methods. Identification of resistant determinants of vancomycin resistance. The presence of VRSA isolates indicate that resistance to vancomycin will appear due to overuse, so we recommend that vancomycin should not be used except if it is highly indicated and only after susceptibility tests

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