Prevalence of multidrug resistant*Staphylococcus aureus* in fish

Eldaly, E. A.[1], Hussein, M. A.[1], Shafik, S[2], and Mishref, M. A. [2]

1-Food Control Dept.,Faculty of Vet. Medicine,Zagazig University,Zagazig,Egypt.

2-Animal Health Research Institute, Mansoura Lab.,Mansoura, Egypt.

Correspondence author: Mohammed Abdel Fattah Mishref, mishref2015@gmail.com

Abstract

A total of 240 samples of raw fish *(T. nilotica, M. cephalus,*shrimpandcrab*)* and processed fish (Fesiekh and smoked herring) (40 of each) were collected from different fish markets in Dakhlia Governorate, Egypt. The collected samples were subjected to organoleptic examination and examined for the presence of *Staph. aureus*. Sensory evaluationof the examined fish samples revealed that 92.5%, 95%, 100%, 100%, 90% and 92.5% of the examined *T. nilotica, M. cephalus*, shrimp, crab, Fesiekh, and smoked herring samples were accepted, respectively. Fesiekh had the highest mean total *Staph. aureus* count ($2.7\times10^3\pm0.78\times10^3$) followed bysmoked herring ($2.4\times10^3\pm0.7\times10^3$), *M. cephalus* ($1.2\times10^3\pm0.38\times10^3$), *T. nilotica* ($7\times10^2\pm2.8\times10^2$), shrimp ($2.1\times10^2\pm1.5\times10^2$), and crab ($1.5\times10^2\pm0.9\times10^2$), respectively. The incidence of *Enterotoxin* production in 16 selected *Staph. aureus* isolates from processed fish was 6.25% for *Enterotoxin A,* 6.25% for*Enterotoxin C*, and 12.5% for*Enterotoxin D*. The antibiotic resistance percentages for the same selected 16 *Staph. aureus* isolates was the highest for Neomycin (100%) followed by Kanamycin (93.8%), Streptomycin (81.3%), Nalidixic acid (81.3%), Penicillin (75.0%), Oxytetracycline **(**62.5%), Cephalothin **(**56.3%), Sulphamethoxazol **(**56.3%), Enrofloxacin **(**50.0%), Erythromycin (43.8%), Gentamicin (31.3%), Ciprofloxacin (25.0%), Oxacillin (12.5%), and Vancomycin (6.3%). Percentage of 81.3% of *Staph. aureus* isolates showed Multiple Antibiotic Resistance for more than 3 antibiotics. PCR identification of erythromycin, gentamicin, methicillin and vancomycin resistance genes in tested *Staph. aureus* isolates were 37.5%, 18.75%, 12.5%, and 6.25%, respectively.

Key words*:* *Staphylococcus aureus*, Fish, Fesiekh, Smoked Herring, Antibiotic Resistance

1. Introduction

Fish considered as a good source of protein rich in essential amino acids, micro and macro elements (calcium, phosphorus, fluorine, iodine), fats that are valuable sources of energy, fat-soluble vitamins, and unsaturated fatty acids that, among other benefits, have a hypocholesterolic effect (anti-arteriosclerosis) **(Usydus *et al.*, 2008).**Moreover,fish is one of the most highly perishable food products and the shelf life of such products is limited in the presence of normal air by the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms. The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in colour, odor and texture **(Özogul *et al.*, 2004).**

Microbiological spoilage of foods may take diverse forms, but all of them are a consequence of microbial growth and/or activity, which manifests itself as changes in the sensory characteristics. Fish products with high salt contents (salted fish) may spoil due to growth of halophillic bacteria or growth of anaerobic bacteria and yeasts **(Gram and Huss, 1996).**

The handling of fish products during the manufacturing process involves a risk of contamination by *Staph. aureus* causing foodborne human intoxication **(Ash, 1997).** These bacteria are salt-tolerant and

therefore can contaminate all cured preparations such as smoked fish **(Shena and Sanjeev, 2007).***Staphylococcus spp.* has pathogenic strains which could cause food poisoning due to the heat stable *Staphylococcus enterotoxin* which is resistant to gastrointestinal enzymes.**(Okareh and Erhahon, 2015)**.

The widespread use of antibiotics has provoked an exponential increase in the incidence of antibiotic resistance in several bacterial groups in recent years. Thus, multi drug resistant *Staph. aureus* are rather common in hospital settings and farms but have been also detected in food animals and food like meat, milk and dairy products and fishery products. Also, the food chain is considered as a potential route of transmission of antibiotic-resistant bacteria to humans. The resistance to antimicrobials, particularly to ß-lactam antibiotics, has also raised high concern as an emerging problem in the food environment **(Hwa Lee, 2003)**. *Tilapia nilotica, M. cephalus,* shrimp, crab, Fesiekh, and smoked herringare popular fishes in Egyptian markets so the present study was planned to carry out the sensory evaluation and microbial examination for presence of *Staph. aureus* in raw (*Tilapia nilotica, M. cephalus,* shrimp, crab) and processed (Fesiekh, and smoked herring) fish.

2. Material and Methods

2.1.Collection of samples:

A total of 240 samples of raw fish *(T. nilotica, M. cephalus,*shrimp, andcrab*)* and processed fish (Fesiekh and, smoked herring) (40 of each) were collected from different fish markets in Dakhlia Governorate, Egypt. The collected samples were packed, identified, transferred immediately as possible in an ice-box and immediately processed in Laboratory of Animal Health Research Institute, Mansoura lab.

2.2.Sensory evaluation:

Samples were evaluated for acceptability according to **Land and Shepherd (1988)**.

2.3.Preparation of fish samples (ICMSF, 1978):

The hands were thoroughly washed and sterilized by alcohol 70% and fish were laid on one side above a sterile piece of aluminum foil. The dorsal, pectoral and ventral fins were removed by sterile scissors and forceps. Fish were held with a sterile forceps and scales were removed from the body surface everywhere by means of a sterile scalpel. Body surface were sterilized by hot spatula. The sterilized surface were removed by sterile scissors and forceps under complete aseptic conditions. Accurately, 10 grams of the back muscle just behind the head were aseptically transferred into a sterile homogenizer flask (Homogenizer type MPW–302, Poland) containing 90 ml of sterile 0.1% peptone water. The contents were homogenized for 2.5 minutes at 14000 r.p.m. to provide a homogenate of 1/10 dilution and then allowed to stand for about 5 minutes. The homogenate was transferred into a sterile test tube and 1ml was transferred into a sterile test tube containing 9 ml of 0.1% peptone water from which tenfold serial dilutions up to 10^6 were prepared.

2.4. Isolation and Identification of *Staphylococcus aureus*:

2.4.1. Isolation of *Staphylococcus aureus* according to Food and Drug Administration "FDA"(2011)

2.4.2. Morphological and Biochemical identification (Cruickshank *et al.*, 1975 and MacFaddin, 2000)

2.4.3. Detection of *Staphy. aureus* enterotoxin (Shingaki *et al.*, 1981):

2.4.4. Antibiotic Resistance of Isolated Pathogens (Antibiogramme) (Deresse *et al.*, 2012):

2.5. Polymerase Chain Reaction (PCR)

2.5.1. Primer sequences of *Staph. aureus* used for PCR identification system:

Application of PCR for identification of erythromycin (*ermA*), gentamicin (*aac 6-aph 2*), methicillin (*mecA*) and vancomycin (*vanA*) resistance genes of *Staph. aureus* was performed essentially by using Primers (Pharmacia Biotech) as shown in the following table:

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
ermA (F)	**5′** TATCTTATCGTTGAGAAGGGATT **′3**	139	**Amghalia *et al.*,(2009)**
ermA (R)	**5′** CTACACTTGGCTTAGGATGAAA **′3**		
aac 6-aph 2(F)	**5′** TTGGGAAGATGAAGTTTTTAGA **′3**	174	
aac 6-aph 2(R)	**5′** CCTTTACTCCAATAATTTGGCT **′3**		
mecA (F)	**5′** AAAATCGATGGTAAAGGTTGGC **′3**	533	
mecA (R)	**5′** AGTTCTGCAGTACCGGATTTGC **′3**		
vanA (F)	**5′**CATGAATAGAATAAAAGTTGCAATA**′3**	1030	
vanA (R)	**5′** CCCCTTTAACGCTAATACGATCAA **′3**		

2.5.2. DNA Extraction using QIA amp kit (Shah*et al.*, 2009):

After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and the following steps were carried out:

Equal volume from the lysate (50-200 µl) was added, addition of 20-50 µl of proteinase K, then incubation at 56 ºC for 20-30 min. After incubation, 200 µl of 100% ethanol was added to the lysate.

The solution was added to the column and centrifuged at 8000 rpm for 1 min. then the filtrate was discarded.

The sediment was washed using AW1 buffer (200 µl), the column was centrifuged at 8000 rpm / 1 min, and the filtrate was discarded.

Washing was applied by using the AW2 buffer (200µl), the column was centrifuged at 8000 rpm / 1 min. and the filtrate was discarded.

The column was placed in a new clean tube then, 25-50 µl from the Elution buffer was added, centrifuged at 8000 rpm/1min. Then the column was discarded. The filtrate was put in clean tube containing the pure genomic DNA. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

2.5.3. DNA amplification reaction (Perez *et al.*, 2001):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). The multiplex PCR was performed in a total volume of 25 µL containing 80 mM $MgCl_2$, PCR buffer, 3.5 mM DNTP mix (Fermentas), 10 picomole µlof each of the primers and 1 unit of Taq polymerase with 1 µl of bacterial suspension obtained from the rapid DNA extraction method described above.

Amplifications were carried out by using a thermal cycler with the following thermal cycling profile: an initial denaturation step at 94°C for 5 min was followed by 25 cycles of amplification (denaturation at

94°C for 45 sec, annealing at 64°C for 30 sec and extension at 72°C for 1 min) ending with a final extension step at 72°C for 10 min.

After amplification, 10 µL of the reaction mixture was loaded onto a 2% agarose gel and electrophoresed to estimate the sizes of the amplification products with a 100-bp molecular size standard ladder. The gel was then stained with ethidium bromide and photographed under UV illumination.

3. Results and Discussion

Safety and quality assurance considered the main problem of modern fish industry, which greatly depends on presence or absence of food borne pathogens in a fish product and is a function of the harvest, environment, sanitary conditions, and practices associated with equipment and personnel in the processing line **(Food and Drug Administration "FDA", 2001).**

Sensory evaluationof the examined fish samples presented in Table (1) showed that 37 (92.5%) and 38 (95%) out of examined *T. nilotica* and *M. cephalus* samples were accepted by panel persons, respectively. On the other hand, all examined samples of shrimp and crabwere accepted by panel persons. Concerningprocessed fish, 90% and 92.5% of examined Fesiekhandsmoked herring samples were accepted by the panelist respectively. Nearly similar results were recorded by **Morshdy *et al.*, (2002)** for raw fish and by**Shafik, *et al.*, (2017)**for processed fish.

Fig. (1) revealed thatFesiekh had the highest mean *Staph. aureus* count followed bysmoked herring, *M. cephalus, T. nilotica,* shrimp, and crab, respectively. Poor personal hygiene of the food handler, no pre-employment and routine medical examination and improper storage of processed fish (Fesiekh and smoked herring) were identified as the contributing factors of *Staph. aureus* illness **(Center for Health protection, 2011).**

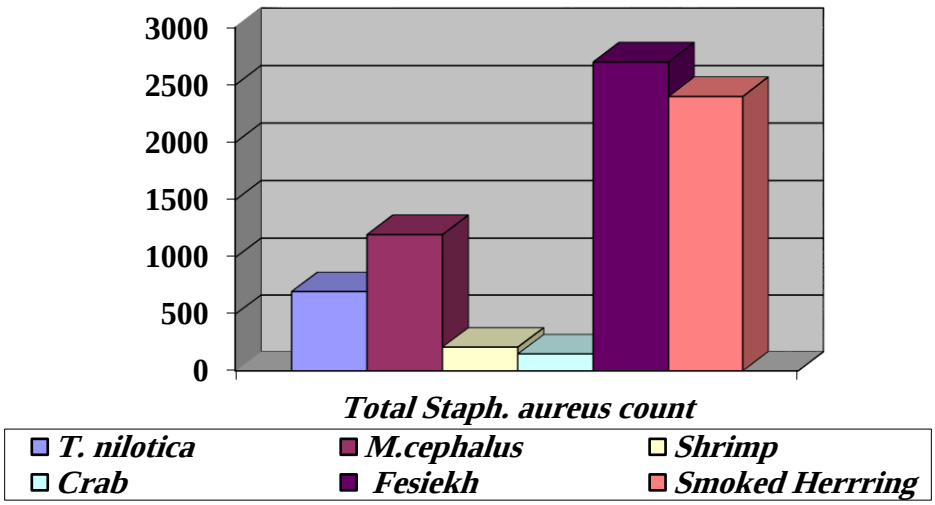

Fig. (1): Mean total *Staph. aureus* count (cfu/g) in the examined fish samples (*T. nilotica*, *M. cephalus*, Shrimp, Crab, Fesiekh and Smoked herring) (No=40 of each).

The obtained results in Table (2) revealed that the mean count of *Staph. aureus* was $7\times10^2\pm2.8\times10^2$ cfu/g with an incidence of 45 % in examined *T. nilotica*, while suchcount was $1.2\times10^3\pm0.38\times10^3$ cfu/g in examined *M. cephalus* with an incidence of 52.5%. On the other hand, the mean count of *Staph. aureus* in examined shrimp was $2.1\times10^2\pm1.5\times10^2$ cfu/g with an incidence of 4 %, Meanwhile such count was $1.5\times10^2\pm0.9\times10^2$ in crab with an incidence of 17.5%.

Nearly similar findings were reported by **Morshdy *et al.*, (2002)**in *T. nilotica* and *M. cephalus*, **Nahla, (2009)**in *M. cephalus*, **Shaimaa Nada *et al.*, (2014)** in shrimp and crab and **Aman *et al.*, (2017)** in *M. cephalus*. However, lower results were obtained by **Ola, (2011)**in *T.nilotica* and *M.cephalus*. On the other hand, higher results were obtained by **Omaima and El-kewaiey (2008)**in *T.nilotica* and *M.cephalus*, **Nahla, (2009)** in *T. nilotica*, and **Aman *et al.*, (2017)** in *T. nilotica*.

On comparing *Staph. aureus* count in Table (2) in relation to **ES, (2005a)** we found that 5 (12.5%) of *T. nilotica*, 7 (17.5%) of *M. cephalus*, 1 (2.5%) of shrimp, and 1 (2.5%) of crab samples considered as refused " more than 10^3 cfu/g". The higher means of *Staph. aureus* counts may be attributed to hand contamination of fish handlers during catching, sorting and selling which in turn contaminates fish, water and ice used for their preparation for selling **(Le Loir et al, 2003).**

The obtained results in Table(3) revealed that the mean count of *Staph. aureus* was $2.7\times10^3\pm0.78\times10^3$ cfu/g with an incidence of 92.5% in examined Fesiekh, while suchcount was $2.4\times10^3\pm0.7\times10^3$ cfu/g in examined smoked herringwith an incidence of 82.5%.

Lower results were obtained by**Shafik, *et al.*, (2017)**. On the other hand, higher result was obtained by **Edris *et al.*, (2014)**. Such variations may be attributed to the level of sanitation applied during marketing of such fish. On comparing *Staph. aureus* count in Table (3) in relation to **ES, (2005b)** and**ES, (2005c)** we found that 9 (22.5%) of Fesiekh, 7 (17.5%) of smoked herring samples considered as refused " more than 10^2 cfu/g".

Table (4) displayed the incidence of *Enterotoxin* production in 16 selected *Staph. aureus* isolates from processed fish which was 6.25% for *Enterotoxin A*, 6.25% for*Enterotoxin C*, and 12.5% for*Enterotoxin D*. This study shows that consumption of these products pose a risk of *Staph. aureus* intoxication. Nearly similar findings were recorded by**Sergelidis *et al.*, (2014)**.

A dose lower than 1 µg of SE (*Staphylococcal Enterotoxins*) has been reported to make symptoms of staphylococcal food poisoning to appear within 1-6 h after consumption of contaminated food in an adult healthy individual **(Pinchuk *et al.*, 2010)**.This toxin level can be reached when cell number exceeds 10^5 cfu/g of food **(Bathia and Zahoor, 2007)**. As a preventive measure, legal limit of 10^2 cfu/g had been set for total *Staph. aureus* count in different fishery products.

Table (1): Sensory evaluationof the examined fish samples (*T. nilotica*, *M. cephalus*, Shrimp, Crab, Fesiekh andSmoked herring) (No=40 of each).

Samples	Odour	Colour	Texture	Taste	Appearance	Overall acceptability	
						No	%
T. nilotica	5.75±0.24	6.4±0.2	5.9±0.29	-	6.15±0.26	37	92.5
M. cephalus	5.93±0.25	6.75±0.26	6.38±0.23	-	6.58±0.25	38	95
Shrimp	7.13±0.22	6.68±0.23	6.5±0.24	-	6.63±0.19	40	100
Crab	6.7±0.27	6.85±0.26	6.68±0.23	-	6.83±0.24	40	100
Fesiekh	6.18±0.27	5.9±0.26	5.7±0.3	6.35±0.25	5.8±0.21	36	90
Smoked herring	6.08±0.26	6.5±0.28	5.8±0.3	6.13±0.28	6.28±0.26	37	92.5

Table (2): Total *Staph. aureus* Count (cfu/g) in the examined raw fish samples (*T. nilotica, M. cephalus,* Shrimp and Crab) (No=40 of each).

Samples	Incidence		Min	Max	Mean ±SE	Accepted samples $< 10^3$		Refused samples $\geq 10^3$	
	No	%				No	%	No	%
T. nilotica	18	45	<10	7.6×10^3	$7\times10^2\pm2.8\times10^2$	35	87.5	5	12.5
M. cephalus	21	52.5	<10	8.7×10^3	$1.2\times10^3\pm0.38\times1^3$	33	82.5	7	17.5
Shrimp	9	22.5	<10	5.9×10^3	$2.1\times10^2\pm1.5\times10^2$	39	97.5	1	2.5
Crab	7	17.5	<10	3.5×10^3	$1.5\times10^2\pm0.9\times10^2$	39	97.5	1	2.5

* Accepted and refused samples according to ***ES (2005a)***.

Table (3): Total *Staph. aureus* Count (CFU/g) in the examined processed fish samples (Fesiekh and Smoked herring) (No=40 of each).

Samples	Incidence		Min	Max	Mean±SE	Accepted samples $< 10^2$		Refused samples $\geq 10^2$	
	No	%				No	%	No	%
Fesiekh	37	92.5	<10	1.8×10^4	$2.7\times10^3\pm0.78\times10^3$	31	77.5	9	22.5
Smoked herring	32	80	<10	1.6×10^4	$2.4\times10^3\pm0.7\times10^3$	33	82.5	7	17.5

* Accepted and refused samples according to ***ES (2005b)***and ***ES (2005c)***

Table (4): Incidence of Enterotoxin production in 16 selected *Staph. aureus* isolates from processed fish.

Enterotoxin		Enterotoxin A	Enterotoxin C	Enterotoxin D
Incidence	No	1	1	2
	%	6.25%	6.25%	12.5%

The same 16 *Staph. aureus* were also assayed for susceptibility to 14 antibiotics commonly used in medical and veterinary practice as displayed inTable (5). The antibiotic resistance percentages for the tested *Staph. aureus* isolates was the highest for Neomycin (100%) followed by Kanamycin (93.8%), Streptomycin (81.3%), Nalidixic acid (81.3%), Penicillin (75.0%), Oxytetracycline **(**62.5%), Cephalothin **(**56.3%), Sulphamethoxazol **(**56.3%), Enrofloxacin **(**50.0%), Erythromycin (43.8%), Gentamicin (31.3%), Ciprofloxacin (25.0%), Oxacillin (12.5%), and Vancomycin (6.3%). Nearly similar findings were reported by**Laniewska-Trokenheim *et al.*, (2006)**in smoked fish,**Vazquez- Sanchez *et al.*, (2012)** in salted fish, and**Sergelidis *et al.*, (2014)** in ready to eat fish.

More so, in Egypt, little is known about the occurrence of antibiotics resistant bacteria in fish. As far as intensive fish culture goes, a large amount of fish food and antibiotics have been used to increase production and to protect the fish from diseases. Consequently, a large portion of feeds and antibiotic enter the water as wastes, causing water pollution.

Table (5): Antimicrobial susceptibility of 16 selected *Staph. aureus* isolates from processed fish.

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Neomycin (N)	-	-	-	-	16	100
Kanamycin (K)	-	-	1	6.3	15	93.8
Streptomycin (S)	1	6.3	2	12.5	13	81.3
Nalidixic acid (NA)	3	18.8	-	-	13	81.3
Penicillin (P)	3	18.8	1	6.3	12	75.0
Oxytetracycline (T)	2	12.5	4	25.0	10	62.5
Cephalothin (CN)	4	25.0	3	18.8	9	56.3
Sulphamethoxazol (SXT)	5	31.3	2	12.5	9	56.3
Enrofloxacin (EN)	7	43.8	1	6.3	8	50.0
Erythromycin (E)	7	43.8%	2	12.5	7	43.8%
Gentamicin (G)	9	56.3%	2	12.5	5	31.3%
Ciprofloxacin (CP)	10	62.5%	2	12.5	4	25.0%
Oxacillin (OX)	11	68.8%	3	18.8	2	12.5%
Vancomycin (V)	14	87.5%	1	6.3	1	6.3%

S: Susceptible, I: Intermediate susceptibility, R: Resistant

Antimicrobial resistance profile ofthe 16 selected *Staph. aureus* isolates was revealed in Table (6). 13 out of 16 isolates (81.3%) showed Multiple Antibiotic Resistance (MAR) for more than 3 antibiotics. It was cleared that MAR index was ranged from 1 to 0.071 with an average of 0.553. Multiple Antibiotic Resistant *Staph. aureus* is recognized as an environmental hazard to the food supply and human health. Nearly similar findings were recorded by **Sergelidis *et al.*, (2014)**, and **Sivaraman *et al.*, (2017)**.

The emergence of multi-drug resistant pathogens is recognized as an environmental hazard to the food supply and human health, as it makes eradication more difficult and incidence to increase **(Popovich *et al.*, 2007).**

Although the use of antibiotics in human medicine has influenced the emergence of resistant bacteria, the use of antibiotic in animals has contributed to the problem of resistance and complicates the choice of treatment in human diseases **(Novotny *et al.*, 2004).** Especially the fact that transfer of resistant bacteria between aquatic animals and humans through food production line has been documented and can pose a threat to public health **(Grema *et al.*, 2015).** Although there is records of antimicrobial resistance from fish and fish handlers, the world organization for animal health (OIE), aquatic animal health code recommends the continuous monitoring and surveillance of antimicrobial resistance in microorganism associated with aquatic animals **(Smith *et al.*, 2013).**

Fig. (2)revealed PCR identification of erythromycin (*ermA*), gentamicin (*aac 6-aph 2*), methicillin (*mecA*) and vancomycin (*vanA*) resistance genes of *Staph. aureus*. The results showed that *ermA* gene was detected in 6 (37.5%) isolates, *aac 6-aph 2* gene was detected in 3 (18.75%) isolates, *mecA* gene was detected in 2 (12.5%) isolates, and *vanA* gene was detected in 1 (6.25%) isolate. Nearly similar findings were reported by**Hammad *et al.*, (2012)** and**Sergelidis *et al.*, (2014)** in ready to eat fish.

However, **Vazquez- Sanchez *et al.*, (2012)** detected no *mecA* gene in salted fish.On the other hand,**Eman F.A. Farag, (2016)** detected higher incidence of *mecA* gene in 2 isolates out of selected 5 *Staph. aureus* isolated from Fesiekh.

Antibiotics used for animals either for therapy, prophylactic or growth promotion purposes at a sub therapeutic dose can result in transfer of resistant genes from animals to humans and thereby establishing a reservoir of resistant microbes **(Angulo *et al.*, 2004).**

Staph. aureus becomes resistant by the acquisition of the *mecA* gene, which encodes a penicillin-binding protein (PBP2a) with a low affinity for b-lactams. Strains producing PBP2a are resistant to all b-lactam antibiotics **(Chambers 1997).**

Table (6): Antimicrobial resistance profile of16 selected *Staph. aureus* isolates from processed fish.

NO	**Strains**	**Antimicrobial resistance profile**	**MAR index**
1	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN, E, G, CP, OX, G	1
2	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN, E, G, CP, OX	0.928
3	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN, E, G, CP	0.857
4	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN, E, G, CP	0.857
5	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN, E, G	0.786
6	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN, E	0.714
7	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN, E	0.714
8	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT, EN	0.643
9	*Staph .aureus*	N, K, S, NA, P, T, CN, SXT	0.571
10	*Staph .aureus*	N, K, S, NA, P, T	0.428
11	*Staph .aureus*	N, K, S, NA, P	0.357
12	*Staph .aureus*	N, K, S, NA, P	0.357
13	*Staph .aureus*	N, K, S, NA	0.286
14	*Staph .aureus*	N, K	0.143
15	*Staph .aureus*	N, K	0.143
16	*Staph .aureus*	N	0.071
Average 0.553			

MAR: Multiple Antibiotic Resistances

N: Neomycin K: Kanamycin S: Streptomycin NA: Nalidixic acid
P: Penicillin T: Oxytetracycline CN: Cephalothin SXT: Sulphamethoxazol
EN: Enrofloxacin E: Erythromycin G: Gentamicin CP: Ciprofloxacin
OX: Oxacillin V: Vancomycin

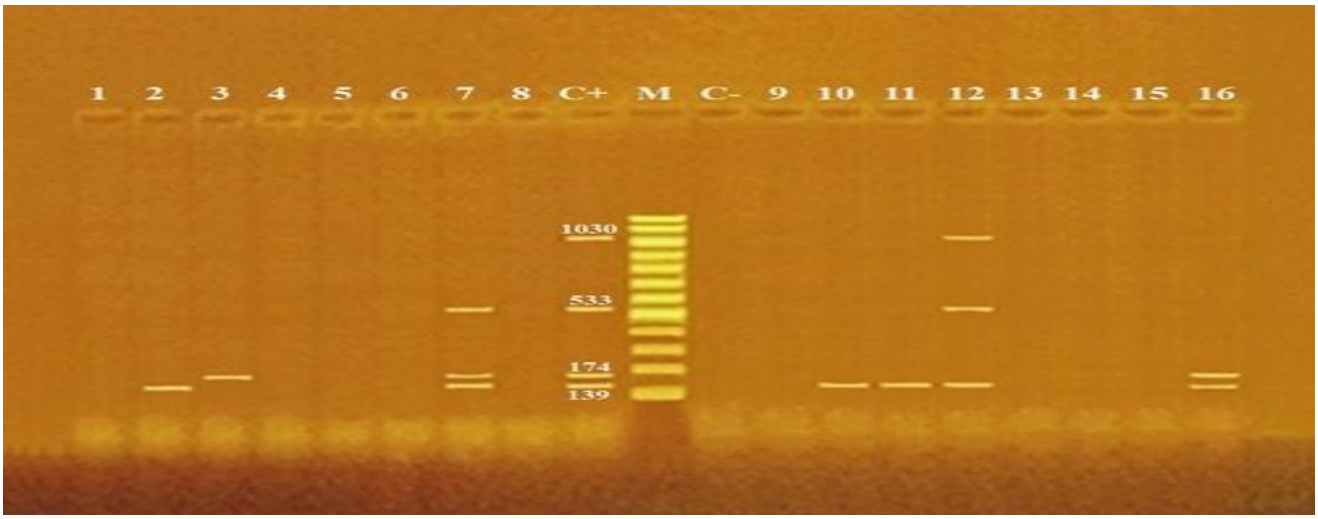

Fig. (2): Agarose gel electrophoresis of multiplex PCR of *ermA* (139 bp), *aac (6)-aph (2)* (174 bp), *mecA* (533 bp) and *vanA* (1030 bp) antibiotic resistance genes of *Staph. aureus.* **Lane M:** 100 bp ladder as molecular size DNA marker, **Lane C+:** Control positivefor*ermA, aac (6)-aph (2),mecA* and *vanA* genes.**Lane C-:** Control negative. **Lanes 2, 10 & 11:** Positive *Staph. aureus* strains for*ermA* gene. **Lane 3:** Positive *Staph. aureus* strain for*aac (6)-aph (2)* gene. **Lane 16:** Positive *Staph. aureus* strain for *ermA* and*aac (6)-aph (2)* genes. **Lane 7:** Positive *Staph. aureus* strain for *ermA,aac (6)-aph (2)* and *mecA* genes. **Lane 12:** Positive *Staph. aureus* strain for *ermA,mecA* and *vanA* genes. **Lanes 1, 4, 5, 8, 9, 13, 14 & 15:** Negative *Staph. aureus* strains for*ermA, aac (6)-aph (2), mecA* and *vanA* genes.

4. Conclusion

In conclusion, the examined raw fish (*T. nilotica*, *M. cephalus*, shrimp, andcrab) were subjected to various sources of contamination during harvesting, transportation, distribution and marketing with *Staphylococcus aureus*. On the other hand, this study indicates that Fesiekh, and smoked herring consumption may pose a risk of food borne intoxication to consumers. Special health warnings for susceptible groups such as pregnant woman, young children, the elderly, immune compromised persons and persons who have decreased stomach acidity.

The widespread presence of antibiotic resistance microorganisms should be a priority to reinforce the importance of basic hygiene for fish. T here is urgent need for a monitoring system of antibacterial drugs that are being used in aquaculture practices.

It is recommended that the hygienic handling of fish from the moment of capture to the point of consumption is crucial to ensure good quality and long shelf life.

Acknowledgement

This study was funded by Faculty of Veterinary Medicine, Zagazig University.

5. References:

Aman, I.M.; Ali, Y.E.; Moustafa, N.Y. and Hamza, A.A. (2017). Quality assessment of *Tilapia nilotica* and *Mugil cephalus* fish from Egypt. J Vet Med Allied Sci. 1(2):1-6.

Amghalia, E.; Al-Hag, N.; Shamsudin, M; Radu, S.; Rosli, R.; Neela, V. and Rahim, R. (2009). Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in *Staphylococcus aureus* isolated from Malaysian hospitals. Res. J. Biol. Sci., 4 (4): 444-448.

Angulo, F.J.; Nunnery, J.A. and Bair, H.D. (2004). Antimicrobial resistance in zoonotic enteric pathogens. Rev. Sci. Tech. Off. Int. Epiz., 23(2): 485-496.

Ash, M. (1997).*Staphyhcoccus aureus* and Staphyioeoccal Enerotoxins. In: Foodborne microorganisms of public health importance, 5th Edition, (Eds) Hocking, A.D., Arnold, *G*,.,Jenson I., Newton, K.; Sutherland. Aifst (NSW Brands, Svdney.313-332.

Bathia, A. and Zahoor, S. (2007).*Staphylococcus aureus* enterotoxins: a review. Journal of Clinical and Diagnostic Research 1 (2), 188-197.

Center for Health protection (2011). Scientific committee on Enteric Infections and Foodborne diseases. http://www.chp.gov.hk/files/pdf/review of Staphylococcal Food poisoning in Hong Kong.pdf

Chambers, H.F. (1997). Methicillin resistance in *Staphylococci*: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 10, 781–791.

Cruickshank, R.; Duguid, J.R.; Marmion, B.D. and Swain, R.H.A. (1975). Medical microbiology. The practice of medical microbiology. VIII 11th and 12th Ed. Churchill Living Stone Edinberg, London and New York.

Deresse D.; Solomon, G. and Dawit, Y. (2012). Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. Ann. Clin. Microbiol. Antimicrobials, 11: 26-34.

Edris, A.A.; Reham, A. Amin; Marionette, Z. Naseif and Ebtsam, M. AbdelFatah (2014). Evaluation of Retailed Salted Fish according to Egyptian Standard. Benha Veterinary Medical Journal, 27(2):168-176.

Egyptian Standard Specifications "ES" (2005a). "Egyptian Standards No. 889-1" for whole frozen fish part 1, A.R.E. Egyptian organization and quality.

Egyptian Standard Specifications "ES" (2005b). Salted fish Es: 1725-3/2005.

Egyptian Standard Specifications "ES" (2005c). Smoked fish Es: 288/2005.

Eman F.A. Farag, (2016). Demonstration of *Staphylococcus aureus* in salted fish with special reference to its enterotoxins**.**M.Sc. Thesis, Food Control, Faculty of Veterinary Medicine, Benha University.

Food and Drug Administration "FDA" (2001). Detection and enumeration of *Staphylococcus aureus* in food. *Staphylococcus aureus* Bacteriological analytical manual. 8th Ed., Chapter 12. Gaithersburg, P. 562.

Food and Drug Administration "FDA", (2011). Bacteriological analytical manual on line. http://www.fda.gov/Food/ScienceResearch/ LaboratoryMethods/Bacteriological Analytical Manual BAM/default.htm2011Accessed 2 March 2012.

Gram, L. and Huss, H. H. (1996). Microbiological spoilage of fish and fish products. Food Microbiology, 33: 121-137.

Grema, H.A.; Geidam, Y.A.; Gadzama, G.B.; Ameh, J.A. and Suleiman, A. (2015). Methicillin Resistant *Staphylococcus aureus* (MRSA): A review. Adv. Anim. Vet. Sci., 3(2): 79-98.

Hammad, A.M.; Watanabe, W.; Fujii, T. and Shimamoto, T. (2012). Occurrence and characteristics of methicillin-resistant and susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococci* from Japanese retail ready-to-eat raw fish. Int J Food Microbiol 156, 286–289.

Hwa Lee J. (2003). Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol. 69, 6489-6494.

International Committee on Microbiological Specification for foods "ICMSF" (1978). Micro-organisms in foods. Their significance and methods of enumeration. 2nd ed., Univ. of Toronto press. Toronto, Canada.

Land, D.G. and Shepherd, R. (1988). Scaling and ranking methods in Piggot, J.R. (Ed.), Sensory Analysis of Foods, Sec. Ed. Else-vier, London, 155-185.

Laniewska-Trokenheim, L.; Sobota, M. and Warminska- Radyko, I. (2006). Resistants to antibiotics of bacteria isolated from smoked fish. In 13th World Congress of Food Science and Technology, p. 721. Nantes, France.

Le Loir, Y.; Baron F., and Gautier M. (2003).*Staphylococcus aureus* and food poisoning. Genetics and Molecular Research, 2(1): 63-76.

MacFaddin, J. F. (2000). Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

Morshdy, A.M.; Eldaly E.A. and Sallam, K.I. (2002). Sanitary evaluation of some marketed fishes in Sharkia Province 6th Veterinary Medicine Zagazig University. Conference 313.

Nahla, A. A. El-Shabasy (2009). Microbiological analysis of farm fishes in Alexandria Governorate. Ph.D Thesis, Meat Hygiene Faculty of Veterinary Medicine, Alexandria University.

Novotny, L.; Dvorska, L.; Lorencova, A.; Beran V. and Pavlik, I. (2004). Fish: A potential source of bacterial pathogens for human beings. Vet. Med. Czech., 49(9): 343-358.

Okareh, O.T. and Erhahon, O.O. (2015). Microbiological Assessment of Food and Hand-Swabs Samples of School Food Vendors in Benin City, Nigeria. Food and Public Health, (5)1: 23-28.

Ola, M. El-Betar (2011). Quality Assessment of Some Fresh Fish. M.Sc. Thesis, Meat Hygiene, Faculty of Veterinary Medicine, Alexandria University.

Omaima A. Saleh, and El-kewaiey, I.A. (2008). Microbial Assessment of Some Marketed Fish in Damanhour City. Assuit Vet. Med. J. 54(118).

Özogul, F.; Polata, A. and Özogul, Y. (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). Food Chemistry, 85: 49–57.

Perez, E.; Claverie, M.; Villar, J. and Alvarez, M. (2001). Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection methicillin and mupirocin resistance. J. Clin. Microbiol., 39 (11): 4037 - 4041.

Pinchuk, I.V.; Beswick, E.J. and Reyes, V.E. (2010). Staphylococcal Enterotoxins. Toxins 2, 2177-2197.

Popovich, K.J.; Hota, B. and Weinstein, R.A. (2007). Treatment of community-associated methicillin-resistant *Staphylococcus aureus*. Current Infectious Disease Reports 9, 398- 407.

Sergelidis, D.A.; Abrahim, T.; Papadopoulos, N.; Soultos, E.; Martziou, V.; Koulourida, A.; Govaris, A.; Pexara, A.; Zdragas, and Papa, A. (2014). Isolation of methicillin-resistant *Staphylococcus* spp. From ready-to-eat fish products. Letters in Appl. Microbiol. 59, 500-506.

Shafik, S.; Ola, Atia; Sania, T. EL-Ghamry; EL-Dosoky, H.F.A. and Ayman, A.E. (2017). Spread of *Staph. aureus* in salted and smoked fish using PCR with studying the effect of Ultra Violet radiation on the reduction of the isolates. Animal Health Research Journal, 5(4): 84-92.

Shah, D.; Shringi, S.; Besser, T. and Call, D. (2009). Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor and Francis group, Florida, USA, Pp. 369-389.

Shaimaa, M. Nada; Dina, I. ELZahaby; El-Sayed, M. El Dahshan and Ola, F.A. Talkhan (2014). Chemical and bacteriological evaluation of some sea foods. Benha Veterinary Medical Journal, 27, (2) :1-8.

Shena, S. S. and Sanjeev, S. (2007). Prevalence of enterotoxigenic*Staphylococcus aureus* in fishery products and fish processing factory workers. Food Control, 18 (12): 1565-1568.

Shingaki, M.; Igarashi, H.; Fujikawa, H.; Ushioda, H.; Terayrna, T. and Sakai, S. (1981). Study on Reversed Passive Latex Aggltination for detection of staphylococcal enterotoxins A, B, and C. Annu.; Rep. Tokyo, metro p. Res.Lab.Public Health; 32(1): 128-131.

Sivaraman, G.K.; Lalitha, K.V.; Ravi Shankar, C.N.; Jha، A.K.; Remya, S.; Vanik, D.; Visnuvinayagam, S.; Kriplani, Y. and Ajeesh (2017).Coagulase gene polymorphism of *Staphylococcus aureus* in fish and its multiple drug resistant pattern. Journal of Environmental Biology, 38, 389-392.

Smith, P.; Alday-Sanz, V.; Matysczak, J.; Moulin, G.; Lavilla-Pitogo, C.R. and Prater, D. (2013). Monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals. Rev. Sci. Tech. Off. Int. Epiz., 32(2): 583-593.

Usydus, Z.; Szlinder-Richert, J.; Polak-Juszczak, L.; Kanderska, J.; Adamczyk, M.; Malesa-Ciecwierz, M. and Ruczynska, W. (2008). Food of marine origin: between benefits and potential risks. Part I. Canned fish on the Polish market. Food Chemistry, 111: 556–563.

Vazquez-Sanchez, D.; Lopez-Cabo, M; Saa-Ibusquiza, P. and Rodriguez Herrera, J.J. (2012). Incidence and characterization of *Staphylococcusaureus* in fishery products marketed in Galicia Northwest Spain. International Journal Food Microbiology, 157: 286-296.

الملخص العربي

مدى تواجد بكتيرياالمكورات العنقودية الذهبيةالمقاومة للأدوية المتعددة فىالأسماك

السعيد أبوزيد الدالى[1]،محمد عبدالله حسين[1]، صالح شفيق محمد[2]، و محمد عبدالفتاح مشرف[2]

1-قسم مراقبة الأغذية-كلية الطب البيطرى - جامعة الزقازيق - الزقازيق - مصر

2- معهد بحوث صحة الحيوان- معمل المنصورة- المنصورة - مصر

تعتبر الأسماك مصدرا جيدا للبروتينات الغنية بالأحماض الأمينية الأساسية والمعادن (كالسيوم ، الفوسفور ، الفلور ، اليود) ، والدهون التي تعتبر مصادر قيِّمة للطاقة ، والفيتامينات التي تذوب في الدهون ، والأحماض الدهنية غير المشبعة.

تتعرض الأسماك إلى الكثير من مخاطر التلوث ، وقد تجد هذه الملوثات طريقها عبر العديد من المصادر ثم تنمو وتتكاثر في الأسماك مما يجعلها غير صالحة للاستهلاك البشري. لذلك ، تم التخطيط لتنفيذ هذه الفجوة باستخدام الأسماك الطازجة (البلطى، البورى، الجمبرى ، والكابوريا) ، الأسماك المجهزة (الفسيخ، والرنجة المدخة) التي كانت أكثر الأسماك استهلاكا وشعبية في محافظةالدقهلية.

تم جمع 240 عينة من الأسماك الطازجة (البلطى، البورى، الجمبرى ، والكابوريا) والأسماك المجهزة (الفسيخ، والرنجة المدخة) (40 لكل منها) من أسواق الأسماك المختلفة في محافظة الدقهلية لتقييمها حسيا وفحصها لمدى تواجد ميكروبات المكور العنقودى الذهبى بها وكذلك الكشف عن مقاومة معزولات هذا الميكروب للمضادات الحيوية.

وكشف التقييم الحسي لعينات الأسماك التي تم فحصها أنه تم قبول البلطى، البورى، الجمبرى ، والكابوريا، والفسيخ، والرنجة المدخة بنسبة 92.5٪، و 95٪، و 100٪، و 100٪ ، و 90٪، و 92.5٪ ، على التوالي.

كان للفسيخ أعلى متوسط للعد الكلى للميكروب العنقودى الذهبى ($2.7 \times 10^3 \pm 0.78 \times 10^3$) متبوعًا بالرنجة المدخنة ($2.4 \times 10^3 \pm 0.7 \times 10^3$) ، البورى($1.2 \times 10^3 \pm 0.38 \times 10^3$) ، البلطى ($7 \times 10^2 \pm 2.8 \times 10^2$) ، الجمبرى ($2.1 \times 10^2 \pm 1.5 \times 10^2$) ، الكابوريا ($1.5 \times 10^2 \pm 0.9 \times 10^2$) ، على التوالي.

كان معدل إنتاج السموم المعوية من 16 معزول منتخب من الميكروب العنقودى الذهبىالمعزولة من الأسماك المجهزة بنسبة 6.25 ٪ للسم المعوى ايه ، و 6.25 ٪ للسم المعوى سى ، و 12.5 ٪ للسم المعوى دى. وتبين هذه الدراسة أن استهلاك هذه المنتجات تشكل خطرا من امكانية التسمم بسم الميكروب العنقودى الذهبى.

كانت أعلى نسبة لمقاومة المضادات الحيوية لنفس معزولات الميكروب العنقودى الذهبى المعزولة من الأسماك المجهزةللنيومايسين (100٪) يليها كانامايسين (93.8٪) ، ستربتوميسين (81.3٪) ، حمض ناليديكسيك (81.3٪) ، بنسيلين (75٪) ، أوكسي تتراسيكلين (62.5٪) ، سيفالوثين (56.3٪) ، وسلفاميثوكسازول (56.3٪)، وانروفلوكساسين (50٪)، واريثرومايسين (43.8٪)، وجينتامايسين (31.3٪)، وسيبروفلوكساسين (25٪)، واوكساسيلين (12.5٪)، وفانكومايسن (6.3٪)، على الترتيب. أظهرت المعزولات المقاومة المتعددةللمضادات الحيوية لأكثر من 3 مضادات حيوية بنسبة 81.3٪.

تم الكشف عن الجينات المقاومةللمضادات الحيوية الاريثرومايسين ، وجنتاميسين ، وميثيسيلين وفانكومايسين في معزولات الميكروب العنقودى الذهبى بنسبة 37.5٪ و 18.75٪ و 12.5٪ و 6.25٪ على التوالي باستخدام تفاعل البلمرة المتسلسل.