Enterotoxin producing *S. aureus* in salted fish

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ABSTRACT

A total of 90 samples of sardine, molouha and feseikh (30 of each) were collected from different retail markets for bacteriological and molecular examination. The average of *Staphylococci* counts (cfu/g) were ranged from1.0 x 10^2 to 1.1x10^4 in sardine, ranged from 1.0x10^2 to 3.4×10^5 in molouha and 1.0×10^2 to 7.8×10^5 in feseikh. with a mean value 2.75×10^3 ± 0.41×10^3, 1.98×10^4 ± 0.28×10^4 and 5.03×10^4 ± 1.12×10^4, respectively. Concerning to *S. aureus* it was detected in 36.67%, 46.67% and 50.00% of the examined salted fish sardine, molouha and fesiekh, respectively. Totally 44.44% of the examined samples of salted fish were contaminated with *S. aureus*. The incidence of enterotoxins (A, B, C and D) produced by *S. aureus* were 20.00%, 40.00%, 60% of sardine, molouha and fesiekh. Modern rapid methods as Polymerase Chain Reaction (PCR) has high sensitivity, specificity and reduce detection time. It offers advantages over conventional diagnostic methods.

Keywords: Salted fish, Staph. aureus, Enterotoxin, PCR.

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1. INTRODUCTION

Fish acts as a vehicle for many types of microorganisms from its natural aquatic environment, sewage, soil, contaminated harvesting areas and contaminated utensils during handling, processing, distribution (Shewan, 1971). Regarding the external contamination of fish, it may be actively contamination of fish, it may be actively infected with human pathogens by exposure to contamination of water and may constitute a public health hazard (Janssen and Meyers, 1968). Feseikh a traditional Egyptian salted fish, has been considered as a popular part of the Egyptian diet especially in certain celebration times as spring day. The handling of fish products during the manufacturing process involves a risk of contamination by *S.aureus*, causing human intoxication (Ash, 1997). These bacteria are salt-tolerant and therefore can contaminate all cured preparations such as cold smoked fish and caviar (Shena and Sanjecv, 2007). *Staphylococcal* enterotoxins (SEs) are toxic compounds excreted mainly by strains of *Staphylococcus aureus*. Among these toxins, enterotoxins A (SEA) and B (SEB) are both of the most prevalent compounds in *staphylococcal* food poisoning, to date more than 20 SEs have been described: SEA to SElV (Soriano et al., 2012). Over the past 15 years there has been an important evolution in molecular approaches for the rapid detection of food borne pathogens. Modern rapid methods as Polymerase Chain Reaction (PCR) has high sensitivity, specificity and reduce detection time. It offers advantages over conventional diagnostic methods. (Guion et al., 2008). The aim of this study is evaluation confirmation of retailed salted fish (Sardine, Molouha and Feseikh) with the Egyptian organization for Standardization and Quality Control.

2. MATERIALS AND METHODS

2.1. Collection of samples:

Ninety random samples of salted fish represented by Sardine, Molouha and Fesiekh (30 of each) were collected from different fish markets in Menoufia government, Egypt. Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. The collected samples were subjected to the bacteriological and chemical examinations to evaluate their safety and fitness for human consumption.

2.2. Preparation of samples (ICMSF, 1996).

To 25 grams of the sample, 225 ml of sterile peptone water were added and thoroughly mixed using sterile blender for 1 – 1.5 minutes, from which tenfold serial dilutions was prepared. The prepared samples were subjected to the following examinations:

2.3. Determination of total Staphylococci count (ICMSF, 1996).

2.4. Isolation, Identification of S.aureus : according to (Cruickshank et al., 1975) , (MacFaddin, 2000) and (Lachia et al., 1971).

2.5. Amplification of enterotoxin genes of S. aureus (Mehrotra et al., 2000).

2.6. Statistical analysis: according to (Feldman et al., 2003)

3. RESULTS

The results recorded in table (1) showed that 76.67% of the examined sardine were positive *staphylococci spp* and at frequency ranged from1.0 x 10^2 to 1.1x10^4 and 73.33% of examined molouha were positive *Staphylococci spp* and at frequency ranged from 1.0x10^2 to3.4×10^5 and 86.67% of the examined feseikh were positive Staphylococci spp and at frequency ranged from1.0×10^2 to 7.8×10^5 with a mean value 2.75×10^3 ± 0.41×10^3, 1.98×10^4 ± 0.28×10^4 and 5.03×10^4 ± 1.12×10^4, respectively. Moreover, there was high significant differences (P<0.01) between the examined samples of salted fish as shown in table (1). Accordingly, 63.33% ,70.00% and 80.00% of the examined salted fish of sardine, molouha and fesiekh were unaccepted according to Eos (2005).

Results in table (3) concerning to *S. aureus* it was detected in 36.67% ,46.67% and 50.00% of the examined salted fish sardine, molouha and fesiekh, respectively. Totally 44.44% of the examined samples of salted fish were contaminated with *S. aureus*. Moreover, examined salted fish were contaminated with other spp of *Staphylococci*. Such as *S.epidermidis* that detected in 26.67% , 30.00% and 20.00% in salted fish (Sardine , Molouha and Fesiekh), respectively. Table (4) showed that 20.00%, 40.00%, 60% of sardine, molouha and fesiekh were positive enterotoxins produced by *S. aureus*, respectively. They were unaccepted according to Egyptian standard (2005) which recommended that salted fish must be free from enterotoxins of *S. aureus*. Results obtained in photo (1) detected that Lane 5 Positive *S. aureus* strain for sec gene in sardine, Lane 10 Positive *S. aureus* strain for sea and seb genes and Lane 11 Positive *S. aureus* strain for sea and sed genes in molouha, Lane 14 Positive S. aureus strain for sea, seb and sec genes, Lane 16 Positive *S. aureus* strain for sea gene and Lane 17 Positive *S. aureus* strain for seb gene in feseikh.

Table (1): Statistical analytical results of total Staphylococci count/g in the examined samples of salted fish (n=30).

	+ve samples				
Meat Products	No.	%	Min	Max	Mean ± S.E*
Sardine	23	76.67	1.0×10^2	1.1×10^4	2.75×10^3 ± 0.41×10^{3++}
Molouha	22	73.33	1.0×10^2	3.4×10^5	1.98×10^4 ± 0.28×10^4
Fesiekh	26	86.67	1.0×10^2	7.8×10^5	5.03×10^4 ± 1.12×10^4

S.E* = Standard error of mean. ++ = High significant differences (P<0.01)

Table (2): Acceptability of the examined samples of salted fish based on their Staphylococci count/g (n=20).

Products	Staphylococci count /g*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Sardine	> 10^2	11	36.67	19	63.33
Molouha	> 10^2	9	30.00	21	70.00
Fesiekh	> 10^2	6	20.00	24	80.00

* Egyptian Organization for Standardization "EOS" (2005). No 1725-1/2005 (Part 1) for salted fesiekh. No 1725-2/2005 (Part 2) for salted sardine. No 1725-3/2005 (Part 3) for salted molouha

Table (3): Incidence of Staphylococcus species isolated from the examined samples of salted fish (n=30).

Salted Fish / Staphylococcus species	Sardine (30)		Molouha (30)		Fesiekh (30)		Total (90)	
	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus	11	36.67	14	46.67	15	50.00	40	44.44
Staphylococcus capitis	0	0	2	6.67	5	16.67	7	7.78
Staphylococcus hominis	1	3.33	1	3.33	0	0	2	2.22
Staphylococcus epidermidis	8	26,67	9	30.00	6	20.00	23	25.56
Staphylococcus intermedius	3	10.00	1	3.33	4	13.33	8	8.89
Staphylococcus saprophyticus	4	13.33	3	10.00	3	10.00	10	11.11

N.B The isolation % was calculated according to the number of samples

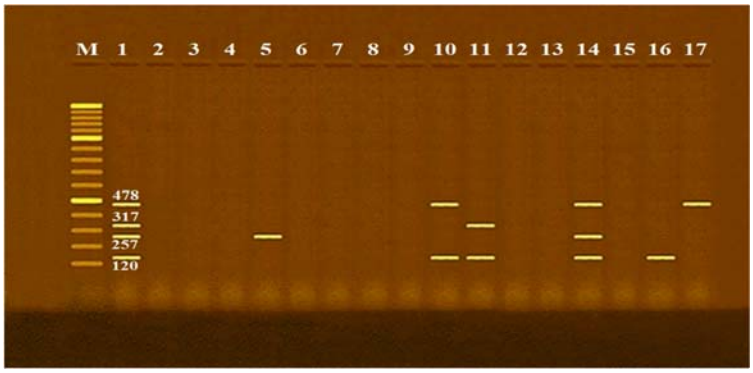

Photo (1): Agarose gel electrophoresis of multiplex PCR of sea. (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes for characterization of *S. aureus*. Lane M: 100 bp ladder as molecular size DNA marker. Lane 1: Control positive for sea, seb, sec and sed genes. Lane 2: Control negative. Lane 5: Positive *S. aureus* strain for sec gene. Lane 10: Positive *S. aureus* strain for sea and seb genes. Lane 11: Positive *S. aureus* strain for sea and sed genes. Lane 14: Positive *S. aureus* strain for sea, seb and sec genes. Lane 16: Positive *S. aureus* strain for sea gene. Lane 17: Positive *S. aureus* strain for seb gene. Lanes 3, 4, 6, 7, 8, 9, 12, 13 & 15: Negative *S. aureus* strains for enterotoxins.

Table (4): Incidence of enterotoxin genes as virulence factors of the isolated *Staphylococcus aureus* by using multiplex PCR.

Enterotoxin	Sardine (5)		Molouha (5)		Fesiekh (5)	
	No.	%	No.	%	No.	%
A	0	0	0	0	1	20
B	0	0	0	0	1	20
C	1	20	0	0	0	0
A & B	0	0	1	20	0	0
A & D	0	0	1	20	0	0
A, B & C	0	0	0	0	1	20
C	0	0	0	0	0	0
-ve strains	4	80	3	60	2	40
Total	5	100	5	100	5	100

4. Discussion

Nearly similar results obtained by (El-Shorbagy et al., 2000) who found that *Staph. aureus* count in feseikh samples was 15x 10^3/gm and in sardine samples was 4.25 x 10^3/gm, also nearly similar results were obtained by (Abdel-Rahman et al., 1988; Morshdy, 1980; Zeidan et al., 1983) and lower results were obtained by (El-kewaiey, 2001). *Staphylococcus aureus* is still a major cause of food poisoning due to ingestion of enerotoxins (Stengel, 1990); the ability to produce such enterotoxins in food is more likely when competing microorganisms were absent (Frazier and

Westhoff, 1984). Presence of *S.arueus* in a food indicates contamination from the skin, mouth and / or nose of food handlers. Inadequately cleaned equipment may be considered as a source of contamination (Thatcher and Clark, 1978). *Staphylococci* can grow best in salty and low water activity-containing foods in which the competing organisms are in reduced numbers (Vishwanath et al., 1998). (Basti et al., 2003) showed that *S.aureus* was the most important genus identified from heavy-salted fish and they assumed that the *S.aureus* isolated was due to the contamination of fish during capture and subsequent unhygienic handling and processing. Accordingly, 63.33% ,70.00% and 80.00% of the examined salted fish of sardine, molouha and fesiekh were unaccepted according to EOS(2005) recommended that shouldn't exceed the permissible limit 10^2 cfu/g. photo (3): showed Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes for characterization of *S. aureus*.

The detection of *staphylococcal* enterotoxin genes by PCR allows the determination of potentially enterotoxigenic *S. aureus* irrespective of whether the strain produces the toxin or not, the inability to detect the enterotoxin by immunological methods may occur due either to low level production of enterotoxin or to mutation in the coding region or in a regulatory region.

In conclusion, feseikh samples were the most contaminated product by *S.aureus* than the other fish samples and may cause a serious hazards in consumption that need more attention for control and prevention.

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