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Bacterial hazards of ready to eat fish products

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ABSTRACT

The aim of this study was to evaluate the bacterial quality of the examined ready to eat (RTE) fish product samples through isolation and identification of Enteropathogenic E. coli, Coagulase +ve S. aureus and Vibrio parahaemolyticus. A total of one hundred random samples of RTE fish products represented by fried Nile tilapia, grilled tilapia, smoked herring and salted sardine (25 of each) were collected from different fish markets and restaurants in Menofia governorate, Egypt. The incidence of Enteropathogenic *Escherichia coli* (*E.coli*) were 24%, 32%, 36% and 50 % in the examined samples of fried tilapia, grilled tilapia, smoked herring and salted sardine, respectively. While, Coagulase +ve *Staphylococcus aureus* (*S. aureus)* was detected in 32%, 44%, 52% and 72 % of the examined samples of fried tilapia, grilled tilapia, smoked Herring and salted Sardine, respectively. Furthermore, *Vibrio parahaemolyticus* (*V. parahaemolyticus*) was isolated only from 20% and 36 % of the examined samples of smoked herring and salted sardine, respectively. In conclusion, bacterial examination of RTE fish products is a sensitive indicator verifying the quality and good hygienic status of RTE fish products

1. INTRODUCTION

Ready to eat (RTE) fish products is a perfect food stuff which is up to standards of national nourishment, in addition it is a source of healthy and good digestible materials i.e. high-quality protein, minerals, vitamins, high amino acids content and high proportion of unsaturated fatty acids (Toth et al., 2012). Nevertheless, there is major evidence that RTE fish products are on the list of foods associated with outbreaks of food borne diseases, where raw or undercooked fish acts as a source to Enteropathogenic *E.coli*, *S. aureus* and *V. parahaemolyticus* (Ahmad et al., 2017). Contamination sources of RTE fish products could come from several ways such as contact with food handlers and asymptomatic carriers suffering from infected skin lesions, poor hand and fingernail hygiene as, the workers during preparation process may touch cooked RTE fish products that are usually eaten without further cooking or re-heating. Also, contacting with inadequate unclean utensils or serving plates, contaminated preparation surfaces, poor sanitation in kitchens, insects, rodents and other animals all can cause this illness along with temperature abuse of food. So, it is necessary to use HACCP system in restaurants for prevention of food borne diseases (Nichols et al., 2002). *E. coli* has been considered as an indicator to sewage pollution and reported as opportunistic pathogen in fish (Fredrick et al., 2015). Some types of *E. coli* can produce Shiga toxins (*stx*), these are called Shiga toxigenic *E. coli* (STEC). Enterohemorrhagic *E. coli* O_{157}:H_7 induces illness secondary to its production of *stx* that causes a range of gastrointestinal and enterohemorrhagic disease start from watery diarrhea to hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Erickson et al., 2019). *S. aureus* is a true food poisoning organism as it produces heat stable Staphylococcal enterotoxins (SEs) when allowed to grow in foods. Even if the food is heated before eating, the poison in the food will cause illness although the heat has killed the bacterial cells (Soriano et al., 2012). Staphylococcal food poisoning is associated with nausea, vomiting, abdominal cramps and diarrhea, which are the most common symptoms appear 3-8 hrs after ingestion (Pinchuk et al., 2010). Vibrio is among the most common surface organisms in surface waters of the world and they considered as part of the indigenous microflora of the marine environment (Hasan et al., 2010). Many halophilic *V.* species such as *V. parahaemolyticus* have been implicated in human enteric infection. Clinical symptoms of *V. parahaemolyticus* poisoning are acute dysentery and abdominal pain, accompanied by diarrhea, nausea, vomiting, fever, chills and water like stools (Guillod et al., 2019). It is better to consume RTE fish products consumed immediately, otherwise they should be hold hot foods at or above 60 °C or cool them quickly in the refrigerator to 4.4 °C for rapid cooling because, bacteria can grow rapidly in the danger zone temperatures between 5 °C and 60 °C. So, keep hot foods "hot" and cold foods "cold" (Shale et al., 2005).

2. MATERIAL AND METHODS

2.1. Collection of samples

A total of one hundred random samples of RTE fish products represented by fried tilapia, grilled tilapia, smoked herring and

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salted sardine (25 of each) were collected from different fish markets and restaurants in Menofia governorate, Egypt. Collected samples were separately kept in sterile plastic bags and preserved in an ice box, transferred to the laboratory under aseptic conditions without any delay and suspected to bacteriological examination for detection of food poisoning bacteria in examined samples and their suitability for human consumption.

2.2. Preparation of samples According to *ICMSF (1996)*
25 grams of the sample, 225 ml of sterile peptone water were added and thoroughly mixed using sterile blender for 1.5 minutes to provide a homogenate 1:10 form which tenth fold decimal dilutions were prepared.

2.3. Screening of pathogenic E. coli
From the original dilution, one ml was inoculated into MacConkey broth tubes supplemented with inverted Durham's tubes. Inoculated tubes were incubated at 37 °C for 24 hours. Suspected colonies were metallic green in color.
2.3.1. Morphological examination, motility test and biochemical identification
The Culture characteristics, motility and all presumptive biochemical identification were done as described by MacFaddin (2000).
2.3.2. Serodiagnosis of E. coli According to Kok et al. (1996)
A rapid diagnostic *E. coli* antisera sets (Denka Seiken Co., Japan) were used for diagnosis of the Enteropathogenic types.
2.4. Determination of S. aureus
From previously prepared dilutions 0.1 ml was spread over duplicated pates of Baird Parker agar plate. The inoculated and control plates were incubated at 37 °C for 48 hrs. Suspected colonies of *S. aureus* appear as black, shiny, circular, smooth and convex with narrow white margin. All the procedures followed FDA, (2001)
2.4.1. Morphological examination, Motility test and Biochemical identification
2.4.2. Detection and typing of enterotoxin
Reverse Passive Latex Agglutination technique "RPLA" kits were used for the detection of staphylococcal enterotoxins A, B, C and D (SET-RPLA, Denka Sekeu LTD, Japan) following MacFaddin (2000).

2.5. Detection of V. parahaemolyticus
As described by ISO (2017) 25 g of each sample were homogenized with 225 ml of sterile alkaline peptone water (3% NaCl and pH 8) and incubated over night at 37 °C. Then streaked onto Thiosulfate citrate bile and sucrose agar and incubated at 37 °C for 24 hrs. Typical colony of *V. parahaemolyticus* appeared as smooth and green (sucrose negative)

3. RESULTS

3.1. Enteropathogenic *E. coli*
The results in table (1 & 2) revealed that, 37 isolates of *E. coli* were isolated from examined samples represented as: 6(24%) from fried tilapia with serotypes O44:H18 (4%), O111:H2 (4%), O119:H6 (4%), O121:H7 (8%) & O128:H2 (4%); 8(32%) from grilled tilapia with serotypes O26:H11 (4%), O111:H2 (8%), O114:H4 (4%), O121:H7 (4%), O124 (4%) & O126:H21 (8%); 9 (36%) from smoked herring with serotypes O44:H18 (4%), O91:H21 (12%), O111:H2 (4%), O114:H4 (4%), O124 (4%), O128:H2 (8%) & O171 (4%) and 14(50%) from salted sardine with serotypes O26:H11 (4%), O55:H7 (4%), O91:H21 (4%), O111:H2 (8%), O121:H7 (4%), O124 (8%), O126:H21 (4%) & O128: H2 (12%).

Table 1 Incidence of Enteropathogenic *E. coli* in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	*E. coli* incidence			Acceptability *			
	No. of +ve samples	% of +ve samples	*E. coli* count /25 g*	Accepted samples		Unaccepted samples	
				No.	%	No.	%
Fried *O. niloticus*	6	24	Free	19	76	6	24
Grilled *O. niloticus*	8	32	Free	17	68	8	32
Smoked Herring	9	36	Free	16	64	9	36
Salted Sardine	14	56	Free	11	44	14	56
Total	37	37		63	63	37	37

*Egyptian Organization for Standardization "EOS" (2005). No 1725-2/2005 for salted fish, No 288/2005 for smoked fish and No 3495/2005 for fish products breaded or in batter.

Table 2 Serotyping of *E. coli* serotypes isolated from the examined samples of ready to eat fish products (n=25).

E. coli strains	Fried *O. niloticus*		Grilled *O. niloticus*		Smoked Herring		Salted Sardine		Strain Characteristics
	No.	%	No.	%	No.	%	No.	%	
O_{26} : H_{11}	-	-	1	4	-	-	3	12	EHEC
O_{44} : H_{18}	1	4	-	-	1	4	-	-	EPEC
O_{55} : H_{7}	-	-	-	-	-	-	1	4	EHEC
O_{91} : H_{21}	-	-	-	-	3	12	1	4	EHEC
O_{111} : H_{2}	1	4	2	8	1	4	2	8	EHEC
O_{114} : H_{4}	-	-	1	4	1	4	-	-	EPEC
O_{119} : H_{6}	1	4	-	-	-	-	-	-	EPEC
O_{121} : H_{7}	2	8	1	4	-	-	1	4	EHEC
O_{124}	-	-	1	4	1	4	2	8	EIEC
O_{126} : H_{21}	-	-	2	8	-	-	1	4	ETEC
O_{128} : H_{2}	1	4	-	-	2	8	3	12	ETEC
O_{171}	-	-	-	-	1	4	-	-	EPEC
Total (100)	6	24	8	32	10	36	14	48	

EPEC = Enteropathogenic *E. coli.* EIEC = Enteroinvasive *E. coli.* ETEC = Enterotoxigenic *E. coli.* EHEC = Enterohaemorrhagic *E. coli*

The acceptability of the examined samples was shown in table (1) as 63 accepted samples represented as 19 (76%), 17 (68%), 16 (64%) and 11 (44%) from fried tilapia, grilled tilapia, smoked herring and salted sardine, respectively. While 37 unaccepted samples represented as 6 (24%), 8 (32%), 9 (36%) and 14 (56%) from fried tilapia, grilled tilapia, smoked herring and salted sardine, respectively.

3.2. Coagulase positive *S. aureus*

The results in tables (3 & 4) revealed that 48 isolates of coagulase positive *S. aureus* were isolated from examined samples represented as 8 (32%) from fried tilapia with enterotoxin C (4%); 11 (44%) from grilled tilapia with enterotoxin A (4%); 13 (52%) from smoked herring with enterotoxins A& B (4%) and A & C (4%) and 16 (72 %) from salted sardine with enterotoxins A (8%), D (4%) and B & D (4%).

The acceptability of the examined samples was shown in table (3) as 42 accepted samples represented as 14(56%), 12 (48%), 9 (36%) and 7 (28%) from fried tilapia, grilled tilapia, smoked herring and salted sardine, respectively; while 58 unaccepted samples represented as 11 (44%), 13 (52%), 16 (64%) and 18 (72%) from fried tilapia,, grilled tilapia,, smoked herring and salted sardine, respectively.

3.3. V. parahaemolyticus

The results in table (5) revealed that 14 isolates of *V. parahaemolyticus* species were isolated from examined samples represented as 5 (20%) from smoked Herring, 9 (36%) from salted Sardine, while fried tilapia, and grilled tilapia, were free from vibrio (0%). The acceptability of the examined samples represented as 90 accepted samples represented as 25 (100%), 25 (100%), 22 (88%) and 18 (72%) from fried tilapia,, grilled tilapia,, smoked herring and salted Sardine, respectively; while 10 unaccepted samples represented as 3 (12%) from smoked Herring and 7 (28%) from salted Sardine. This according to *Egyptian Organization for Standardization "EOS" (2005)* (No 1725-2/2005 for salted fish, No 288/2005 for smoked fish and No 3495/2005 for fish products breaded or in batter).

Table 3 Incidence of coagulase +ve *S. aureus* in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	S. aureus incidence			Acceptability*			
	No. of +ve samples	% of +ve samples	S. aureus count /25 g	Accepted samples		Unaccepted samples	
				No.	%	No.	%
Fried *O. niloticus*	8	32	Free	14	56	11	44
Grilled *O. niloticus*	11	44	Free	12	48	13	52
Smoked Herring	13	52	Free	9	36	16	64
Salted Sardine	16	72	Free	7	28	18	72
Total	48	48		42	42	58	58

* Egyptian Organization for Standardization "EOS" (2005). No 1725-2/2005 for salted fish, No 288/2005 for smoked fish and No 3495/2005 for fish products breaded or in batter.

Table 4 Incidence of enterotoxins producing *S. aureus* isolated from the examined samples of ready to eat fish products (n=25).

Enterotoxin	Fried *O. niloticus*		Grilled *O. niloticus*		Smoked Herring		Salted Sardine		Total (100)	
	No.	%	No.	%	No.	%	No.	%	No.	%
A	0	0	1	4	0	0	2	8	3	3
C	1	4	0	0	0	0	0	0	1	1
D	0	0	0	0	0	0	1	4	1	1
A & B	0	0	0	0	1	4	0	0	1	1
A & C	0	0	0	0	1	4	0	0	1	1
B & D	0	0	0	0	0	0	1	4	1	1
Total	1	4	1	4	2	8	4	16	8	8

Table 5 Incidence of *V. parahaemolyticus* in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	*V. parahaemolyticus* incidence		*V. parahaemolyticus* count /25 g*	Acceptability*			
	No. of +ve samples	% of +ve samples		Accepted samples		Unaccepted samples	
				No.	%	No.	%
Fried *O. niloticus*	8	32	Free	25	100	0	0
Grilled *O. niloticus*	11	44	Free	25	100	0	0
Smoked Herring	13	52	Free	22	88	3	12
Salted Sardine	16	72	Free	18	72	7	28
Total	48	48		90	90	10	10

*Egyptian Organization for Standardization "EOS" (2005). No 1725-2/2005 for salted fish, No 288/2005 for smoked fish and No 3495/2005 for fish products breaded or in batter.

4. DISUCSSION

Contamination of fish with organisms of public health significant remains as a major global concern that affects the consumer. Therefore, a serious attention has to be given to RTE fish products that act as a vector for human pathogenic bacteria (Jacxsens et al., 2009). Therefore, the present study was carried out on fried tilapia, grilled tilapia, smoked herring and salted sardine (25 of each), collected from Menofia governorate, Egypt to evaluate the bacterial hazards for them.

The incidence of Enteropathogenic *E. coli* in the examined samples and their acceptability was showed that *E. coli* was isolated from 37 samples represented as: 6(24%) fried *O. niloticus*, 8 (32%) grilled *O. niloticus*, 9 (36%) smoked herring and 14 (50%) salted sardine. The acceptability of the examined samples were 63 accepted samples represented as 19 (76%), 17 (68%), 16 (64%) and 11 (44%) from fried *O. niloticus*, grilled *O. niloticus*, smoked herring and salted sardine, respectively; while 37 unaccepted represented as 6 (24%), 8(32%), 9 (36%) and 14 (56%) from fried *O. niloticus*, grilled *O. niloticus*, smoked herring and salted

sardine, respectively. This according to Egyptian Organization for Standardization "EOS" (2005).

Serotyping of *E. coli* serotypes isolated from the examined samples of ready to eat fish products and results showed that fried *O. niloticus* with serotypes O44:H18 (4%), O111:H2 (4%), O119:H6 (4%), O121:H7 (8%) & O128:H2 (4%); grilled *O. niloticus* with serotypes O26:H11 (4%), O111:H2 (8%), O114:H4 (4%), O121:H7 (4%), O124 (4%) & O126:H21 (8%); smoked herring with serotypes O44:H18 (4%), O91:H21 (12%), O111:H2 (4%), O114:H4 (4%), O124 (4%), O128:H2 (8%) & O171 (4%) and salted sardine with serotypes O26:H11 (4%), O55:H7 (4%), O91:H21 (4%), O111:H2 (8%), O121:H7 (4%), O124 (8%), O126:H21 (4%) & O128: H2 (12%). The obtained results are being higher than those obtained by Ramadan (2009) who isolated *E. coli* from 20% of RTE samples of tilapia. The current results are being lower than those recorded by Hassan (2013) isolated *E. coli* from 57.10% of RTE samples of tilapia. These results came in accordance with those obtained by Atanassova et al. (2014) isolated *E. coli* from 56% of salted fish samples. According, the increased incidence of *E. coli* in the examined samples may be due to mishandling during production and distribution, leading to gastroenteritis characterized by vomiting, abdominal pain, paralysis and low fever (Olaleye and Abegunde, 2015).

S. aureus is considered a major problem worldwide as it can be found in respiratory passages, skin and superficial wounds of man. Thus, Contamination with *S. aureus* is important risk index in evaluation of safety and hygienic quality of food (Jyhshiun et al., 2009). The results of the incidence of coagulase +ve *S. aureus* in the examined samples and their acceptability revealed that coagulase +ve *S. aureus* was isolated from 48 samples represented as 8(32%) fried tilapia, 11(44%) grilled tilapia, 13(52%) smoked herring and 16 (72 %) salted sardine. Acceptability of the examined samples was 42 accepted samples represented as 14 (56%), 12 (48%), 9 (36%) and 7 (28%) from fried tilapia,, grilled tilapia,, smoked herring and salted sardine, respectively; while 58 unaccepted samples represented as 11 (44%), 13 (52%), 16 (64%) and 18 (72%) from fried tilapia,, grilled tilapia, smoked herring and salted sardine, respectively. This according to Egyptian Organization for Standardization "EOS" (2005).

The results of the incidence of enterotoxins producing *S. aureus* isolated from the examined samples of ready to eat fish products and results showed that fried tilapia, with enterotoxin C (4%); grilled tilapia, with enterotoxin A (4%), moked herring with enterotoxins A&B (4%) and A&C (4%) and salted sardine with enterotoxins A (8%), D (4%) and B&D (4%). The obtained results are being higher than those obtained by Vazquez-Sanchez (2012) isolated *S. aureus* from 27% of salted fish and 26% of smoked fish samples. The current results are being lower than those recorded by Grigoryan et al. (2010) detected *S. aureus* in 74% of the analyzed samples of smoked fish. Nearly similar results were recorded by Subramanian (2007).

The presence of specific human pathogenic vibrio species can serve as an indicator of public health safety of food destined for human consumption. Vibriosis is characterized by diarrhea, primary septicemia and wound infections (Shimohata and Takahashi, 2010). The results of the incidence of *V. parahaemolyticus* in the examined samples and their acceptability revealed that *V.* species were isolated from 14 samples represented as 5 (20%) smoked Herring and 9 (36%) salted Sardine, and failed to be detected in grilled and fried tilapia. Acceptability of the examined samples represented as 90 accepted samples represented as 25 (100%), 25 (100%), 22 (88%) and 18 (72%) from fried tilapia, grilled tilapia, smoked Herring and salted Sardine, respectively. While 10 unaccepted represented as 3 (12%) smoked Herring and 7 (28%) salted Sardine, this according to Egyptian Organization for Standardization "EOS" (2005). The obtained results are being higher than those obtained by Vigano et al. (2007) couldn't isolate *V. parahaemolyticus* from the examined samples of fried sea foods. The current results are being lower than those recorded by Abd Allah (2010) isolated *vibrio* organisms from 20% of the examined samples of sardine. These results were agreed with those reported by Al-Sunaiher et al. (2010) detected *V. parahaemolyticus* in 16.8% of the total examined samples of RTE fish. The factors associated with post heat treatment contamination of RTE fish products may be attributed to direct contact with poor personal hygiene food handlers, no pre-employment and routine medical examination for *S. aureus*, contaminated utensils or serving plates, contamination of preparation surfaces, poor sanitation in kitchens and improper storage of cooked food. So, to avoid the post heat treatment contamination of RTE fish products we should protect cooked sea food from insects, rodents and other animals, application of HACCP system in all fish restaurants to ensure food safety and keep kitchens and food-serving areas clean and sanitized Center for Health Protection "CHP" (2010). Cross- contamination between raw sea food containing pathogens and cooked RTE fish products should be avoided as possible as we can by separating from raw food, avoid preparing RTE fish products dishes and raw foods at the same time, prevent cross-contamination between fish tank water and RTE fish products and cooked sea food should be stored in cleaned sealed containers to protect them from contamination Donald et al. (2001). Heat treatment process of fish considers one of the most suitable methods to contain the risk of food born disease illness by decreasing and preventing the growth and the presence of pathogenic microorganism such as *E.coli*, *S. aureus* and *V. parahaemolyticus* in RTE fish products. So, it is be recommended to using the following heat treatments processes to get the best results in reaching to RTE fish products free from pathogenic microorganism as possible as we can: frying 190 °C/10 min, roasting for 30 min. and in oven 120 °C/35min or 150 °C/45 min in microwave Lahmer et al. (2017).

5. CONCULOSIONS

The current study allow to conclude that the examined samples of fried tilapia, grilled tilapia, smoked herring and salted sardine proved to be contaminated with various microorganisms such as (Enteropathogenic *E. coli*, coagulase +ve *S. aureus* and *V. parahaemolyticus*).

Therefore, RTE fish products constitute, at times, a public health hazard. Consequently, illness can be prevented by proper cooking, avoiding post-cooking contamination and good hygienic practices.

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