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Enterobacteriaceae in Some Imported Fish

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Abstract

A total of 200 random samples of imported fishes represented by *Bangasius hypophanmus (Basa), mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel) and *Sparus aurata* (Denise) (50 of each) which were collected from different markets at Alexandria province, for detection of enteropathogenic *E.coli*, Salmonellae, Shigella, *Yersinia enterocolitica, Vibro parahaemolyticus*, and Aeromonas. The current study had been done from March 2014 to February 2015. *E. coli* incidence was (21) 42%, (18) 36%, (19) 38%, and (13) 26% in examined samples of *Basa*, Barboni, Mackerel and Denis, respectively. Incidence of Salmonella was (3) 6%, (5) 10%, (4) 8% and (2) 4% of the examined samples of *Basa*, Barboni, Mackerel and Denise, respectively. Incidence of Shigella which was (13) 26%, (11) 22%, (14) 28% and (9) 18% of the examined samples of *Basa*, Barboni, Mackerel and Denise, respectively. Incidence of Yarsinia which was (23) 46%, (21) 42%, (18) 36% and (16) 32% of the examined samples of Basa, Barboni, Mackerel and Denise, respectively. Incidence of *Vibrio spp*. was (15) 30%, (14) 28%, (13) 26% and (11) 22% of the examined samples of *Basa*, Barboni, Mackerel and Denise, respectively. Finally, incidence of *Aeromonas hydrophilia* was (8) 16%, (11) 22%, (8) 16% and (6) 12% of the examined samples of *Basa*, Barboni, Mackerel and Denise, respectively. Also, serological tests had been done for each microorganism to identify the incidence of specific strains. The public health significance of contaminants and the possible sources of contamination of imported fishes with these organisms as well as suggestive hygienic measures to improve the quality of such fishes were discussed.

Keywords: Imported fish; Enteropathogenic *E.coli*; Salmonellae; Shigella; *Yersinia enterocolitica; Vibro parahaemolyticus;* Aeromonas

Introduction

Unlike meat and poultry, fish are more liable to contamination with pathogenic bacteria from human reservoir which may contaminate the water depending on the fishing and also may be further contaminated during handling, processing and packaging. While the muscle flesh of fish, which is the main edible part is normally sterile but microorganisms can penetrate from the skin and the gut to the flesh, the penetration and contamination increase in case of fish caught from polluted area where there are high densities of bacteria. Singh & Kulshrestha [1] isolated 17 strain of *E.coli* from fresh and marine fish, shrimp and mollusk fish which positive for enterotoxogenicity. Edris [2] Samples collected from Cairo and Giza supermarkets included (Mackerel, Sardine, Sardinella, fish burger and breaded shrimp). Such quality control was evaluated through organoleptical, microbiological and chemical examinations of 30 random samples of each fish and fish products. The present investigation proved that the examined random samples of the imported fish and fish products were quite safe for human *E. coli* and Salmonella were not isolated from all the examined samples. Ahmed [3] 62 samples of chilled fish fillets, 50 samples of iced peeled shrimp and 15 samples each of frozen imported and local peeled shrimp samples were collected from Cairo and Giza markets. Examined samples for isolation and identification of specific pathogens *Vibrio spp., E. coli, Listeria spp., A. hydrophila, S. aurerus* and *Y. enterocolitica,* Salmonella and Shigella. About 82.3% of filets samples were accepted according to the ESS (3494/2005), while 72% of peeled shrimp samples were accepted according to ESS (516/1993), whereas, 100 and 93% of frozen imported and local shrimp samples were accepted. *E. coli* were isolated from fillets and peeled shrimp samples. Khadega [4] isolated Salmonella from10% and 16% from Mullus and *Basa* while she failed to isolate Salmonella from Barboni. Elhadi [5] examined 35 samples and found 11 samples contaminated with Salmonella (31.4%). Morris et al. [6] examined samples of fish immediately after catching where they failed to isolate Salmonella. But after they arrived to the plants, they could isolate this organism. Based on Morris's research imported fish contaminated during transportation, packaging and handling process. Onyango [7] among 120 imported *Basa*, 63 (52.5%) were infected with Enterobacteriaceae. Out of these, 25 (39.7%) were *Shigella spp*, 9 (14.3%) *Salmonella typhimurium,* 7 (11.1%) *S. typhi,* 4 (6.3%) *S. enteritidis,* 16 (25.4%) *Escherichia coli,* 1 (1.6%) *Proteus spp*. and Enterobacter aerogenes, respectively. Ten fish collected from

open-air markets yielded *E. coli* (50%), *S. typhimurium* (20%), *S. paratyphi* (10%) and *S. typhi* (20%). El-Leboudi [8] examined 15 imported fish samples, the author isolated 2 of *Yersinia enterocolitica* strains (13.3%) from imported fish. Harydi [9] a total of 675 imported frozen fish samples from different origins were collected on arrival to Sokhna port, Suez city, Egypt. The prevalence of *Salmonella spp.* were found to be (1.6%) in whole shrimp, (3.7%) in peeled shrimp, (2.0) in whole fish fillets, and (1.67%) in sepia, respectively. Meanwhile in calamari; Salmonella prevalence was found to be 4.0%. Alzainy [10] examined 60 samples of frozen imported fish and live fish, 65% of samples were found to be positive for *Aeromonas hydrophila* isolation 76.6% were in life fish samples and 53.3% in frozen fish 94.87% exhibited α and β hemolysis, 100% of life fish isolates show β hemolysis while frozen fish isolates show 85.7% β hemolysis and 14.3% α hemolysis, 97.43% of isolates show cytotoxic effect on Vero cells the highest frequency occur in the isolates of life fish group 60.50%. Farag [11] Sixty two samples of chilled fish fillets, 50 samples of iced peeled shrimp and 15 samples each of frozen imported and local peeled shrimp samples were collected from Cairo and Giza markets bacteria counts for *Aeromonas spp* and *vibrio spp*. Were positive. Sharma [12] the research has shown that imported fish is most frequently and most extensively contaminated with bacteria from the Aeromonas genus (positive samples ranging from 37% to 93%). El Noby [13] Sixty samples (20 each of *Tilapia sp., Mugil cephalus* and frozen mackerel fish samples) were randomly collected from different local shops of fish sailing and fish retailers of different sanitation levels at Zagazig city. Collected samples were examined bacteriologically for determination of the incidence as well as the count of psychrotrohic microorganisms using the rapid method (25 pOCI 24 hours) following by pour plate technique. The obtained results revealed that. The mean count of Aeromonas was 5.7x10)±3.0x102, 1.0x105±4.9x104 and 3.3x105±2.7x104cfu/g of examined *Tilapia nilotica, Mugil cephalus* and frozen Mackerel samples, respectively.

Materials and Methods

Materials

Collection of samples: A total of 200 random samples of imported fishes represented by *Bangasius hypophanmus (Basa), mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel) and *Sparus aurata* (Denise) (50 of each) were collected from different fish markets in Alexandria city. Each sample (250g) was kept in a separate plastic bag and transferred directly with a minimum of delay to the laboratory of food hygiene department, Faculty of Veterinary Medicine, Alexandria University in an insulating refrigerated container under complete aseptic condition to avoid any changes in the quality of the sample. Samples were examined bacteriologically immediately after arrival to the laboratory for isolation and identification of Salmonella, *Escherichia coli*, Shigella, *Vibrio parahaemolyticus, Yersinia enterocolitica* and Aeromonas.

Culture media: EMB medium (HIMEDIA), XLD agar (Acumedia), MacConkey, Agar (BBL), Nutrient Agar (Difco), Semisolid agar (Difco), Thiosulfate-citrate-bile salt-sucrose agar (TCBS Agar) (BBL), M-Aeromonas Selective Agar Base (Havelaar) (Himedia) with ampicillin vial, Cefsulodin-irgasan-novobiocin (CIN) agar (Oxoid), Trypticase Soy Broth, Bile-Oxalate-Sorbose broth and MacConkey's broth.

Methods

25 grams of each fish fillet sample were aseptically transferred into sterile blender flask containing 225ml of sterile peptone water 0.1% and homogenized at 1400rpm for 2-5 minutes to provide a homogenate 1/10 dilution and then allowed to stand for about 6 minutes at room temperature. The contents of the flask were thoroughly mixed by shaking and one ml of the homogenate was transferred with sterile pipette to another tube containing 9 ml of sterile peptone water, from which tenth fold serial dilutions were prepared up to 10-6 (APHA, 1985) [14].

1.Isolation and identification of Salmonellae: According to (Rappaport & Harvy and Price) [15,16], (Collins & Cruickshank) [17,18], (Simmon) [19], (Kovacs) [20], (Ljutov) [21], (MacFaddin) [22], (Hugh & Leifson) [23], (Edwards & Ewing) [24], (Kauffman) [25] for serological examination, and (ICMSF, 1996) [26] for pre-enrichment.

2. Isolation and identification of Escherichia coli: According to (Rappaport & Harvy) [15,16], (Collins & Cruickshank) [17,18], (Simmon) [19], (Kovacs) [20], (Ljutov) [21], (MacFaddin) [22], (Hugh & Leifson) [23], and (Edwards & Ewing) [24].

3. Isolation and identification of Shigella: According to (Rappaport & Harvy) [14,15], (Collins & Cruickshank) [17,18], (Simmon) [19], (Kovacs) [20], (Ljutov) [21], (MacFaddin) (Hugh & Leifson) [23], and (Edwards & Ewing) [24].

4. Isolation and identification of Yersinia: According to (Schiemann, 1983)[27] for Pre-enrichment, Krieg & Holt [28] and (MacConkey) [29] for selective enrichment and plating.

5.Isolation and identification of Vibrio parahaemolyticus: According to (APHA) [30].

6. Isolation and identification of Aeromonas: According to (Havelaar) [31].

7. The obtained results were statistically evaluated according to the guidelines recommended by Feldman et al. [32].

Discussion

Literature extended over many years pointed out that fish and its products are liable to contamination with various kinds of micro-organisms from different sources. Such contamination may render the fish unsafe to the consumers or impair its utility, especially in undeveloped countries, where the hygienic measures are still underway. Many efforts were done to keep the fish free from pathogens of public health hazard.

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Isolation of enteropathogenic *E. coli*

Table 1: Incidence of Enteropathogens in the examined samples of imported fishes (N=50).

	Basa		Barboni		Mackerel		Denise	
	No.	%	No.	%	No.	%	No.	%
Salmonella	3	6	5	10	4	8	2	4
E.coli	21	42	18	36	19	38	13	26
Shigella spp.	13	26	11	22	14	28	9	18
Yersiniaspp.	23	46	21	42	18	36	16	32
Vibrio spp.	15	30	14	28	13	26	11	22
Aeromonas hydrphilie	8	16	11	22	8	16	6	12

It was evident from the results recorded in (Table 1), enteropathogenic *E. coli* was isolated from (21) 42%, (18) 36%, (19) 38% and (13) 26% of the examined samples of *S Bangasius hypophanmus (Basa), mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel) and *Sparus aurata* (Denise), respectively. This result was not compatible with the Egyptian standards (E.S889/2009) [33] of frozen fish, part 1 whole fish that stated that fish must be free from *E. coli.* The current result of isolation of *E. coli* from the examined samples of imported fish was higher than those obtained by Singh & Kulshrestha [1] who could isolate 17 strains from all examined samples. Also, our results for Mackerel is contradict the result obtained by Edris [2] who stated that *E.coli* was quite safe for human consumption according to his results. As well as Ahmed [3] who examined 15 samples of imported fish and stated that it is accepted for (E.S889/2009) [32]. Serotyping of enteropathogenic *E.coli* isolated from the examined samples of imported fish was declared in Table 2.

Table 2: Serotyping of *E.coli* isolated from the examined samples of imported fishes (n=50).

	Basa		Barboni		Mackerel		Denise		Strain character
	No.	%	No.	%	No.	%	No.	%	
O86:K61(B7)	4	8	6	12	3	6	3	6	EPEC
O111:K58(B9)	3	6	2	4	6	12	-	-	EHEC
O124:K72(B17)	4	8	1	2	3	6	4	8	EIEC
O26:K60(B6)	5	10	4	8	3	6	2	4	EHEC
O128:K67(B12)	5	10	5	10	4	8	4	8	ETEC
Total	21	42	18	36	19	38	13	26	

Table 2 Serotyping of *E.coli* isolated from the examined samples of imported fishes stated 5 strains of *E. coli* isolated from *Basa*, Barboni and Mackerel, as O86:K61 (B7), O111:K58 (B9), O124:K72 (B17), O26:K60(B6), and O128:K67(B12). Furthermore, 4 strains were serologically isolated from Denise as O86:K61 (B7), O124:K72 (B17), O26:K60 (B6), and O128:K67 (B12). The result in (Table 2) shows the percentage of the incidence for each strain as follow, for O86:K61(B7) it was (4) 8%, (6) 12%, (3) 6%, and (3) 6% of the examined samples of *S Bangasius hypophanmus (Basa), Mullus surmuletus* (Barboni), Saurida undosquamis (Mackerel) and *Sparus aurata* (Denise), respectively. Secondly, incidence of O111:K58(B9), was (3) 6, (2) 4, (6) 12, and zero in the examined samples of *S Bangasius hypophanmus (Basa), Mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel) and *Sparus aurata* (Denise), respectively. Thirdly, incidence of O124:K72 (B17) was (4) 8, (1) 2, (3) 6, and (4) 8 in the examined samples of *S Bangasius hypophanmus (Basa), Mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel) and *Sparus aurata* (Denise), respectively. As well as, the incidence of O26:K60 (B6), was (5) 10, (4) 8, (3) 6, and (2) 4 in the examined samples of *S Bangasius hypophanmus (Basa), Mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel) and *Sparus aurata* (Denise), respectively. Finally, incidence of O128:K67 (B12) was (5) 10, (5) 10, (4) 8, and (4) 8 in the examined samples of *S Bangasius hypophanmus (Basa), Mullus surmuletus* (Barboni), *Saurida undosquamis* (Mackerel) and *Sparus aurata* (Denise), respectively. Nearly similar results were reported by Donenberg & Kaper [33,34].

Salmonella

Table 3: Serotyping of Salmonella organisms isolated from the examined samples of imported fishes (No.50).

	Basa		Barboni		Mackerel		Denise	
	No.	%	No.	%	No.	%	No.	%
S.enteritidis	1	2	1	2	2	4	1	2
S.typhimurium	-	-	1	2	1	2	-	-
S.paratyphi	-	-	1	2	1	2	-	-
S.haifa	2	4	2	4	-	-	1	2
Total	3	6	5	10	4	8	2	4

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The recorded results in Table 3 stated incidence of Salmonella which was (3) 6%, (5) 10%, (4) 8% and (2) 4% of the examined samples of Basa, Barboni, Mackerel and Denise, respectively. This result was not compatible with the Egyptian standards (E.S889/2009) [33] of frozen fish that stated that fish must be free from Salmonella in 25g. These results were in harmony with that of Khadega [4] who could isolate Salmonella from10% and 16% from Mullus and Basa while she failed to isolate Salmonella from Barboni. Also, Salmonella organisms were previously isolated from imported fish by Stevens et al. [35], Baquar et al. [36] and Dalsgaarg [37]. Elhadi [5] his result higher than our result in Mackerel, he examined 35 samples and found 11 samples contaminated with Salmonella (31.4%), whereas, we found only 4 out of 50 Mackerel samples (8%). We found Salmonella in 4 samples 8% of Mackerel, while Edris [2] couldn't find Salmonella in (30) examined Mackerel samples. As well as Ahmed [3] who stated that 15 samples of imported fish were free from Salmonella. It is important to mention that Morris et al. [6] examined samples of fish immediately after catching where they failed to isolate Salmonella. But after they arrived to the plants, they could isolate this organism. Based on Morris's research imported fish contaminated during transportation, packaging and handling process.

Accordingly, the presence of Salmonella as enteropathogens in imported fish may reflect the unsatisfactory hygienic conditions during handling, packaging and marketing of the fish. Serological identification of the obtained Salmonella isolates was tabulated in Table 2. It reflected that *Sal. enteritidis* (1) 2% and *Sal. haifa* (2) 4% were serologically identified from the examined samples of *Basa.* On the other hand, Barboni was contaminated by 4 different strains of Salmonella as follow, *Sal. enteritidis* (1) 2%, *S. typhimurium* (1) 2%, *S. paratyphi* (1) 2% and *Sal. haifa* (2) 4%. In addition, Denise was contaminated by 3 different strains of Salmonella as follow, *Sal. enteritidis* (2) 4%, *S. typhimurium* (1) 2%, and *S.paratyphi* (1)2%. Lastly, Serotyping of the isolated Salmonella indicated that *Sal. enteritidis* (1) 2% and *Sal. haifa* (1) 2% were serologically identified from the examined samples of Mackerel.

Shigella

The illustrated data in Table 4 showed that the incidence of Shigella species in the examined imported fish samples was (13) 26%, (11) 22%, (14) 28% and (9) 18% of the examined samples of Basa, Barboni, Mackerel and Denise, respectively. In addition, the serotyping identification of the recovered isolates Table 3 revealed the examined samples of Basa were contaminated by Shigella flexeneri (4) 8%, Shigella desenteriae (5) 10%, Shigella boydii (2) 4% and Shigella sennei (2) 4%. Also, Barboni samples were contaminated by Shigella flexeneri (2) 4%, Shigella desenteriae (5) 10%, Shigella boydii (1) 2% and Shigella sennei (3) 6%. Furthermore, Barboni samples were contaminated by Shigella flexeneri (2) 4%, Shigella desenteriae (5) 10%, Shigella boydii (1) 2% and Shigella sennei (3) 6%. Furthermore, Mackerel samples were contaminated by Shigella flexeneri (6) 12%, Shigella desenteriae (4) 8%, Shigella boydii (3) 6% and Shigella sennei (1) 2%. Finally, Denise samples were contaminated by Shigella flexeneri (2) 4%, Shigella desenteriae (2) 4%, Shigella boydii (2) 4% and Shigella sennei (2) 6%. According to (E.S889/2009) [38] which assumed that frozen fish must be free from Shigella in 25g. Consequence, these examined fish is unaccepted, on the other hand Ahmed [3] who stated that 15 samples of imported fish were free from Shigella and accepted. Imported Basa fish was contaminated by Shigella 26%, Onyango [7] among 120 imported Basa out of these, 25 (39.7%) were contaminated by Shigella spp.

Table 4: Incidence of Shigella strains isolated from the examined samples of imported fishes (n=50).

Shigella strains	*Basa*		Barboni		Mackerel		Denise	
	No.	%	No.	%	N0.	%	No.	%
Shigella flexeneri	4	8	2	4	6	12	2	4
Shigella desenteriae	5	10	5	10	4	8	2	4
Shigella boydii	2	4	1	2	3	6	2	4
Shigella sennei	2	4	3	6	1	2	3	6
Total	13	26	11	22	14	28	9	18

Yersinia

Table 5: Incidence of Yersinia strains Isolated from the examined samples of imported fish (No=50).

	Basa		Barboni		Mackerel		Denise	
	No.	%	No.	%	No.	%	No.	%
Yersinia enterocolitice	14	28	9	18	6	12	9	18
Yersinia frederiksenii	7	14	8	16	5	10	6	12
Yersinia ruckeri	2	4	1	2	4	8	1	2
Yersinia intermedia	-	-	3	6	3	6	-	_
Total	23	46	21	42	18	36	16	32

The illustrated data in Table 3 described Incidence of Yarsinia which was (23) 46%, (21) 42%, (18) 36% and (16) 32% of the examined samples of *Basa,* Barboni, Mackerel and Denise, respectively. In addition, the serotyping identification of the recovered isolates (Table 5) revealed the incidence of different strains of Yarsinia as follow, the examined samples of *Basa* contaminated by were *Yarsinia enterocolitice* (14) 28%, *Yarsinia frederiksenii* (7) 14%, *Yarsinia ruckeri* (2) 4% and *Yarsinia intermedia* 0%. Also, Barboni samples contaminated were *Yarsinia enterocolitice* (9) 18%, *Yarsinia frederiksenii* (8) 16%, *Yarsinia ruckeri* (1) 2% and *Yarsinia intermedia* (3) 6%. Furthermore, Mackerel samples were Yarsinia enterocolitice (6)

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12%, *Yarsinia frederiksenii* (5) 10%, *Yarsinia ruckeri* (4) 8% and *Yarsinia intermedia* (3) 6%. Finally, Denise samples were Yarsinia enterocolitice (9) 18%, *Yarsinia frederiksenii* (6) 12%, and *Yarsinia ruckeri* (1) 2%. El-Leboudi [8] examined 15 imported fish samples, the author isolated 2 of *Yersinia enterocolitica* strains (13.3%) from imported fish. It is of great concern to record that *Y. enterocolitica* was one of human pathogens that can grow at refrigeration temperature and its presence in food constitutes a public health hazard. In this respect, *Y. enterocolitica* has been implicated in several outbreaks of food illness during the past 20 years in numerous countries all over the world [37].

Vibrio spp

V. cholera has long been known to be responsible for the life threatening secretary diarrhea termed as Asiatic cholera or epidemic cholera Ryan & Ray [38]. The illustrated data in Table 3 explained the incidence of Vibrio spp was (15) 30%, (14) 28%, (13) 26% and (11) 22% of the examined samples of *Basa,* Barboni, Mackerel and Denise, respectively. In addition, the serological identification of the recovered isolates (Table 4) showed the incidence of different strains of Vibrio as follow, the examined samples of *Basa* contaminated by *Vibrio parahaemolyticus* were (11) 22% and (4) 8% for *Vibrio cholera.* Also, Barboni samples contaminated were *Vibrio parahaemolyticus* were (13) 26% and (1) 2% for *Vibrio cholera.* Furthermore, Mackerel samples were *Vibrio parahaemolyticus* were (11) 22% and (2) 4% for *Vibrio cholera.* Finally, Denise samples were *Vibrio parahaemolyticus* were (8) 16% and (3) 6% for *Vibrio cholera.* Egyptian standards (E.S889/2009) [32] of frozen fish stated that fish must be free from *V. parahaemolyticus. V. parahaemolyticus* was previously isolated from imported fish by Abdelnoor & Roumani [39], Binta et al. [40] and Harydi [9].

Aeromonas hydrophilia

Table 6: Incidence of Vibrio strains Isolated from the examined samples of imported fish (No=50).

	Basa		Barboni		Mackerel		Denise	
	No.	%	No.	%	No.	%	No.	%
Vibrio parahaemolyticus	11	22	13	26	11	22	8	16
Vibrio cholera	4	8	1	2	2	4	3	6
Total	15	30	14	28	13	26	11	22

The illustrated data in Table 6 incidence of *Aeromonas hydrophilia* was (8) 16%, (11) 22%, (8) 16% and (6) 12% of the examined samples of *Basa*, Barboni, Mackerel and Denise, respectively. Also, *Aeromonas hydrophilia* founded in frozen imported fish samples by El-Noby [13], Vila et al. & Farag [11], Sharma [12] and Alzainy [10].

Finally, it is important to mention that the results show that the higher bacterial count (Salmonella) was found in Barboni and gently went down in Mackerel, *Basa* and Denise, respectively. The higher bacterial count (E. coli) was found in Basa and gently went down in Mackerel, Barboni and Denise, respectively. Moreover, results show that the higher bacterial count (Shigella) was found in Mackerel and gently went down in, *Basa,* Barboni and Denise, respectively [41,42]. Also, results show that the higher bacterial count (Yarisina) was found in Basa and gently went down in Barboni, Mackerel and Denise, respectively. The higher bacterial count (*Vibro spp)* was found in *Basa* and gently went down in Barboni, Mackerel and Denise, respectively. Finally, the higher bacterial count (Aeromonas hydrphilie) was found in Barboni and gently went down in, (*Basa* and Mackerel) and Denise, respectively. To sum up, it is obvious that Denise spp. was the lowest contaminated imported fish for all kind of examined Enteropathogens. On the opposite, *Basa* was the most contaminated spp. of imported fish, except contamination by Salmonella it is clear that Barboni and mackerel higher than *Basa.* Accordingly, the consumption of such contaminated imported fish may, at times, induce public health hazard. The obtained results in the current work, clarified that imported fish possess a higher number of enteric pathogens with significant public health risk. These results may be attributed to unsanitary conditions, cross contamination, fecal pollution and bad personal hygiene conditions during handling, storage, distribution and selling.

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