



Enterobacteriaceae in Some Marine Fish Fillet

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Key

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ABSTRACT:

A total of 100 random samples of marine fish fillet represented by *Epinephelus alexandrinus* fillet, *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet (25 of each) were collected from different fish markets in Alexandria city. The sample weight was 100 grams. Each sample was kept in a separate sterile plastic bag and transferred in an ice box to the laboratory under complete aseptic conditions without undue delay. All collected samples were subjected to bacteriological examination for isolation and identification of Enterobacteriaceae. The results revealed that the total Enterobacteriaceae count in the examined samples of marine fish fillet were varied from 1.6×10^2 to 6.3×10^4 with an average of $2.1 \times 10^4 + 3.1 \times 10^3$ cfu/g for *Epinephelus alexandrinus* fillet, 2.1×10^2 to 4.3×10^4 with an average of $9.1 \times 10^3 + 1.1 \times 10^3$ cfu/g for *Dicentrarchus labrax* fillet, 3.1×10^2 to 6.7×10^4 with an average of $1.3 \times 10^4 + 7.3 \times 10^3$ cfu/g for Stingray fish fillet and 2.3×10^2 to 1.1×10^5 with an average of $6.3 \times 10^4 + 5.2 \times 10^3$ cfu/g for *Scomberomorus commerson* fillet, respectively. Accurately, all examined samples (100%) out of *Epinephelus alexandrinus* fillet, *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet were contaminated with Enterobacteriaceae. Also, the total coliform count in the examined samples of marine fish fillet were ranged from 1.3×10 to 2.1×10^3 with a mean value of $9 \times 10^2 + 7.5 \times 10$ cfu/g for *Epinephelus alexandrinus* fillet, 3.1×10 to 3.2×10^3 with a mean value of $1.1 \times 10^3 + 2.3 \times 10^2$ cfu/g for *Dicentrarchus labrax* fillet, 1.9×10 to 7×10^3 with a mean value of $2.6 \times 10^3 + 1.6 \times 10^2$ cfu/g for Stingray fish fillet and 6×10 to 1.3×10^4 with a mean value of $6.4 \times 10^3 + 7.1 \times 10^2$ cfu/g for *Scomberomorus commerson* fillet. The enteric bacteria isolated were *Enterobacter aerogenes* (44 %) was the most frequent bacterial species isolated from *Epinephelus alexandrinus* fillet samples, followed by *Proteus vulgaris* (28 %), *Citrobacter diversus* and *Shigella* species (20 % of each) then *Klebsiella ozanae* and *Providencia* spp (16 % of each).

In regard to *Dicentrarchus labrax* fillet samples, the most frequent bacterial species isolated was *Enterobacter aerogens* (40 %), followed by *Klebsiella pneumoniae* (32 %), *Citrobacter diversus* (28 %), *Proteus vulgaris* and *Providencia* species (20 % of each), *Citrobacter freundii* (16 %) then *Enterobacter cloacae*, *Klebsiella ozanae* and *Shigella* species (12 % of each). Concerning Stingray fish fillet samples, *Serratia liquifaciens* (44 %) represented the highest rate of isolation followed by *Enterobacter aerogens* and *Enterobacter cloacae* (36 % of each), *Citrobacter freundii* (28 %), *Citrobacter diversus* and *Enterobacter cloacae* (24 % of each), *Shigella* species (20 %) and *Proteus vulgaris* (16 %).

On the other hand, *Citrobacter freundii* (28 %), *Enterobacter aerogens* (24 %), *Proteus vulgaris* (20 %) then *Klebsiella ozanae*, *Proteus rettgeri* and *Shigella* species (12 % of each) were isolated from the examined samples of *Scomberomorus commerson* fillet. The Enteropathogenic *Escherichia coli* was isolated from 8 %, 16 %, 20 % and 28 % of the examined samples of *Epinephelus alexandrinus* fillet, *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet, respectively. Also, *Salmonella* species were recovered from 8 %, 12 % and 8 % of the examined samples of *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet, respectively. The serotypes of *Salmonella* organisms isolated from *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet were *S. enteritidis* (4 % for each) and *S. Haifa* (4 % for each). However, only one strain (4 %) isolated from Stingray fish fillet were serologically identified as *S. typhimurium*.

Enterobacteriaceae count, Coliform count, the isolated enteric bacteria, the isolated enteropathogenic *E. coli* and the isolated *Salmonella* organisms as well as the public health significance were discussed and the prophylactic measures to reduce the counts and the isolates in marine fish fillet were recommended.

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1- INTRODUCTION

Fish is a food of excellent nutritional value, providing high-quality protein rich in essential amino acids, and a wide variety of minerals, including phosphorus, magnesium, iron, zinc, and iodine in marine fish (Ariño et al., 2013).

Sea foods harvested from the marine water reflect the types of bacteria growing in it. An increase in total colony count in sea foods was expected in those suffering from unsatisfactory conditions of handling during which multiplication of organisms could occur, and then fish might cause food poisoning (Wood, 1976).

The fish flesh is generally sterile immediately after catching, however, it may become contaminated with different microorganisms during subsequent handling (Brock et al., 1984 and Etzel et al., 1998).

The flesh of healthy live or newly-caught fish is sterile as the immune system of the fish prevents the bacteria from growing in the flesh. When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely (Ruskol and Bendsen, 1992).

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 micro-organisms can penetrate from the skin and the gut to the flesh (FAO, 1983). The penetration and contamination increase in case of fish caught from polluted area where there are high densities of bacteria (Howgate, 1998).

The Enterobacteriaceae is a large family of Gram-negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens,

such as Salmonella, Escherichia coli, Yersinia pestis, Klebsiella and Shigella (Brenner et. al, 2005).

Enteropathogenic Escherichia coli may constitute a public health hazard as it may give rise to severe diarrhea in infants and young children as well as food poisoning and gastroenteritis among adult consumers (Banwart, 1989).

Salmonellosis is a worldwide problem responsible for food poisoning outbreaks but it is mainly difficult to determine whether the contamination of fishes occurred in their aqueous habitat or during their handling and marketing (Etzel et al., 1998).

Therefore, the present study was planned to determine the contamination level of some marine fish fillet with Enterobacteriaceae to protect the health of the consumers.

2- MATERIAL and METHODS

A total of 100 random samples of marine fish fillet represented by *Epinephelus alexandrinus* fillet, *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet (25 of each) were collected from different fish markets in Alexandria city. The sample weight was 100 grams.

Each sample was kept in a separate sterile plastic bag and transferred in an ice box to the laboratory under complete aseptic conditions without undue delay. All collected samples were subjected to bacteriological examination for isolation and identification of Enterobacteriaceae.

Preparation of samples:

To 5 grams of the sample, 45 ml of sterile peptone water 0.1% were added and thoroughly mixed using sterile blender for 1 - 1.5 minutes, from which tenth fold serial dilutions were prepared.

The prepared samples were subjected to the following examinations:

Determination of Total Enterobacteriaceae Count (ICMSF, 1996) .

Total Coliform count (ICMS, 1996). Identification of family Enterobacteriaceae:

Members belonging to Enterobacteriaceae were further identified according to Cowan and Steel (1974) and MacFaddin (1976). Isolation and identification of Enteropathogenic *Escherichia coli* (ICMSF, 1996). Serodiagnosis of *Escherichia coli* (ICMSF, 1996).

The isolates were serologically identified by using diagnostic sera "welcome E.coli" agglutinating sera for diagnosis of the Enteropathogenic types.

Isolation and identification of *Salmonellae* (Rappaport et al., 1956; Harvey and Price, 1981). Serological identification of *Salmonellae* (Kauffman, 1974) .

3- RESULTS and DISCUSSION

Table (1): Statistical analytical results of total Enterobacteriaceae count (TEC) cfu/g in the examined samples of marine fish fillet (n= 25 of each type).

Fish species	Minimum	Maximum	Mean	+ S.E.M [*]
<i>Epinephelus alexandrinus</i> fillet	1.6×10^2	6.3×10^4	2.1×10^4 a	3.1×10^3
<i>Dicentrarchus labrax</i> fillet	2.1×10^2	4.3×10^4	9.1×10^3 b	1.1×10^3
Stingray fish fillet	3.1×10^2	6.7×10^4	1.3×10^4 a	7.3×10^3
<i>Scomberomorus commerson</i> fillet	2.3×10^2	1.1×10^5	6.3×10^4 c	5.2×10^3

*S.E.M = Standard error of mean.

Means with different letters within the same column are significantly different at (P < 0.05)

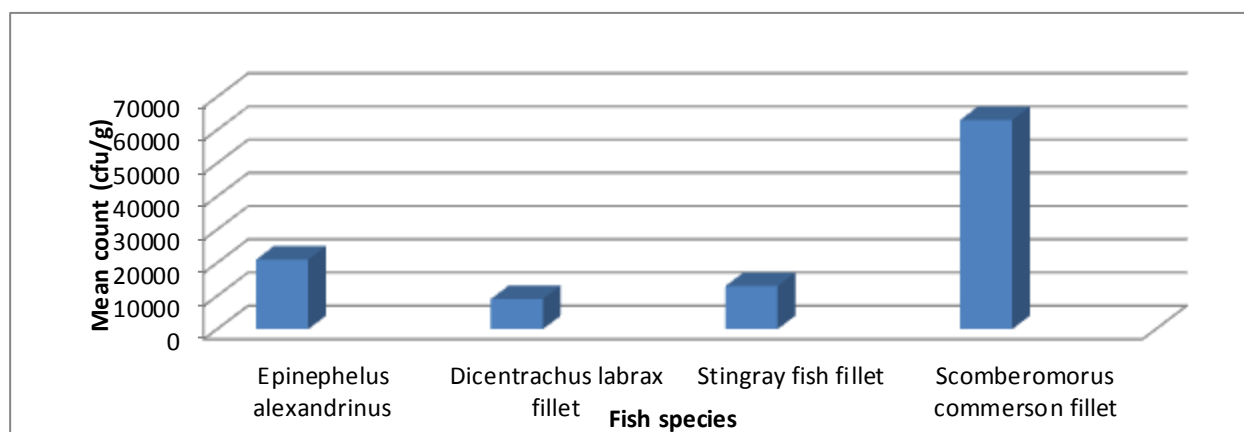


Figure (1): Mean values of total Enterobacteriaceae count in the examined marine fish fillet (cfu/g).

Table (2): Statistical analytical results of total coliform count in the examined samples of marine fish fillet (n = 25 of each type).

Fish fillet species	Minimum	Maximum	Mean	+ S.E.M [*]
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Epinephelus alexandrinus fillet	1.3×10	2.1×10^3	9×10^2 a	7.5×10
Dicentrarchus labrax fillet	3.1×10	3.2×10^3	1.1×10^3 b	2.3×10^2
Stingray fish fillet	1.9×10	7×10^3	2.6×10^3 b	1.6×10^2
Scomberomorus commerson fillet	6×10	1.3×10^4	6.4×10^3 c	7.1×10^2

*S.E.M = Standard error of mean.

Means with different letters within the same column are significantly different at $P < 0.05$

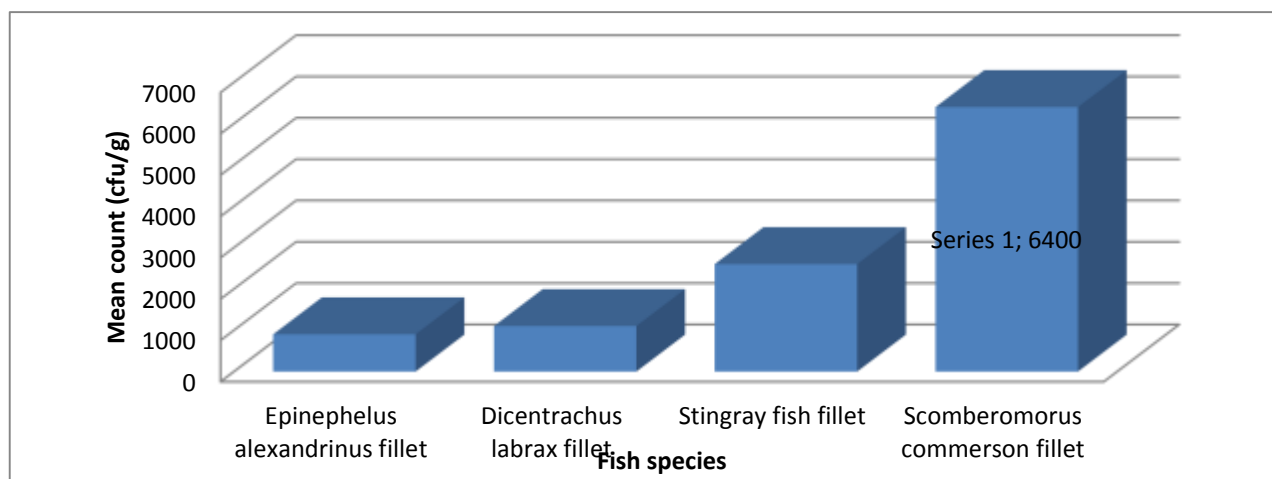


Figure (2): Mean values of total coliform count in the examined samples of marine fish fillet (cfu/g).

Table (3): Incidence of enteropathogenic E.coli in the examined samples of marine fish fillet (n = 25).

Marine fish fillet	No.	%
Epinephelus alexandrinus fillet	2	8
Dicentrarchus labrax fillet	4	16
Stingray fish fillet	5	20
Scomberomorus commerson fillet	7	28

Table (4): Serotyping of E.coli isolated from the examined samples of marine fish fillet (n = 25).

Fish species Serotype	Epinephelus alexandrinus fillet		Dicentrarchus labrax fillet		Stingray fish fillet		Scomberomorus commerson fillet		Strain character
	No.	%	No.	%	No.	%	No.	%	

O ₂₆ : k ₆₀ (B ₆)	-	-	1	4	2	8	2	8	EHEC
O ₈₆ : k ₆₁ (B ₇)	1	4	-	-	1	4	-	-	EPEC
O ₁₁₁ : k ₅₈ (B ₉)	-	-	1	4	-	-	2	8	EHEC
O ₁₂₄ : k ₇₂ (B ₁₇)	1	4	1	4	1	4	1	4	EIEC
O ₁₂₈ : k ₆₇ (B ₁₂)	-	-	1	4	1	4	2	8	ETEC
Total	2	8	4	16	5	20	7	28	
EHEC = Enterohaemorrhagic E.coli.					EIEC = Enteroinvasive E.coli.				
EPEC = Enteropathogenic E.coli.					ETEC = Enterotoxogenic E.coli				

Table (5): Incidence of Enterobacteriaceae isolated from the examined samples of marine fish fillet (n = 25).

Fish species Bacteria	Epinephelus alexandrinus fillet		Dicentrarchus labrax fillet		Stingray fish fillet		Scomberomorus commerson fillet	
	No.	%	No.	%	No.	%	No.	%
Citrobacter diversus	5	20	7	28	6	24	-	-
Citrobacter freundii	-	-	4	16	7	28	7	28
Enterobacter aerogenes	11	44	10	40	9	36	6	24
Enterobacter agglomerans	-	-	-	-	2	8	-	-
Enterobacter cloacae	3	12	3	12	9	36	-	-
Enterobacter hafniae	-	-	-	-	6	24	-	-
Klebsiella azanae	4	16	3	12	-	-	3	12
Klebsiella pneumoniae	-	-	8	32	-	-	2	8
Proteus mirabilis	-	-	-	-	-	-	-	-
Proteus rettgeri	3	12	2	8	7	28	3	12
Proteus vulgaris	7	28	5	20	4	16	5	20
Serratia liquefaciens	-	-	-	-	11	44	-	-
Serratia marcescens	-	-	2	8	3	12	-	-
Providencia spp	4	16	5	20	-	-	2	8
Shigella species	5	20	3	12	5	20	3	12

Table (6): Incidence of Salmonella in the examined samples of marine fish fillet (n = 25).

Marine fish fillet	No.	%
Epinephelus alexandrinus fillet	-	-
Dicentrarchus labrax fillet	2	8

Stingray fish fillet	3	12
Scomberomorus commerson fillet	2	8

Table (7): Serotyping of Salmonella isolated from the examined samples of marine fish fillet (n = 25).

Fish species Serotype	Epinephelus alexandrinus fillet		Dicentrarchus labrax fillet		Stingray fish fillet		Scomberomorus commerson fillet	
	No.	%	No.	%	No.	%	No.	%
S. enteritidis	-	-	1	4	1	4	1	4
S. typhimurium	-	-	-	-	1	4	-	-
S. Haifa	-	-	1	4	1	4	1	4
Total	-	-	2	8	3	12	2	8

Concerning the fish fillet species, it is clear that Scomberomorus commerson fillet had the highest Enterobacteriaceae count followed by Stingray fish fillet, Epinephelus alexandrinus fillet and Dicentrarchus labrax fillet.

These variations could be attributed to the fish species, environment, extent of handling during transportation, slicing and distribution as well as marketing (Wang et al., 1994).

Generally, the Enterobacteriaceae count in all types of marine fish fillet seems to be high and this may be attributed to enteric contamination from different sources during transportation, storage, bad handling, slicing from the intestinal tract of the fish during evisceration and marketing.

In conclusion, to avoid contamination of the fish fillet with enteric microorganism, the fish must be caught far away from sewage drainage area in the sea, the vehicles and boxes used for transportation must be cleaned and sanitized, the workers handling fish must be educated about personal hygiene as their hands must be washed after using the toilet, the knives used for evisceration must not be used for slicing to avoid cross contamination and the workers

whom eviscerate the fish must not slice the fish, the water used for washing the fillet must be bacteriologically examined and avoid retailing fish fillet in open air.

Coliform count:

Table (2) and Figure (2) indicated that the total coliform count in the examined samples of marine fish fillet were ranged from 1.3×10^1 to 2.1×10^3 with a mean value of $9 \times 10^2 + 7.5 \times 10^1$ cfu/g for Epinephelus alexandrinus fillet, 3.1×10^1 to 3.2×10^3 with a mean value of $1.1 \times 10^3 + 2.3 \times 10^2$ cfu/g for Dicentrarchus labrax fillet, 1.9×10^1 to 7×10^3 with a mean value of $2.6 \times 10^3 + 1.6 \times 10^2$ cfu/g for Stingray fish fillet and 6×10^1 to 1.3×10^4 with a mean value of $6.4 \times 10^3 + 7.1 \times 10^2$ cfu/g for Scomberomorus commerson fillet.

Also, Table (2) showed that there is no significant difference between the mean values of the examined samples of Dicentrarchus labrax fillet and Stingray fish fillet. But, there is significant difference between the mean values of both Dicentrarchus labrax fillet and Stingray fish fillet and each of Epinephelus alexandrinus fillet and Scomberomorus commerson fillet ($P < 0.05$).

Coliform count reflects inadequate sanitation during catching and handling of the raw fish, contact surface of fish and workers. However, the occurrence of large number of coliform in marine fish fillet is highly undesirable and mostly suggests faecal contamination and severe hazards. From their original faecal, soil, water or plant environment, coliform bacteria can reach the food handlers hands, or preparing fish fillet environment where they may be spread via equipment and utensils or by employees. The presence of Coliform in marine fish fillet indicates a potable faecal source of contamination (National Academy of Sciences, 1985).

Generally, the presence of Coliform infection in marine fish fillet serves as an index of sanitation under which the fish is handled. Thus their significance is directly associated with the faecal contamination, handling and storage of fish.

Enteropathogenic E.coli:

The results recorded in Table (3) showed that the Enteropathogenic E.coli was isolated from 8 % , 16 % , 20 % and 28 % of the examined samples of *Epinephelus alexandrinus* fillet, *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet, respectively.

Serotyping of enteropathogenic E.coli isolated from the examined samples of marine fish fillet was declared in Table (4) and Figure (4).

Accurately, out of 2 strains of E.coli isolated from samples of *Epinephelus alexandrinus* fillet, one was recorded as O₈₆ : K₆₁ (B₇) and the other strain was O₁₂₄ : K₇₂ (B₁₂).

While, O₂₆ :K₆₀ (B₆) strain, O₁₁₁ :K₅₈ (B₉) strain, O₁₂₄ :K₇₂ (B₁₇) strain, O₁₂₈ :K₆₇ (B₁₂) strain (4 % of each) were identified from *Dicentrarchus labrax* fillet samples.

Furthermore, O₂₆ :K₆₀ (B₆) strain (8 %), O₈₆ :K₆₁ (B₇) , O₁₂₄ :K₇₂ (B₁₇) and O₁₂₈ :K₆₇ (B₁₂) strains (4 % of each) were detected in Stingray fish fillet samples.

Regarding *Scomberomorus commerson* fillet, out of 7 strains of E.coli , 2 strains were serologically identified as O₂₆ :K₆₀ (B₆), 2 identified as O₁₁₁ :K₅₈ (B₉) , 2 were O₁₂₈ :K₆₇ (B₁₂) and the last strain was O₈₆ :K₆₁ (B₇) .

In general, EPEC strains are the major cause for many infantile diarrheas. In typical cases, symptoms appear within 12 to 36 hours. Clinically, EPEC illness is characterized by fever, nausea, vomiting and watery stools which occasionally contain mucus, but without gross blood (Scotland et al., 1990). Furthermore, EPEC was implicated in case of gastroenteritis, cystitis, colitis, pyelonephritis, peritonitis and puerperal sepsis as well as food poisoning outbreak (Bryan, 1980).

Enterotoxigenic E. coli (ETEC) strains (O₇₈ and O₁₂₈) are considered the common cause of traveler's diarrhea and/or children diarrhea. In this respect, 4 out of 11 American traveler's suffering from diarrhea was infected with (ETEC) strains producing either heat labile (LT) and/or heat stable (ST) toxins (FAO, 1993).

In summer of 1976, more than 2200 visitors at a national park in USA suffered from diarrhea. The causative organism was ETEC O₁₂₈ producing LT (Rosenberg et al., 1977).

In 1971 ETEC (O₁₂₄) was responsible for gastroenteritis and dysentery in 387 persons. The severity of the disease may vary from a mild form resembling *Shigella sonnei* infection to an extreme form resembling classical dysentery. Clinically, the illness due to ETEC is marked by fever; severe abdominal cramps and watery diarrhea followed by gross bloody diarrhea (Hoepflich et al., 1994).

On the other hand, EHEC (O₁₁₁) was implicated in 16 outbreaks of diarrhea in young children and infants (Evans et al., 1979). In illness caused by EHEC is typically quite severe and characterized by sudden onset of severe crampy abdominal pain followed by watery diarrhea, which later becomes grossly bloody. Typically

there is little or no fever and the duration of illness is 2 to 9 days. Death rate in some reported outbreaks may reach 36% while in other no death had occurred (Griffin, 1991). Since 1982, more than 100 outbreaks of EHEC were reported in USA (WHO, 1997).

Isolated Enterobacteriaceae:

Table (5) summarized the different enteric bacteria isolated from the examined samples of marine fish fillet.

Enterobacter aerogenes (44 %) was the most frequent bacterial species isolated from *Epinephelus alexandrinus* fillet samples, followed by *Proteus vulgaris* (28 %), *Citrobacter diversus* and *Shigella* species (20 % of each) then *Klebsiella ozanae* and *Providencia* spp (16 % of each).

In regard to *Dicentrarchus labrax* fillet samples, the most frequent bacterial species isolated was *Enterobacter aerogenes* (40 %), followed by *Klebsiella pneumoniae* (32 %), *Citrobacter diversus* (28 %), *Proteus vulgaris* and *Providencia* species (20 % of each), *Citrobacter freundii* (16 %) then *Enterobacter cloacae*, *Klebsiella ozanae* and *Shigella* species (12 % of each) as shown in Table (5).

Concerning Stingray fish fillet samples, *Serratia liquifaciens* (44 %) represented the highest rate of isolation followed by *Enterobacter aerogenes* and *Enterobacter cloacae* (36 % of each), *Citrobacter freundii* (28 %), *Citrobacter diversus* and *Enterobacter cloacae* (24 % of each), *Shigella* species (20 %) and *Proteus vulgaris* (16 %).

On the other hand, *Citrobacter freundii* (28 %), *Enterobacter aerogenes* (24 %), *Proteus vulgaris* (20 %) then *Klebsiella ozanae*, *Proteus rettgeri* and *Shigella* species (12 % of each) were isolated from the examined samples of *Scomberomorus commerson* fillet.

The *Enterobacter* organisms can be found in soil, water, sewage and intestinal tract of man and animals. The organisms are important in food as a potential health

hazard, and indicator organism and spoilage. Some strains of *Enterobacter* spp. had been implicated in acute and chronic diarrhoeal disease and in several cases of food poisoning (Banwart, 1989). *Klebsiella* organisms are responsible for food borne outbreaks of gastroenteritis (Rahkia et al., 1998). *Klebsiella pneumoniae* was incriminated in cases of lobar pneumonia and other affections of respiratory tract (Cruickshank et al., 1975). Also, *Klebsiella pneumoniae* cause bronchopneumonia of one or several lung lobes producing fused foci and lung abscesses. They added that the organism may be responsible for meningitis, appendicitis, pyaemia, mastoiditis and cystitis (Pyatkin and Krivoshein, 1980).

Certain numbers of *Citrobacter* have been suspected to cause enteric infection (Sugita et al., 1995). However, *Citrobacter* is responsible for food borne outbreaks of gastroenteritis (Rusul et al., 1991). Moreover, *Citrobacter freundii* have been found among intestinal, urinary and pyogenic infection (Krieg and Holt, 1984).

Proteus organisms are widely distributed in various foods. The importance of *Proteus* in foods includes potential health hazards and spoilage. Also, certain strains of *Proteus* can cause enteric infection in human (Bryan, 1980).

Proteus species have been incriminated in cases of summer diarrhea in infants, sinusitis, otitis as well as urinary tract infection (Varnam and Evans, 1991). *Proteus* species was involved in the spoilage of seafood.

Salmonella:

Salmonellosis is considered as one of the most important zoonotic disease in which the main source of infection is food of animal origin and the mortality due to Salmonellosis is relatively low and occurs only in very old individuals and infants (Sharma et al., 1996).

Result given in Table (6) indicated that *Salmonella* organisms were recovered

from 8 %, 12 % and 8 % of the examined samples of *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet, respectively.

In contrast, all examined samples of *Epinephelus alexandrinus* fillet were free from *Salmonella*.

Table (7) revealed that the serotypes of *Salmonella* organisms isolated from *Dicentrarchus labrax* fillet, Stingray fish fillet and *Scomberomorus commerson* fillet were *S. enteritidis* (4 % for each) and *S. Haifa* (4 % for each). However, only one strain (4 %) isolated from Stingray fish fillet were serologically identified as *S. typhimurium*.

However, *Sal. typhimurium* is the commonest *Salmonella* isolated from cases of food poisoning and represents about 50 – 60% of such cases (WHO, 1997). Furthermore, FAO (1993) reported that the cases of food poisoning outbreaks due to *Sal. typhimurium* were 407 cases in Spain (1981), 237 in Poland (1980), 227 in Denmark (1981), 130 in Sweden (1981), 84 in Scotland (1981), 80 in Ireland (1981), 37 in Yugoslavia (1984), 22 in England and 3 cases in Belgium (1981).

Despite the fact that *Salmonella* organisms exist all over the world, it does not mean that *Salmonellosis* should be accepted as inevitable but every defense must be considered through application of efficient sanitation to control such serious organisms.

Accordingly, the presence of *Salmonella* as enteropathogens in fish fillet may reflect the unsatisfactory hygienic condition during handling the fishes, slicing, packaging and marketing.

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