Molecular detection of Shiga toxin producing *Escherichia coli*, *Yersinia enterocolitica* and *Salmonellae* in fresh and frozen *Saurus* fish in Damietta, Egypt

Mohammed Reda EL- Bediwi *
Food Inspection Lab., Damietta Sea’ Port, Animal Health Research Institute (AHRI), Egypt

*Corresponding Author: Mohammed Reda EL- Bediwi
Food Inspection Lab., Damietta Sea' Port, Animal Health Research Institute (AHRI), Egypt
Tel: 0020507927888, E-mail: dr.m.elbediwi@gmail.com

**Abstract**

The objectives of this study were to determine the incidence of non STEC, STEC, *Y. enterocolitica* and *Salmonellae* isolated from frozen and fresh *Saurus* fish samples obtained from fish markets in Damietta governorate. A total of 200 fish samples comprising 100 samples from each frozen and fresh *Saurus* were collected from different public fish markets at Damietta Governorate. STEC, non STEC isolation were performed according to FDA (2011). While, and *Y. enterocolitica* was performed according to FDA (2001).While, isolation of *Salmonellae* was performed according to (ISO 6579, 2002). Positive samples of standard microbiological techniques were confirmed with polymerase chain reaction (PCR)by detecting a fragment of Prs genes. Results revealed that, non STEC was isolated from fresh and frozen *Saurus* fish as 10 (10%) and 2 (2%), respectively. Meanwhile, STEC and *Y. enterocolitica*were only isolated from fresh samples as 2(2%) and 11(11%), respectively. However, *Salmonellae* could not be detected in all fish samples. According to E.S.S (2005) frozen and chilled fish samples must be free from *E. coli, Y. enterocolitica* and *Salmonellae*. The study showed that *E. coli* and *Y. enterocolitica* were common contaminant of fish obtained from Damietta fish markets, and this may pose serious public health implications.

**Key words:** non STEC, STEC, *Y. enterocolitica, Salmonellae*, Saurus fish, PCR, Egypt.

**Introduction**

Fish constitute an essential food constituent for a large section of world population and as one of the most important source of high quality animal protein, easy digestibility and high nutritional value [1, 2]. *E. coli* is a normal inhabitants in the small intestine and colon and non-pathogenic, meaning they do not cause disease in the intestine. Although, these *E. coli* are non-pathogenic, they can cause disease if they spread outside the intestine. The pathogenic strains of *E. coli* may cause diarrhoea by releasing toxins (enterotoxigenic *E. coli* or ETEC) and cause of food poisoning from fish. The presence of
*E. coli* in higher range suggests that contamination of the samples before or during handling, processing and marketing [3]. STEC are the cause of worldwide outbreaks of haemorrhagic colitis and led in 10% of the cases to life-threatening haemorrhagic uremic syndrome (HUS) with a case-fatality rate ranging from 3% to 5%. HUS is characterized by acute renal failure, haemolytic anaemia and thrombocytopenia [4]. It often produces two antigenically distinct types of Stx, stx1 and stx2 [5]. Salmonellosis is a worldwide health problem. The non-typhoidal *Salmonella* is a common cause of a number of different disease syndromes including bacteraemia and gastroenteritis, which is often characterized by abdominal pain, nausea, vomiting, diarrhoea, and headache [6]. Isolation of *Salmonella* and *E. coli* indicate faecal and environmental pollution. The organism *E. coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large numbers it is an indicator of mishandling [7]. The genus *Yersinia* comprises of 11 species, of which *Y. enterocolitica* was most important as a causative agent of human foodborne illness. Both of *Y. enterocolitica* and *Y. pseudotuberculosis* are transmitted through the faecal oral route. *Y. enterocolitica* has been detected in environmental sources, such as soil and water (e.g., ponds and lakes) and has the ability to grow at temperatures below 4°C. *Y. enterocolitica* could grow over a pH range of 4 to 10, generally with an optimum pH of 7.6 [8]. The contamination by *Y. enterocolitica* might be due to the poor hygienic conditions of the water bodies, improper handling and storage practices [9]. *Y. enterocolitica* commonly causes enteritis in children younger than 5 years old with symptoms include fever, abdominal pain and diarrhoea, which is often bloody. These sequelae include glomerulonephritis; endocarditis; erythema nodosum (which occurs mainly in women); uveitis; thyroid disorders, such as Graves’ disease; hyperthyroidism; nontoxic goitre; and Hashimoto’s thyroiditis [10]. The present study was undertaken to investigate the prevalence of *E. coli*, *Y. enterocolitica* and *Salmonella* in fresh and frozen Saurus fish samples collected from retail fish markets in Damietta governorate, Egypt.

### Materials and Method

#### Sampling Area

Damietta Governorate is situated in the north eastern part Egypt. Damietta city is the capital city of it. It is situated at the junction between the Mediterranean Sea and Nile. The whole area of Damietta Governorate is 910, 30 km², equal to about 0.10% of Egypt's total area and its population density is 1.40 per km². Damietta governorate is divided into five cities: Damietta, El-Zarka, Faraskor, KafraSaad, and Kafir Al-Buteekh.

#### Sampling

Between January 2014 and November 2015, two hundreds local fresh and imported frozen *saurus* (*Synodus saurus*) (100 each) were collected randomly from different fish markets in the morning between 7.00 a.m. and 9.00 a.m. local time, with different hygiene levels from four different cities (Damietta city (84) – Faraskur (44) – KafrSaad (38) – AzZarqa (34)) in Damietta Governorate. Each sample represented by (75 grams) were collected, identified and labelled in sterile bags to prevent any contamination and transported in an ice box as quick as possible to food inspection laboratory, Damietta sea port. Fresh samples were inspected at once while; frozen samples were kept at –20°C until inspection.

#### Isolation and Identification of non STEC and STEC [11]

This method is a general procedure for the isolation of non STEC. It is recommends pre-enrichment of a 25 g food sample in 225 ml brain heart infusion (BHI) broth at 35 °C for 3 h to facilitate resuscitation of sub lethally injured cells. The pre-enrichment is then transferred to 225 ml of tryptone phosphate (TP) broth and incubated at 44 °C for 20-24 h. A volume of enriched broth is then plated onto Levine’s eosin-methylene blue (L-EMB; colonies produce a green metallic sheen) agar
and MacConkey agar plates (colonies are brick red in color). These plates should be incubated at 37 °C for 24 h. 

**STEC** was enriched in Trypticase soy broth, incubated overnight at 37°C, spread on plates with sorbitol MacConkey agar (SMAC), and incubated again overnight at 37°C.

**Microscopic examination**

**Staining:** Films from the suspected colony was stained by Gram stain and examined microscopically for Gram negative short rods.

**Biochemical reactions:** According to [12, 13]:-

- Motility, Indole production test, Voges-Proskauer test, Methyl red test, Citrate utilization test, Triple sugar iron agar test.

**Interpretation:** All cultures that (a) appear as Gram-negative non spore forming rods (b) ferment lactose with gas production without H2S within 48 h at 37°C and (c) give IMVC patterns of ++-- are considered to be *E. coli*.

**Isolation and Identification of Salmonellae** [14]

**Pre-enrichment:**

Twenty five grams samples were homogenized with 225 ml of buffered peptone broth in a stomacher for one minute. The samples were pre-enriched in buffered peptone broth; incubated for 18- 20h at 35°C to provide available nutrients required for the survival and repair of stressed and injured *Salmonella* cells.

**Selective Enrichment**

About 0.1 ml of the pre-enriched sample was transferred to 10 ml of Rappaport-Vassiliadis soya broth (R.V.S) and incubated at 41.5°C for 18±2 hours. In addition 1ml of the pre-enriched sample was transferred to 10 ml of Muller Kuffman tetraionate broth (TT) *(Oxoid, CM0671)*; and incubated at 35-37°C for 24 hours.

**Plating on Solid Selective Media:**

Each selective enrichment broth was shaken and then a loopful from each of them was streaked onto plates of Hekton enteric agar medium and Xylose lysine desoxycholate (XLD) agar *(Oxoid, CM0469)*. All plates were incubated at 35°C for 24-48 hours and then inspected for typical *Salmonella* colonies.

**Identification**

*Salmonella* strains were identified by microscopic examination and biochemically. Suspected *Salmonella* colonies; pink or reddish colour with or without black centre on XLD, and dark green circular Hekton enteric agar medium colonies. Isolates were differentiated and identified according to various biochemical characteristics including: urease test, triple sugar iron test inoculated on nutrient agar slants and incubated at 37°C for 24 hours. About 3 to 4 suspected colonies were emulsified in one drop of sterile normal saline, on a glass slide. A drop from polyvalent *Salmonella* O -antisera poly A–I & VI were added, and agglutination was observed. Colonies that showed agglutination considered positive.

**Staining reaction:** Films from suspected colony stained with Gram stain for Gram negative non spore forming rods.

**Biochemical tests** [12, 13]

**The following tests were performed:** Triple sugar iron agar (TSI), Fermentation of sugars, Citrate utilization, Indole production test, Vogesprokaeur test, Methyl red test, Urea hydrolysis test.

**Interpretation:** All cultures that (a) appear as Gram negative non spore forming rods (b) not ferment lactose (c) Give negative urea hydrolysis test (d) Give IMVC patterns of -+- + are considered to be *Salmonella* species (e) agglutination with polyvalent Salmonella O -antisera poly A–I & VI.
Isolation and Identification of *Yersinia enterocolitica* [15]

**Enrichment**

Twenty five grams of sample was aseptically inoculated into 225 ml Peptone sorbitol bile broth, homogenized and incubated at 10°C for 10 days. On the tenth day, enrichment broth removed from incubator and mixed accurately. 0.1 ml of enrichment was transferred to 1ml of 0.5% potassium hydroxide in 0.5% saline, and mixed for 2–3s. Successively one loopful was streaked onto MacConkey agar plate and one loopful to Cefsulodin-irgasan-novobiocin agar plate (CIN) (CM 653, Oxoid). Additionally, 0.1 ml of enrichment was transferred to 1ml 0.5% saline and mixed for 5–10s before streaked as above. The agar plates were incubated at 30°C for 1-2 days.

**Isolation**

CIN plates were examined after 1 day incubation. Small (1-2 mm diameter) colonies having deep red centre with sharp border surrounded by clear colourless zone with entire edge “bull’s eye” appearance were selected. MacConkey agar plates were examined after 1-2 days incubation. Small flat, colourless, or pale pink colonies were selected.

**Identification**

**Microscopic examination**

**Staining:** Films from the suspected colony were stained and examined microscopically for Gram negative rod shaped bacteria.

**Motility:** Colonies were examined on semi solid medium for motility at 22°C.

**Biochemical reactions:** Citrate utilization, Indole production test, Vogesprokaeur test, Triple sugar iron agar (TSI), Fermentation of sugars, Oxidase test.

**Interpretation:** All the isolates which were Gram-negative rods and motile at 25 °C but non-motile at 37 °C, and give alkaline slant and acid butt, no gas and no H2S (KA- -) Reaction in Lysine arginine iron agar, which were also urease- positive.

**Confirmation of positive of non STEC, STEC and *Y. enterocolitica* by PCR**

For non STEC, the primer sequence was based on the gene sequence of afa. This gene is responsible for pathogenicity and is specific to *E. coli* [16]. The primer sequence for the amplification of the afa gene from *E. coli* is:

- forward primer, 5’ GCT GGG CAA ACT GAT AAC TCT C 3’;
- reverse primer, 5’ CAT CAA GCT GTG TGG TCC GCC G 3’ [17].

The afa gene PCR yields a product of 400 bp. Strains identified as pathogenic *E. coli* by afa gene. PCR were further characterized to determine the presence of the Shiga toxin gene (stx1).

Primers used for stx1 gene were 5'ATA AAT CGC CAT TCG TTG ACT AC 3’ and 5' AGA ACG CCC ACT GAG ATC ATC 3’.

While, Primers used for (stx2) gene were 5'GCC ACT GTC TGA AAC TGC TCC 3’ and 5’ TCG CCA GTT ATC TGA CAT TCT G 3’ [18]. These primers were used to amplify a 180bp and 225 bp product specific to the (stx1) and (stx2) genes. *Y. enterocolitica* confirmation PCR primer was selected which target the ailgene of *Y. enterocolitica* [19].

The 5'-Biotin (BIOT) labelled primer (5'-BIOT-TTAATGTGTAAGCTGAGTG-3') and the 3' (3'-CTGGAAATATTTATGAGG-5') were commercially synthesized (Operon Technologies Alameda, CA) these primer amplify a 425(bp) product of the ailgene [19].

PCR was conducted using a thermal cycler (Biorad, Hercules, USA). The reaction mix consisted of an assay buffer, dNTP mix, 0.075 μM of each of the primers, 0.65 units of TaqDNA polymerase. Ten μL of the supernatant from the processed homogenates was taken in a sterile 0.5 mL microcentrifuge tube. The reaction volume was adjusted to 25 μL with sterile nuclease-free water. The reaction mixture tubes were placed in a thermal cycler and the reaction was performed for 40 cycles of PCR, with each cycle consisting of 1 minute at 94.8°C (denaturation), 30 seconds at 58°C (annealing) and 1 minute at 72°C (primer extension). An additional step of 5 minutes
at 72°C was also included for primer extension at the end of the reaction. After the reaction was complete, the PCR products were detected on agarose gel by electrophoresis, followed by visualization under a UV trans illuminator. For the 400 bp afa gene product and the 513 bpsx gene product, 1% agarose gel was used; for the 425bpail gene product, 3% agarose gel was used.

Results
Phenotypic characteristics of non STEC, STEC and Y. enterocolitica isolates
Results revealed that E.coli and Y. enterocolitica isolates showed typical characteristics by microbiological tests. In addition, E. coli isolates were further confirmed by PCR amplification of 400bp of the downstream of afa gene and 180 bp, 225 bp of the downstream of (stx1 and stx2) genes for confirmation of shiga toxin producing E. coli. While Y. enterocolitica isolates confirmed by PCR amplification of 425 bp of the downstream of ail gene.

Incidence of non STEC, STEC and Y. enterocolitica isolates
The present data tabulated in Table (2) and Figures (2, 3, 4 and 5) revealed that the overall incidence of non STEC in was 12/200 (6%) and STEC was 2/200 (1%), whereas the overall incidence of Y. enterocolitica was 11/200 (5.5%). The incidence of non STEC, STEC and Y. enterocolitica contamination of fresh Saurus fish was much higher than frozen one collected from markets. Non STEC was detected in 10% in fresh samples, 2% in frozen one and STEC was only detected in 2% in fresh samples. While, Y. enterocolitica was detected in 11% in fresh samples could not be detected in frozen one. Salmonellae was failed to be detected in all examined samples.

Incidence of non STEC, STEC and Y. Enterocolitica isolates in Damietta governorate
As tabulated in Table (1), the total incidence of non STEC was highest in KafrSaad (7.8%), nearly similar in Damietta city and AzzZarqa (5.9, 5.8%) and lowest in Faraskur (4.5%). While, STEC was only detected in Damietta city (2%). Whereas, the highest incidence of Y. enterocolitica was nearly similar in Dameitta city and Faraskur (9.5, 9%), which was much higher than AzzZarqa and KafrSaad (5.8, 5.25%).

Differences of non STEC, STEC and Y. enterocolitica incidences among between fresh and frozen samples
As illustrated in Table (1) and Figure (1) results showed higher isolation rates of non STEC from fresh samples 10/100 (10%) compared with isolation rate from frozen samples 2/100 (2%). Consequently, Y. enterocolitica and STEC was only detected in fresh samples 11/100(11%) and 2/100 (2%), respectively.

Discussion
Egypt has two coastlines on the Mediterranean Sea and the Red Sea. The landings in the Mediterranean Sea represented about 62% of the total marine catch in 2009. Marine fisheries produce a wide diversity of species. There are found that Saurus comprise about (4.7 % of landings in 2009), shrimp (8.9 %), anchovy (5.8%), mullets (3.1%), bogue (2.7%), and round scade (6.2%) [20].

There were two forms of E. coli diseases. The first caused by toxigenic strains, which characterized by excessive loss of fluid from profuse diarrhoea, while the second form caused by invasive strains produced a syndrome closely resembling dysentery [21].

Table (1) illustrated that the incidence of both non STEC was 10 (10%) in examined fresh Saurus and 2 (2%) in examined frozen Saurus, while, STEC was only detected in fresh samples 2(2%).

According to [22] frozen and chilled fish samples must be free from E. coli. E. coli in fish is considered as an indicator of potential sewage pollution [23].The organism E. coli is recognized as the reliable indicator of faecal contamination and mishandling. Our results revealed that incidence of E. coli in fresh samples were higher than frozen samples and this may be due to faecal
Table 1: Incidence of total isolated non STEC, STEC, *Y. enterocolitica* and *Salmonellae* among fresh and frozen *Saurus* fish in Damietta Governorate

<table>
<thead>
<tr>
<th>Fish samples (No =100 each)</th>
<th>Non STEC No. (%)</th>
<th>STEC No. (%)</th>
<th><em>Y. enterocolitica</em> No. (%)</th>
<th><em>Salmonellae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fresh Saurus</strong></td>
<td>10 (10%)</td>
<td>2 (2%)</td>
<td>11 (11%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Frozen Saurus</strong></td>
<td>2 (2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Total No=200</strong></td>
<td>12 (6%)</td>
<td>2 (1%)</td>
<td>11 (5.5%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 2: Incidence of isolated non STEC, STEC, *Y. enterocolitica* and *Salmonellae* from *Saurus* fish markets in Damietta governorate (No.200)

<table>
<thead>
<tr>
<th>Samples</th>
<th>N.</th>
<th>Non STEC No. (%)</th>
<th>STEC No. (%)</th>
<th><em>Y. enterocolitica</em> No. (%)</th>
<th><em>Salmonellae</em> No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Damietta city</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh samples</td>
<td>84</td>
<td>5 (5.9%)</td>
<td>2 (2.3%)</td>
<td>4 (9.5%)</td>
<td>0 (0%)</td>
<td>9 (10.7%)</td>
</tr>
<tr>
<td>Frozen samples</td>
<td>42</td>
<td>4 (9.5%)</td>
<td>0 (0%)</td>
<td>4 (9.5%)</td>
<td>0 (0%)</td>
<td>8 (19%)</td>
</tr>
<tr>
<td><strong>Faraskur</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh samples</td>
<td>44</td>
<td>2 (4.5%)</td>
<td>0 (0%)</td>
<td>4 (9%)</td>
<td>0 (0%)</td>
<td>6 (13.6%)</td>
</tr>
<tr>
<td>Frozen samples</td>
<td>22</td>
<td>2 (9%)</td>
<td>0 (0%)</td>
<td>4 (18.1%)</td>
<td>0 (0%)</td>
<td>6 (27%)</td>
</tr>
<tr>
<td><strong>KafrSaad</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh samples</td>
<td>38</td>
<td>3 (7.8%)</td>
<td>0 (0%)</td>
<td>2 (5.25%)</td>
<td>0 (0%)</td>
<td>5 (13.1%)</td>
</tr>
<tr>
<td>Frozen samples</td>
<td>19</td>
<td>2 (10.5%)</td>
<td>0 (0%)</td>
<td>2 (10.5%)</td>
<td>0 (0%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td><strong>AzZarqa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh samples</td>
<td>34</td>
<td>2 (5.8%)</td>
<td>0 (0%)</td>
<td>1 (5.8%)</td>
<td>0 (0%)</td>
<td>3 (8.8%)</td>
</tr>
<tr>
<td>Frozen samples</td>
<td>17</td>
<td>2 (11.7%)</td>
<td>0 (0%)</td>
<td>1 (5.8%)</td>
<td>0 (0%)</td>
<td>3 (17.6%)</td>
</tr>
</tbody>
</table>

Figure 1: Incidence of non STEC, STEC, *Y. enterocolitica* and *Salmonellae* from *Saurus* fish markets in Damietta governorate
Figure 2: The electrophoretic pattern of PCR products from tested fish samples by using primer found in the downstream of afa gene which showed positive amplification at 400bp.

1- (1, 12) positive non STEC strains from samples, 2- (N) control negative

Figure 3: The electrophoretic pattern of PCR products from tested samples by using primer found in the downstream of stx1 gene which showed positive amplification at 180 bp.

1- (1, 2) positive STEC strains from samples, 2- (N) control negative
Figure 4: The electrophoretic pattern of PCR products from tested fish samples by using primer found in the downstream of \textit{stx2} gene which showed positive amplification at 225bp.

1- (2, 3) positive STEC from samples, 2- (1) control negative

Figure 5: The electrophoretic pattern of PCR products from tested fish samples by using primer found in the downstream of \textit{ail} gene which showed positive amplification at 425bp.

1- (1, 2) positive \textit{Y. enterocolitica} strains from samples, 2- (3) control positive, 3- (4) control negative

contamination, improper handling and improper transportation of fresh samples. Besides, the process of freezing mainly reduces the initial bacteriological load. There were found that prevalence of \textit{E. coli} in frozen fish have been found to fall during freezing and frozen storage [24]. Our data of fresh samples were coincided with that recorded by [25]. Lower data were recorded with those

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recorded by [26]. While, higher data were recorded by [27]. Meanwhile, results of frozen samples were coincided with that recorded by [28, 29]. Lower results were observed by [30] While, higher results were observed by [31, 32]. Y. enterocolitica is mainly a gastrointestinal pathogen, the main symptoms is severe abdominal pain usually localized in the right lower quadrant due to acute terminal ileitis and mesenteric lymphadenitis, which misdiagnosed as appendicitis, and has been associated with reactive arthritis, which may occur even in the absence of obvious symptoms [33].

From Table (1) the incidence of isolated Y. enterocolitica was 11(11%) in fresh Saurus. While Y. enterocolitica failed to be detected from frozen samples. According to [22] frozen and chilled fish samples must be free from Y. enterocolitica. Y. enterocolitica in fresh samples might be due to the poor hygienic conditions of the water and inappropriate handling and storage practices.

Our present data of fresh Saurus fish samples were coincided with [34]. Higher results were reported by [35, 36] While, frozen Saurus samples results were nearly similar to those obtained by [37, 38].

As illustrated in Figure (1) results showed that the isolation rates of non STEC, STEC and Y. enterocolitica isolates from fresh samples were higher than from frozen one whereas STEC and Y. enterocolitica were not detected in frozen Sardines. Salmonella is a leading food borne pathogen; causes both typhoid fever and salmonellosis illnesses in humans [39]. Table (1) also showed that Salmonellae was failed to be detected in all fresh and frozen samples. According to [22] frozen and chilled fish samples must be from Salmonellae.

In concern to the absence of Salmonella species in frozen and fresh fish samples in our study it could be due to the unfavourable effect of the salinity of marine water on the viability of bacterial pathogens. Besides, Salmonella species are delicate bacteria, and found in few numbers in fish. The present data of fresh samples illustrated in Table (1) were coincided with [40]. While, higher results were reported by [41]. Our findings were of frozen samples in good harmony with to those obtained by [29, 42, 43]. In contrary to our results, higher finding of Salmonella species incidence rates in frozen samples were obtained by [44].

Conclusion

Results of the study indicated that fresh samples were more contaminated than frozen one as an effect of exposure to cross contamination beside faecal contamination of water taken in by the fish samples in their ecosystem, rather than the application of unhygienic practices during storage, handling and transportation.

Also constitute an indicator of bacteriological contamination of a wide range of analysed fish samples. The danger associated with marketed fresh and frozen fish, determined as the occurrence of specific bacterial pathogens and indicators. Also, it is always needed to notify consumers of the possible health hazards related with the consumption of fish since careful handling of fishes, prevention of cross contamination in preventing infections associated with pathogens, and consumer health is adequately protected. Our results could serve as a basis for future testing of fishes, therefore, for public health, it was recommended to eviscerate fish as fast as possible after harvesting to avoid bacterial attack other tissues. Furthermore, transportation, cross contamination, handling and processing of fish and fish products should be performed in an extreme hygienic condition. Danger zone is between 4°C and60°C. Avoid possible recontamination during post cook handling. Therefore, it is important to improve consumer education regarding food safety practices during the purchase, transport, storage, and handling of food.

References

1. Sichewo PR, Gono RK, Muzvondiwa JV, Sizanobuhle N. 2013. Isolation


28. Harydi AM. 2010. Quality evaluation of the imported frozen fish’s consignment. For Master Degree. Food Hygiene & Control. Faculty of Veterinary Medicine, Suez Canal University
epidemiology of \textit{Yersinia enterocolitica} infections,” FEMS Immunology and Medical Microbiology, 47:315–329
37. El-Kelany KGH. 2014. Microbiological Quality of Imported Fish. For PhD of veterinary sciences. Food Hygiene and Control. Faculty of Veterinary Medicine, Alexandria University
40. Abd-El-Ghaflar AH. 2013. Quality Assurance of Imported Fish. PhD of Veterinary Science. Food Hygiene and Control Department. Faculty of Veterinary Medicine, Alexandria University

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