Occurrence of Escherichia coli O157 in Some Marine Fishes and Shellfish Sold in Isfahan markets, Iran

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EScherichia coli O157 is a food-borne pathogen that attributed to the contamination of some sea foods including fish and shellfish. This study was aimed to identification of Escherichia coli in some marine fish and shellfish such as, lobster, crab and shrimp, and to investigate the occurrence of Escherichia coli O157 as a food born bacterial pathogen. The study was carried out in Isfahan markets (Iran) during spring and summer 2011. A total of 190 samples (90 fish, 40 lobster, 30 shrimp and 30 crabs) were collected, Samples were randomly obtained from the fish markets and examined based on their growth characteristics on specific culture media and molecular (PCR) tests. Among all samples, 49 cases (25.78%) contaminated by E.coli, that among these isolates two cases were distinguished as an E.coli O157. In these samples E.coli O157:H7 was not identified. The highest and lowest contamination rate was observed among fishes and crab with 34.44 and 10 percents, respectively. Also the contamination rates of E.coli in different seasons were 15.78 and 10 percent in spring and summer, respectively.

It can be concluded that marine fish and shellfish can carry some pathogenic bacteria as E.coli O:157 that have risks for human health.

Key Words: Escherichia coli O157, fish, lobster, crab, shrimp.
parahaemolyticus, and Vibrio vulnificus were isolated from different organs of fishes (Ristori et al., 2007). It was found that the hygienic quality and freshness of fish and shellfish decreased in summer, especially for clam and mussel (Hwang et al., 2004). The microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform were Escherichia coli is the most common contaminant and is often encountered in high numbers (Thampuran et al., 2005). Bacterial microbiota associated with fresh raw shrimp was Aeromonas, Pseudomonas, Vibrio, Flavobacterium and Serratia (Jeyasekaran et al., 2006). Heinitz et al. (2000) found that 10% of imported and 2.8% of domestic raw seafood was positive for Salmonella. Also Enterococcus sp, Aeromonas sp, fecal and total coliform, the presence of Listeria sp and Salmonella spp from the external surface of tilapias were shown by Morales et al. (2004). The hazards and challenges associated with handling fish during farming and capture and the environmental contaminants in seafood may pose a risk to human health (Hästein et al., 2006). In the most cases microbiological changes occur when shrimps are insufficiently iced and improperly stored at elevated temperatures (Reilly et al., 1986).

The present purpose is to investigate the occurrence of any human bacterial pathogens in the marine fishes and shellfish taken for examination and also exploring the possible route of entry into food chain through the sea foods. Thus this study was designed to detect E. coli O157 in fish and shellfish, and to characterize these isolates by means of bacteriology, biochemical and PCR tests.

MATERIAL AND METHODS

Study area

Fish and shellfish were collected randomly from Isfahan fish markets during the spring and summer 2011. A total of 190 samples (90 fishes, 30 shrimps, 40 lobsters and 30 crabs) were collected. The species of the fish and shellfish included Tigertooth croake (Otolithes ruber), mackerel (Scomberomorus commerson), white shrimp (Panaeus indicus), lobster (Panulirus penicillatus), Black pomfret (Parapristipoma niger) and crab (Nurisia plicata). Specimens placed on sterilized bags and, under standard condition, transported to the laboratory on ice within one hour.

Sampling and Isolation of E. coli

To examine the bacterial organisms in the samples, the methods of culture and plating as described by Venkataraman and Sreenivasan (1952) were followed. The muscles of fish and shellfish were dissected and processed as described by Hossain (2008). Also Enterococcus sp, Aeromonas sp, fecal and total coliform, the presence of Listeria sp and Salmonella spp from the external surface of tilapias were shown by Morales et al. (2004). The hazards and challenges associated with handling fish during farming and capture and the environmental contaminants in seafood may pose a risk to human health (Hästein et al., 2006). In the most cases microbiological changes occur when shrimps are insufficiently iced and improperly stored at elevated temperatures (Reilly et al., 1986).

PCR for detection of E. Coli O157:H7

DNA extraction

For each sample, DNA was extracted from enrichment broths before or after an 8-0.22 μm filtration step. One milliliter of the 20-24 h-sample broth was centrifuged at 12000 g for 3 min. The pellet was washed twice with PBS with Tween 20 (2%) and three times in PBS alone. After centrifugation, 100 μl or 400 μl of InstaGene Matrix (Bio-Rad, Hercules, USA) were added to the pellet from filtered broths or not, respectively. The mixture was incubated at 56°C for 30 min, vortexed for 15s and then incubated at 100°C for 15 min. After incubation, these suspensions were vortexed for 15s, centrifuged at 12 000 g for 10 min and the supernatant was conserved at −80°C for PCR analysis.

Primers

The oligonucleotide primers that specifically amplified 259bp fragments (for O157) and 625-bp fragments (for O157:H7) were used for
PCR amplification, based on method of Gannon et al. (1997); Paton and Paton (1998). Target primers for amplifying segments of genes are listed in Table 1.

**PCR amplification test**

The amplification reactions were performed in 50 µl reaction mixtures containing 0.1 mM of each deoxynucleotide, 15 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH = 9), 2 mM MgCl2, 10% dimethyl sulfoxide (DMSO, Sigma), 1.5 U of Taq DNA polymerase (Sigma) and 40 ng of template DNA. The PCR reaction was carried out in a PCR programmed thermocycler (Eppendorf, Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany Co.) using the thermal profiles: initial cycle 94°C for 5 min, followed by a further 35 cycles: denaturation at 94°C for 60 s; annealing at 58°C for 60 s, and extension by polymerase at 72°C for 60 s. A final extension step of 5 min at 72°C was performed. The negative control (no template DNA) was distilled water. The PCR products were detected by electrophoresis of 20 µL of each amplification mixture in 2% agarose gel in 1% Tris-acetate-EDTA buffer, after which the gel was stained with ethidium bromide (0.5 µg.mL-1).

**Statistical analysis**

Data were analyzed to determine if there was a relationship between type of specimen and presence of *E. coli* and *E. coli* O157.

**RESULTS**

In the present study 190 samples of fish and shellfish were evaluated for detection of *E. coli* O157H:7. Contamination rates of *E. coli* and *E. coli* O157 in the evaluated specimens are shown in Table 2. Our results indicated that 49 samples (25.78%) and 2 samples (4.08%) contaminated to *E. coli* and *E. coli* O157, respectively. From bacteriological, biochemical and molecular tests that were performed on the all samples, two cases were identified as *E. coli* O157. Out of 190 samples no

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**Table 1. Oligonucleotide primers sequences used for PCR amplification**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5-3)</th>
<th>Fragment size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fliCh7</td>
<td>H7-F</td>
<td>GCGCCTGTTCAGTCTATCGAGC</td>
<td>625</td>
<td>Gannon et al (1997)</td>
</tr>
<tr>
<td></td>
<td>H7-R</td>
<td>CAACGCTGACTTTATCGCCATTCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rfb O157 - R</td>
<td>TTGCTATGTACAGCTAATCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Occurrence of *E. coli* and *E. coli* O157 in the different specimens**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample number</th>
<th><em>E. coli</em> Contaminated</th>
<th><em>E. coli</em> Non contaminated</th>
<th><em>E. coli</em> O157</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>90</td>
<td>31 (34.44%)</td>
<td>57 (63.33%)</td>
<td>2</td>
</tr>
<tr>
<td>Lobster</td>
<td>40</td>
<td>7 (17.50%)</td>
<td>33 (82.50%)</td>
<td>-</td>
</tr>
<tr>
<td>Shrimp</td>
<td>30</td>
<td>8 (26.66%)</td>
<td>22 (73.33%)</td>
<td>-</td>
</tr>
<tr>
<td>Crab</td>
<td>30</td>
<td>3 (10.00%)</td>
<td>27 (90.00%)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>190</td>
<td>49 (25.78%)</td>
<td>139 (74.21%)</td>
<td>2 (4.08%)</td>
</tr>
</tbody>
</table>

**Table 3. Distribution of *E. coli* in the different seasons**

<table>
<thead>
<tr>
<th>Season</th>
<th>Sample</th>
<th>Fish(90)</th>
<th>Lobster(40)</th>
<th>Shrimp(30)</th>
<th>Crab(30)</th>
<th>Total(190)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td></td>
<td>20(22.22%)</td>
<td>4(10.00%)</td>
<td>5(16.66%)</td>
<td>1(3.33%)</td>
<td>30(15.78%)</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td>11(12.22%)</td>
<td>3(7.50%)</td>
<td>3(10.00%)</td>
<td>2(6.66%)</td>
<td>19(10.00%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31(34.44%)</td>
<td>7(17.50%)</td>
<td>8(26.66%)</td>
<td>3(10.00%)</td>
<td>49(25.78%)</td>
</tr>
</tbody>
</table>
E. coli O157:H7 were detected. The highest and lowest contamination of E. coli were seen in fish and crab with 34.44 and 10.00 percent, respectively. The contamination rates of E. coli in the different seasons were shown in Table 3. There were the highest and lowest contamination rates in spring and summer with 15.78 and 10.00 percent, respectively.

**DISCUSSION**

Fishery products, which are of great importance for human nutrition worldwide and provide clear health benefits can act as a source of food borne pathogens and may be a potential source of infection (Kromhout et al., 1985). Bacterial flora of marine fish, sediments and sea water has been studied world over with a view to explain the spoilage of fish (Yagoub, 2009). Among all samples taken from Isfahan markets, 49(27.2%) was contaminated to E. coli which is important because of role of its pathogenesis in human. But in the positive samples of E. coli only two cases were detected as E. coli O157. With considering to table -2 the highest prevalence of E. coli with 31(34.4 %) were seen in fish and the lowest in crab with 3(10%). It should be noted that because of low catch rates of the samples especially lobster and crab, the sample size in the present study wasn’t the same. In the present study E. Coli O157 wasn’t isolated from crab, but Matulkova et al (2012) reported crab meat as a new possible vehicle of E. Coli O157 infection. Guyon et al (2000) from 150 oysters that tested for faecal coliform bacteria, Salmonella, E. coli and E. coli O157, recovered one strain of E. coli O157 from one sample, which in this strain’s stx genes were detected. Fish and shellfish can function as carriers of several microbial and other health hazards. Therefore maintenance of quality is of utmost importance in production and trade of fishery products. The first isolation of Shiga toxin-producing E. coli (STEC) stx1d strains in shellfish were collected from French coastal environments (Gourmelon et al., 2006). This study showed that raw fish and shellfish sold in fish market in Isfahan state could be a source of food- borne bacterial pathogens. Improvements in handling and processing are needed to minimize the prevalence of the pathogenic bacteria. Presence of E. coli in these seafood strongly suggests the urgent need to improve the quality control systems. The results may be considered as additional knowledge to enhance proper controlling of the storage life of fish and shellfish, and its products. The potential of seafood to harbor microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Wright et al., 2004). E. coli levels correlated very strongly with fecal coliform levels in both fresh and stored oysters and clams, suggesting that there is no advantage in replacing fecal coliforms with E. coli as an indicator of shellfish quality. In comparison between different food borne pathogens include Vibrio cholerae, Salmonella, Ecoli and Listeria monocytogenes, Significant difference (p <0.05) was observed in E. coli of the shrimps collected from different seafood processing plants at different months (Antony et al., 2012).

Prevalence of E. Coli during the summer is lower than spring (Table 3). These results are different from other studies, but they can vary depending on the season. This is agree with Iyer and Joseph (1995), they have also stated that seasons play a role in controlling the bacterial quality of fresh shrimps and observed that the bacterial counts were higher in certain specific seasons. They have recorded a high incidence of E. coli in raw shrimps during rainy seasons, which is probably because of the high degree of fecal pollution of water during that period.

Culture of shrimp is one of the fastest growing industries in Iran in the recent years, and the majority of water resources in southern region of Iran have been allocated to shrimp farming. Therefore we should pay much attention to its health management. Immediately after the procurement, there is a significant amount of data on the microbiology of sea food produced or imported in different countries. Therefore maintenance of quality is of utmost importance in production and trade of fishery products. Most of current quality control techniques are time consuming and cumbersome (Yagoub, 2009). Both bacteria and fungi are common flora of frozen fish and fish related products during packaging. The frozen fish samples were heavily contaminated which may be as a result of poor sanitary practices employed by the vendors (Adedapo et al., 2012). Recovery of typical E. coli O157:H7 was done from fish or shellfish in India, it was proved that strict
adherence to hygienic handling methods and proper cooking or processing is needed before consumption of these products. Human infections caused by pathogens transmitted from fish are quite common. The isolation of enteric pathogenic bacteria from fish that might be transmitted to humans after the handling or consumption of fish was studied in Nile tilapia and 39.5% were Shigella sp.; 11.1% were Salmonella typhi; 25.4% were Escherichia coli. Ten fishes collected from open-air markets revealed E. coli (50%) and S. typhi (20%) (Onyango et al., 2009). The multiplex PCR was compared for simultaneous detection of E.coliO157:H7 Salmonella spp and Listeria monocytogenes in some seafood from enrichment cultures of various types of artificially inoculated and naturally contaminated foods. This assay was a valuable method for simultaneous rapid screening for the three pathogens in food, even after frozen storage. Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Yagoub, 2009).

Results of the present study showed that marine aquatic animal, including fish and shellfish can carry bacteria which are dangerous for human health. According to the molecular detection of E. coli O157 by PCR in E. coli isolates, paying much attention to manufacturing, distribution and packaging of these raw seafood is of great importance.

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REFERENCES


