Incidence of *Aeromonas hydrophila* and *Plesiomonas shigelloides* in Seafoods

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Received 25 January 2017
Accepted 12 April 2017

**Abstract**

The present study was conducted to investigate the incidence of the pathogens *Aeromonas hydrophila* and *Plesiomonas shigelloides* in 700 seafoods (400 raw fish, 100 raw shrimps and 200 raw mollusks) collected from retailers. Isolations were performed by conventional culture methods. The isolates were also confirmed by polymerase chain reaction (PCR) assays. *A. hydrophila* and *P. shigelloides* were detected in 5.71% and 0.86% seafood samples, respectively. The highest rate of *A. hydrophila* (15%) was found in shrimp samples. *P. shigelloides* were only isolated from fish samples. The study is showed that effective methods to eliminate the *Aeromonas* and *Plesiomonas* species are needed.

**Keywords:** Fish, mollusk, pathogen, shrimp.

**Introduction**

The most of dietary guidelines recommend eating fish and other seafood, because of the positive health effects related to consumption (Silverstein, 2014). Seafood products which refers not only fish but also of shellfish, which includes crustacea (shrimp) and mollusks (mussel and calamari), contribute significantly to human food consumption (Venugopal, 2006). Seafood are highly perishable products that the growth of pathogenic bacteria is a potential source of risks for human health. *A. hydrophila* and *P. shigelloides* are considered as the main threats to the food safety (Shahidi, Jones, & Kitts 1997).

*A. hydrophila* is an aquatic pathogen implicated in foodborne bacterial outbreaks that include bacteremia, meningitis, septicemia, pulmonary, and wound infections (Daskalov, 2006). It is a psychrotroph, gram negative, facultative anaerobic, rod shaped bacteria of *Aeromonadaceae* family that express a range of virulence factors under refrigerated storage conditions. These characteristics make *A. hydrophila* difficult to control. Also, *Aeromonas* species secretes extracellular proteins known as virulence factors that cause disease in human and fishes (Robinson, Batt, & Patel, 2000).

*P. shigelloides* is gram negative, facultative anaerobic, rod shaped bacteria of *Enterobacteriaceae* family. The primary habitats of *P. shigelloides* are fresh-water ecosystems (rivers, lakes, and surface waters) and marine estuaries in tropical and temperate climates (Levin, 2008). Symptoms associated with gastroenteritis caused by *P. shigelloides* are diarrhea, abdominal pain, nausea, chills, headache, fever and vomiting (Hui, Kitts, & Stanfield, 2001).

There have been some reports on the incidence of *A. hydrophila* in Switzerland, in Taiwan, in Malaysia, in Italy, in India, in China; and *P. shigelloides* in Japan, USA, in Czech Republic, Spain, in Greece (Arai ., 1980; Tsai & Chen, 1996; Bardon, 1999; Gonzalez, Lopez-Diaz, Prieto, & Otero 1999; Radu, Ahmad, Ling, & Reezal 2003; Ottaviani et al., 2006; Papadopoulou et al., 2007; Nagar, Shashidhar, & Bandekar 2011; Shao-wu, Di, Hong-bai, & Tong-yan 2013). In Turkey the incidence of these pathogens in seafood products have not been extensively investigated.

Turkey has a favorable potential in terms of production and consumption of marine products due to surrounded by sea on three sides. Seafood consumption has been increasing in Turkey that ranks (8.2 kg per capita per year) in the 7th place among European countries (Can, Günlü, & Can 2015). The present study was undertaken to determine the presence of *A. hydrophila* and *P. shigelloides* in seafood obtained from retail markets in Istanbul which has approximately an area of 5.343 km² with a population of 14,377,018 (18.66 % of the country).
Materials and Methods

A total of 700 seafoods (400 raw fish, 100 raw shrimps and 200 raw mollusks) were collected from retailers in Istanbul. Raw fish samples consist gilt-head sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax) farmed in Muğla province, salmon (Salmo salar) imported from Norway, bluefish (Pomatomus saltatrix) and Mediterranean horse mackerel (Trachurus mediterraneus) caught in the Marmara sea in 2015. All samples were kept in sterile polyethylene bags and immediately transferred to the laboratory in cold boxes.

Conventional culture-based study of samples was performed. 25 g was taken from each sample and was transferred into 225 ml of alkaline peptone water (APW), and then a pre-enrichment process was performed. The enriched cultures were streaked on Aeromonas Starch DNA Agar Base (HiMedia Laboratories Ltd, Mumbai, India) plates for recovery of Aeromonas species and on Mac Conkey agar, Salmonella – Shigella (SS) agar (HiMedia Laboratories Ltd, Mumbai, India) plates for recovery of P. shigelloides. The plates were incubated at 37 °C for 24 h. After incubation, growing colonies of selective media were followed by the streaking method for isolation of pure cultures. The isolates were identified morphologically (Gram stain, motility test and colony morphology) and biochemically using API 20E system (bioMérieux UK Ltd., Basingstoke, UK) (Jeppesen, 1995; Vitovec, Aldova, Vladik, & Krovacek, 2001).

The DNAs of all the isolates was extracted by Roche High Pure PCR Template Preparation Kit (Roche, France), according to the manufacturer's instructions. All the isolates were confirmed as A. hydrophila and as P. shigelloides by specific PCR assays (Gonzalez-Rey et al., 2000; Yogananth, Bhakyaraj, Chanthuru, Anbalagan, & Nila, 2009). Negative (double-distilled water) and positive (A. hydrophila ATCC 23213, P. shigelloides, ATCC 51903) controls were included in the PCR procedure.

Results

A. hydrophila and P. shigelloides were detected in 5.71% and 0.86% seafood samples, respectively (Table 1).

Discussion

The incidence of A. hydrophila in fish samples investigated in this study was lower in comparison to those detected in U.S. by Wang & Silva (1999) (36.1%), in U.K. by Fricker & Tompsett (1989) (19.0%), in New Zealand Hudson et al. (1992) (28.0%), and in Turkey by Saglam, Isik, Arslan, & Hudaverdi. (2006) (35.0%). On the other hand, Castro-Escarpulli et al. (2003) reported the incidence rate of A. hydrophila was 2.6% in 250 fish samples. In another study, Nagar et al. (2011) stated the presence of A. hydrophila in 3.85% of 52 freshwater fish samples. In the current study, the incidence of A. hydrophila in fish was 3.75%. Our findings showed similarity with the results of Castro-Escarpulli et al. (2003) and Nagar et al. (2011). The differences from the studies with higher rates may be originated from research seasons, detection methods, sampling procedures and the sanitation applications.

In the present study, A. hydrophila was detected in 15.0% of shrimp samples. This result was in acceptance to the findings reported by Tsai & Chen (1996), Hanninen, Oivanen, & Hirvella-Koski (1997) and Vivekanandan, Savithamani, Hatha, & Lakshmanaperumalsamy (2002). Higher results were found by Thayumanavan, Vivekanandan, Savithamani, Subahl Kumar, & Lakshmanaperumalsamy (2003) in South India and Colakoglu, Sarmask, & Koseoglu (2006) in Turkey at rates of 35.6% and 29.1% in shrimp samples, respectively. The incidence was related with different hygiene applications and poor manufacturing processes.

The present study demonstrated that A. hydrophila was isolated from 5.0% of mollusks. Regarding the contamination rate, our result was slightly similar to the study obtained by Mus and Cetinkaya (2013) in Turkey (8.3%). Contrary to this, the studies which had higher results (78.0%, 50.0% and 15.27%) than ours were reported by Colburn et al. (1989), Tsai and Chen (1996), and Ortaviani et al. (2006) respectively. The reason for high contamination rate could be due to the geographical conditions such as grown in a waterfront, climate conditions etc..

Fish samples have been examined in several countries for the presence of P. shigelloides. In Japan (Ara et al., 1980), 10.2% of fish samples and in Zaire (Van Damme & Vandepitte, 1980) 59.0% of fish samples were reported to be contaminated with P. shigelloides. In this study, P. shigelloides was detected in 1.5% of fish samples. According to the results, no P. shigelloides was isolated in shrimp and mollusk samples. Similar results in all seafood samples were reported by Papadopoulou et al. (2007). In contrary, Miller et al. (1986) demonstrated that P. shigelloides was isolated from 8.70% of 46 mussel samples. Differences between the findings obtained from several studies can be related to the contaminations during catching and transportation, preservation conditions and inadequately personal hygiene.

The result of this study confirmed that fish and seafood products collected from sales points in Istanbul may be contaminated with Aeromonas and
Plesiomonas species which can cause public health problems. In Turkey, fish is usually eaten after being cooked. However, the trend of consuming ready to eat raw foods is getting popular. Therefore, it is essential to ensure improving the quality of process from fishing to retail outlet and developing the sanitation conditions of food contact surfaces and handling areas. Because, improper handling and cross-contamination might pose a health hazard, especially to susceptible populations such as the immunosuppressed, children and elderly people. El-Shafai et al. (2004) stated that the major public health concern could be the risk of P. shigelloides entering the wound of people who handled and processed the infected fish. Daskalov (2006) recommended that Aeromonas species can be eliminated by hurdle technology (pH, NaCl, temperature, NaNO₂), smoking, modified atmosphere packaging, heating or cooking and plant extracts (eugenol and pimento extracts). Also, Papadopoulou et al. (2007) reported that Aeromonas and Plesiomonas species are not resistant to food processing regimes and is readily killed by heat treatment.

In addition, food safety training should be provided for all staff to increase the level of awareness and the sense of responsibility regarding food hygiene including handling, storage, transport and the sale points hygiene as well as the prevention of marine pollution. Further studies should be performed to find out the most attractive method to eliminate the Aeromonas and Plesiomonas species.

<table>
<thead>
<tr>
<th>Products</th>
<th>Number of Samples</th>
<th>A. hydrophila</th>
<th>P. shigelloides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>400</td>
<td>15 (3.75%)</td>
<td>6 (1.5%)</td>
</tr>
<tr>
<td>Shrimp</td>
<td>100</td>
<td>15 (15.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Mollusk</td>
<td>200</td>
<td>10 (5.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>700</td>
<td>40 (5.71%)</td>
<td>6 (0.86%)</td>
</tr>
</tbody>
</table>


Gonzalez, C. J., Lopez-Diaz, T. M., Prieto, M., & Otero, A. (1999). Bacterial microflora of wild brown trout (Salmo trutta), wild pike (Esox lucius), and aquacultured rainbow trout (Oncorhynchus mykiss). J Food Protect., 62(11), 1270-1277.


