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SHORT PAPER

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

Beren Basaran Kahraman[1], Emek Dumen[2], Ghassan Issa[3], Tolga Kahraman[2,*], Serkan Ikiz[1]

[1] Istanbul University, Department of Microbiology, Faculty of Veterinary Medicine, Avcılar, 34320, Istanbul, Turkey.
[2] Istanbul University, Department of Food Hygiene and Technology, Faculty of Veterinary, Medicine, Avcılar, 34320, Istanbul, Turkey.
[3] Avrupa Vocational School, Culinary Program, Zeytinburnu, 34020, Istanbul, Turkey

* Corresponding Author: Tel.: +90 212 473 7070; Fax: +90 212 473 7241;
E-mail: tolgakah@istanbul.edu.tr

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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, in 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 km^2 with a population of 14.377,018 (18.66 % of the country)

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and 434 km total shoreline.

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, Isık, 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, & Hirvela-Koski (1997) and Vivekanandhan, Savithamani, Hatha, & Lakshmanaperumalsamy (2002). Higher results were found by Thayumanavan, Vivekanandhan, Savithamani, Subahkumar, & Lakshmanaperumalsamy (2003) in South India and Colakoglu, Sarmasik, & 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 Ottaviani *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 (Arai *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

Table 1. Incidence of *A. hydrophila* and *P. shigelloides* in various seafoods

Products	Number of Samples	*A. hydrophila*	*P. shigelloides*
Fish	400	15 (3.75%)	6 (1.5%)
Shrimp	100	15 (15.0%)	0
Mollusk	200	10 (5.0%)	0
Total	700	40 (5.71%)	6 (0.86%)

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, $NaNO_2$), 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.

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