Isolation of some human pathogens from fresh and smoked shad (*Alosa kessleri*) and silver carp (*Hypophthalmichthys molitrix*)

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The consumption of sea-foods especially different kinds of fish has been increased in many countries such Iran in recent years (Akhonzade Basti et al 2006, Feldhusen, 2004). However, there is substantial evidence that fish and seafood are high on the list of foods associated with outbreaks of food borne diseases (Huss, 2003). Safety of fish products and their quality assurance is one of the main problems of food industry today. The presence or absence of foodborne pathogens in a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment (FDA, 2001; Huss, 2003). The handling of fish products during process involves a risk of contamination by pathogenic bacteria such as *V.parahaemolyticus* and *S. aureus*, causing foodborne human intoxication **(**Shena et al., 2007**);** These bacteria are salt-tolerant and therefore can contaminate all cured preparations such as cold smoked fish, caviar and fish-based preserves**.** *Staphylococcus* is not found in the normal microflora of fish. This microorganism could be associated with salt (Herrero et al., 2003, Hansen et al., 1995) or the raw fish (Ferreira et al., 2007)**.** According to Basti et al, (2006) some kinds of salt smoked fish may be considered as risk of *E.coli* and *S. aureus* infection and intoxication for Iranian consumers, respectively. An assessment on the potential microbiological hazard associated with smoked fish fillets under refrigerated storage was made. *S. aureus* survived better at both storage temperatures (T= -100°C - 50°C) (Mariappan et al., 2010). According to some studies contamination of fish by pathogens particularly such as *S.aureus, E. coli, V. parahaemolyticus* and *Listeria monocytogenes*, may occur prior to harvest (Enclund, 2004). Fish and seafood may also be a vector for many bacterial human pathogens. In assessing the risks from fish, it is important to have information on the incidence of these pathogens (Davis et al., 2003, Hosseini et al., 2004, Khodaeeyan, 2008). Therefore the microbial status of seafood after being caught and during the rest of the processing is closely related to environmental conditions and the microbiological quality of the water. Water temperature, salt content, distance between localization of caught and polluted areas (containing human and animal feces), natural occurrence of bacteria in the water, ingestion of food by fish, methods of catching and chilling, and post- harvest handling or processing conditions are some of the important factors associated with pathogenic bacteria and cause of food born diseases (Feldhusen, 2004, Khodaeeyan, 2008).

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Smoked salted fish is one of the seafood products which its consumption has been increased in Iran and Northern people of Iran (Gilan and Mazandaran provinces) are interested in its consumption (Nikpay 2004). These products are prepared by traditional and inhealth methods and are also consumed under cooked temperature, therefore consumption of these products could lead to food infection or food intoxication such as botulism and other foodborne diseases (shena et al., 2007). There aren't any similar studies on these fish from Iran or other countries. The aim of this study is a survey of three bacterial human pathogens (*S.aureus, V.parahaemolyticus, E.coli*) in fresh (non-smoked) and smoked fish (shad and silver carp).

In this cross-sectional study totally one-hundred samples of fish including 50 wild caught Caspian anadroum shad and 50 cultivated silver carp were taken aseptically from the Caspian Sea and a fish farm. All samples of fish were collected and placed in sterile polyethylene bags, transported to the laboratory and analyzed immediately upon arrival.

Twenty five of each fish species were eviscerated and traditionally salted using 30-35 kg salt per 100 kg fish sample in a watertight container. The samples were then smoked using the routine cold smoking method at a temperature not exceeding 32°C for one week. The process was undertaken according to the practices used in a commercial processing facility in Mazandaran province.

At the end of the smoking process both non-smoked and smoked fish were tested for three human pathogenic bacteria including *S. aureus, V. parahaemolyticus* and *E.coli* using the American Public Health Association (Vanderzant and Splittstoesser, 2008) and the U.S Food and Drug Administration (FDA, 2001) methods. *S. aureus* was isolated by enrichment of 1g sample in 10 ml cooked meat media (Difco) plus 9% NaCl (W/V) and streaking a loopful of the 24 h enrichment culture on Baird-parker agar (Merck) containing egg yolk agar and potassium tellurite (Merck). The subsequent confirmatory test was followed by a coagulase test of lipase-positive jet-black colonies (Horwitz and George, 2010). The APHA method for isolation of *V. parahaemolyticus* was as followed by adding 25g of the homogenized sample in 225 ml alkaline peptone water (APW, Quelab) using a stomacher and incubated at 35°C for 6-8 h. A loopful of APW was then streaked onto thiosulfate citrate bile salt sucrose agar (TCBS, Merck). The plates were then incubated at 35°C for 24 h and confirmation tests were then made by observation of the blue-green colonies tested for acid production from cellobiose, sucrose, maltose, mannitol, trehalose and lactose, Gram staining, motility, Vogues- proskauer, oxidase, nitrite, growth at 43°C and in 6 and 8% NaCl (Vanderzant and Splittstoesser, 2008). Standard methods were used for detection of *E. coli* using a violet red bile agar (VRBA, Merck). To confirm the suspected coliform colonies ten of the purple-red colonies, 0.5 mm in diameter or larger, surrounded by a zone of precipitated bile acids on VRBA were transferred to tubes of brilliant-green lactose bile broth (Merck) at 35°C for 24-48 h. The confirmed coliform colonies were also assessed for detection of *E. Coli* using eosin-methylene-blue lactose and serological tests. The pour plate and surface plate counts were used for coliform and *S. aureus* counting, respectively(HorwitzandGeorge, 010, Vanderzant and Splittstoesser, 2008). After data collection, results were analyzed using SPSS and Fisher statistical test.

The results of this study have been shown in table 1 and figure 1. Based on results *S. aureus* was isolated from 4 samples (8%) of non-smoked fish including 3 silver carp and 1 shad. Also, *S. aureus* was isolated from 10 (20%) smoked samples including 6 silver carp and 4 shad samples. The analysis of data by

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Fisher statistical test, showed that there was a significant difference between smoked *silver carp* and non-smoked *shad* (P= 0.04) but no significant difference between other species.

V. parahaemolyticus was isolated from 3 samples (6%) of non-smoked samples including 1 silver carp and 2 shad. The bacterium was also recovered from 7 samples (14%) of smoked samples including 4 silver carp and 3 shad. No significant difference was found between smoked and non-smoked *shad* and *silver carp* samples (P> 0.04). Coliform bacteria were isolated from 2 samples (12%) of non-smoked samples including 2 silver carp and 1 shad. However, no coliform bacteria were recovered from the smoked samples (Table 1). Coliforms especially *E. coli* from salty smoked fish.

Table 1: Contamination rate of examined fish species to three bacterial pathogens (positive cases and percent)

Type of sample	N. of Samples	*S. aureus* (%)	*V. parahaemoliticus* (%)	*E. coli (%)*
Fresh silver carp	25	3(12)	1(4)	2(8)
Smoked silver carp	25	6(24)	4(16)	0
Fresh shad	25	1(4)	2(8)	1(4)
Smoked shad	25	4(16)	3(12)	0
Total	100	14(14)	10(10)	3(3)

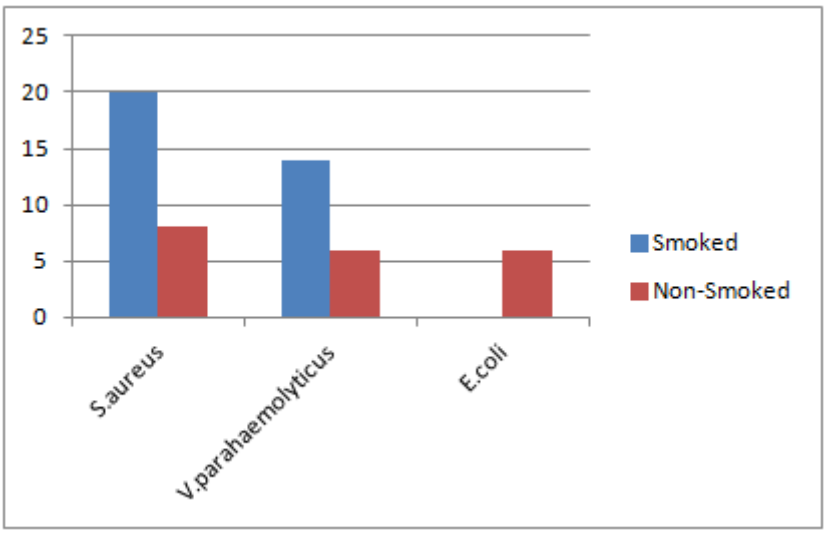


Figure 1: Comparison of contamination smoked and non-smoked fish to *S. aureus , V. parahaemoliticus and E. coli*

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Our study showed that *S. aureus* was the more frequent genus identified in salty cold- smoked fish, as the *S. aureus* does not appear as a part of the natural microflora of newly caught marine and cultivated fish (Herrero et al., 2003). *S.aureus* is one of the hallophilic bacteria, which can grow in 10-12% salt. For preparation of smoked fish in north of Iran 3-3.5% of salt is used, but it can't prevent the growth of S.aureus in this product. (Akhonzade Basti et al., 2006, Shena et al., 2007**)**.6

We assume that the isolated *S.aureus* were due to the contamination of fish during capture and subsequent unhygienic handling and processing (Shena et al., 2007). Isolated *S.aureus* in fishery products and fish processing factory workers. Small numbers of this bacterium in fishery products is not a serious problem but food poisoning may occur if the product is handled carelessly during processing, resulting in high multiplication ($>1\times10^5$cfu/g) (Varnam and Evans, 2001; Vishwanath, et al., 1998). In this study also a plate count above 1×10^3 cfu /g *S.aureus*, a maximum acceptable concentration rate of this bacterium for the smoked and salted fish according to ICMSF was obtained. Therefore, consumption of such products may cause a risk of *S.aureus* intoxication in consumers. As the *S. aureus* is an indicator of hygiene and sanitary conditions, the presence of this organism indicates the unhygienic condition during processing, storage etc. The contamination of the product could be due to a food poisoning. It is recommended to use sanitary gloves for handling ready-to-eat foods to reduce the problem of *S. aureus* contamination.

V. parahaemolyticus is an indigenous bacterium in the marine environment and can also grow in 1-8% salt (Khodaeeyan, 2008, Akhonzade Basti et al., 2006). Therefore, a very low level of *V. Parahaemolyticus* numbers was found for silver carp and this was due to the fish coming from the freshwater fish farms rather than from the Caspian Sea. Our study also indicates that the number of fish contaminated with *V. parahaemolyticus* after salting and cold-smoking process was higher than non-smoking fish, this may be due to the cross-contamination.

Certain strains of bacteria cause diarrhea and are responsible for many deaths each year. The presence of Coliforms especially *E. coli* in salty smoked fish was negative in this study. The absence of coliforms especially *E. coli* in smoked fish may be due to the high concentration levels of NaCl (>7%) in these products. Although some scholars believe that the smoked products have a longer shelf life than fresh products (Shiau, 2006, Serkan and Bekir, 2008) there are some considerable problems with smoked products.

In conclusion, the results of this study showed that the rate of contamination with both *S. aureus* and *V. parahaemolyticus* in smoked silver carp and shad is more frequent. Since these products are commonly consumed as raw or under semi-cooked conditions in Iran, therefore their consumption may pose a risk of food borne infection and intoxication in the consumers (Tavakoli and Riazipour, 2008, Shanley et al., 2009).

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