



Examine the contamination of *Epinephelus coioides* with *Listeria* and *Salmonella* bacteria in Khuzestan coastal zone

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ABSTRACT

Listeria and *Salmonella* species are high risk for human through contaminated foods; these bacteria are psychrophilic and could grow at low temperature even at refrigerator. Fish and shrimp and other aquatic organisms are very good resources for these kinds of bacteria. These bacteria are known as food-borne bacteria because infected of human by foods. These bacteria have been isolated from fresh and processing aquatic foods in the most countries. In this research, two different organs including intestines and gills from each fish were prepared at sterile condition for isolation of salmonella and *Listeria*. *Listeria* was indentified according to FDA and also salmonella was characterized according to impedance method. From total examined 60 fish, 18 fish was contaminated to salmonella and 3 fish was contaminated to *Listeria* respectively. The frequency of *Salmonella* and *Listeria* in Mahshahr fish was 0.30 and 0.05 and in Hendijan fish contamination with salmonella was 0.05 and contamination with *Listeria* was not reported. *S. paratyphi*, *S. typhimurium* B and *S. cholera-suis* C were identified in Mahshahr fish, but in Hendijan, fish was characterized *S. paratyphi* and *S. typhimurium* B. *L. murrayi* was only isolated specie from Mahshahr fish. These two pathogenic bacteria have contaminated Khuzestan coastal zone through human resources, animal waste, waste water and chemical industries. Therefore people receive these kind bacteria through consumption of aquatic contaminated marine foods.

Keywords: *Epinepheelus coioides*, *Listeria* bacterium, *Salmonella* bacterium, intestine, gill

Introduction

Microbes are responsible for wide section of the food poisoning. Most microbes were not considered an agent of the food poisoning in the past and recently they have been identified as Emerging Food borne Pathogens which their importance in food poisoning becomes greater (Mohammad Khani, 2007). In particular, some microbes are able to grow and survive inside the refrigerator and where there is no oxygen even a small number of microbes cause illness and this may be a serious alarm for the health of the consumers of foods. Microorganisms including bacteria are seen in aquatic ecosystems such as free and fresh waters and estuaries and the pathogens consume them as a host (Parihar et al, 2008). Bacteria are transmitted to humans through consumption of the contaminated food and contact with these creatures and cause to diseases, these diseases threaten public health and costs resulting from the treatment of the people is high. *Listeria* and salmonella are the most important marine food borne pathogenic bacteria gained the attention of the scientists due to their capability in transmitting and causing to illness and epidemics (Laciar et al, 2002, Pal et al, 2009). *Listeria* and salmonella are capable of

growth in a variety of foods. The temperature range for growth of *Salmonella* is 1-35 °C and for *Listeria* is 1-45 °C. Fish, shrimp and other aquatic foods are contaminated with these pathogens and can transfer bacteria and cause to disease (Huntsman et al, 1999, Musa et al, 2008). With regard to increasing population needs, especially in Third World countries and nutrition problems feeding, need to production of food, especially proteins is felt. In this regard, need to protein, particularly animal resource protein has led to increase the efficacy and prevention of wasting these resources particularly fish containing Omega 3 resources which meet significant part of human needs. Due to importance of these bacteria in human health they have been considered and research has carried out on isolation, pathogenesis, transmission and epidemic studies on different food resources including marine food (Akhondzadeh Basti, 2006). Investigations show that in our country these bacteria have been isolated from various foods such as fish and marine foods (Ebrahimiyan, 2006, Sershik Khabazi, 2006, Nikpaei, 2002). Mahshahr and Hendijan are the two poles of production and export of the aquatic foods in Khuzestan province and given that the sewage contamination in these two zones can prepare growing conditions for these bacteria, so study

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on contamination of bacteria such as Salmonella and Listeria could offer valuable information for researchers and individuals responsible for the health. Hamoure fish is one of the most important commercial fish in the Persian Gulf zone and due to being delicious and relatively easy fishing (Heinitz et al,2000) the bacteria contamination in this species is necessary and the results can be effective and beneficial.

2. Materials and Methods

The research was conducted for 5 months (April to September 2009) in the Mahshahr and Hendijan. The fish was caught directly from Hendijan and Khoriyat in Mahshahr randomly with hook and the prepared fish was placed in nylon bags and boxes including ice with a layer of fish and a layer of ice and transferred to the laboratory of Public Health in Faculty of Veterinary at Shahid Chamran University.

2.1. Culture of the species and isolation of Listeria and salmonella

At first the bags including the fish was opened and besides flame the gills and intestines were removed with forceps and separately crushed in the sterile mortar and for enrichment, 10 grams were separated and placed in the bottles containing 90 ml Listeria Enrichment Broth. The bottles were incubated for up to 48 hours at 35 ° C in the oven. Then, at 24 and 48 hours, the linear culture on the plates containing Palcam Listeria-Selective agar was begun and then the plates were placed in the oven at 35 ° C oven for 48 hours. Then the clones suspected for Listeria were identified by dark gray green or black. They were cultured on the agar BHI plate and incubated for 24 hours and the colonies were used for diagnosis and testing (14, 30).

2.2. Listerial confirmatory experiments

In these studies to ensure reliability as randomly, the colonies were tested for approval including following steps: Hot staining, catalase test, movement test at temperature 25 ° C, hemolysis, Sugar fermentation of mannitol and xylose, wet lam test. 10 g of the gills and intestines colonies were placed in 90 ml buffered peptone poured in the flask for 24 hours at temperature of 30 ° C and in the flasks passed through non-selective enrichment stage and 100 micro litter was incubated in the specific ducts prepared by impedance method containing 9.9 ml Bimedia 201 C and Bimedia A 205, and the Bactrace 4300 impedance analysis system was set at 24 hours which has an inverse ratio with electrical

conductivity and the resistance or impedance was measured at intervals of 10 minutes and finally, the positive specimens were identified. The maximum time was announced by the system during 24 hours (International Standard Of the thigh, No. 7727). Then the positive samples announced by the impedance system were cultured linearly and separately besides flames on the hood inside plates containing Salmonella agar (SS),(MA) and (BGA) and (XLD). The plates were placed in the oven after culturing for 24 hours at 37 ° C, and in some cases, the time of placement in oven increased 37 ° C for up to 48 hours. Finally, the results colonies were used for diagnosis and confirmation (Iran Industrial Research Standards Institute, 2005).

2.3. Confirmatory experiments of Salmonella

Test TSI, Urease, nodule test, Simon citrate test, red methyl, D-lysine carboxylic test and serologic test were carried out which determine existence of Salmonella and used to identify the group and types of the bacteria. Specimens of gills and intestines with presence of Salmonella bacteria in Mahshahr and Hendijan were transferred to Tehran Veterinary Medicine for typing.

For statistical analysis, software Excel and the Chi-Square test (SPSS) was used for examining the relationship between the presence or absence of bacteria in Mahshahr and Hendijan following formulas were used:

$$\hat{p} \pm 1.96 \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

$$(\hat{p}_1 - \hat{p}_2) \pm 1.96 \sqrt{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}}$$

3. Results

In this research from both Mahshahr and Hendijan zones 30 Hamoure ordinary fish was taken as an example (Table 1). The amount of contamination in the gills and intestines to Listeria in Mahshahr fish was respectively of 10% and Zero and the amount of contamination of Salmonella was 23.3% and 26.7% respectively. Also the amount of Salmonella contamination in the gills and intestines in Hendijan fish was 6.7% and 3.3% and contamination with Listeria was not reported. To investigate the relationship between bacteria and infected organ, x test was done which assumption of independence was rejected and as a result there is a significant relationship between the two factors (P<0.05).

3.1.The relationship between the bacteria and infected organs

To investigate the relationship between bacteria and infected organ, x2 test was done and assumption of independence was rejected and as a result there is a significant relationship between the two factors (P<0.05) (table 2).

According to the formula 1, the amount of contamination in each type with two kinds of bacteria was obtained. In the case of Listeria bacteria (intestine and gills contamination) contamination was not observed that depicts the ratio of zero, and it can be concluded that be that the amount of these two types is approximately zero. According to the formula (Johnson,2005) the amount of contamination in t gills and intestines with salmonella and Listeria was calculated separately. The amount of contamination of gills with Salmonella was 13% and Listeria was 0.05. Therefore, the difference between specimens was 0.08 and confidence level was 95% and difference between the two ratios is 0.185 and -0.019. Therefore, it can be said that there is no significant difference between gill infection with Salmonella and Listeria and in the intestine, contamination with Salmonella (P) was 0.17 and confidence level of 95% (0.08 and 0.26) respectively. But there was no contamination with Listeria in intestine. Consequently, contamination of the intestine with Salmonella is greater. If the types of contamination are taken into account, as the observations divided into both infected and non-

infected, contamination with Salmonella (P) is 0.35 and for Listeria is 0.05. The difference between these two ratios is 0.30 and confidence level is 0.17 and 0.43 and in general and in error level 0.5, contamination of Salmonella has been greater. These differences were seen in the intestine depicting significant differences. To investigate the relationship between the region and bacteria, x 2 test was done and by this assumption the independence of these two factors was rejected. In other words, there is a relationship between the region and the type of bacteria (P<0.05).

3.2.Comparison of contamination of Salmonella and Listeria in Mahshahr and Hendijan

The amount of Salmonella contamination in Mahshahr was 0.30 and in Hendijan was 0.05. The difference between these two ratios was 0.25 and confidence level 95% for these ratios was 0.12 and 0.38. Therefore there is a significant difference between the two ratios and contamination of Salmonella was high in Mahshahr. The amount of contamination of Listeria in Mahshahr was 0.05 and zero in Hendijan. The difference between these two ratios was 0.05. But since confidence level for Mahshahr contamination contains 0- 0.05 therefore the contamination with Listeria had significant difference in the two regions and it cannot be concluded that contamination in Mahshahr was higher than Hendijan.

Table 1: percentage and number of data according to region, bacteria and infected organs

Region				infected organs				
				Healthy	Intestine	Gills	Both	Total
Mahshahr	Bacterium	Salmonella	No/ %	12 40%	8 7/26%	7 3/23%	3 10%	30 100%
		Listeria	No/ %	27 90%	0 0%	3 10%	0 0%	30 100%
	Total	No/ %	39 65%	8 3/13%	10 7/16%	3 5%	30 100%	
Hendijan	Bacterium	Salmonella	No/ %	27 90%	2 7/6%	1 3/3%	-	30 100%
		Listeria	No/ %	30 100%	0 0%	0 0%	-	30 100%
	Total	No/ %	57 95%	2 3/3%	1 7/1%	-	30 100%	



Table 2: relationship between type of bacterium and infected organ

Bacterium	Infection				Total
	Non-infected	Intestine	Gills	Both	
Expected number % Salmonella	39 48 65%	10 5 7/16%	8 5/5 3/13%	3 5/1 5%	60 60 100%
Expected number % Listeria	57 48 95%	0 5 0%	3 5/5 5%	0 5/1 5	60 60 100%
Expected number % total	96 96 80%	10 10 3/8%	11 11 2/9%	3 3 5/2	60 60 100%

Table 3: relationship between the region and bacteria

Bacterium	Bacterium contamination			Total
	Non-infected	Salmonella	Listeria	
Expected number % Salmonella	39 48 65%	18 5/10 30%	3 5/1 5%	60 60 100%
Expected number % Listeria	57 48 95%	3 5/10 5%	0 5/1 0%	60 60 100%
Expected number % total	96 96 80%	21 21 5/17%	3 3 5/2%	60 60 100%

4. Discussion

The amount of frequency (P) of Salmonella and Listeria bacteria in 60 fish was, 0.35, 0.05 and the percentage of Salmonella bacteria and Listeria was 35% and 5%. In America of 153 catfish, 84% of infections of Salmonella were reported (Flores-Abuxapqui et al,2003). Contamination with Listeria in salmon in Finland reported averagely 35-14% respectively (Flores-Abuxapqui et al,2003). In America the contamination of the catfish with salmonella in 2006 reported 1.5-4.5% (Gonzales et al,1993).

Akhundzade et al.(2006) reported contamination with Listeria and E.coli in *Liza aurtrata* about 10% in the Caspian Sea. Boari and Cleube (2008) in 20 tilapias reported contamination of Salmonella and Staphylococcus aureus bacteria. The amount of isolation of Listeria, Salmonella, E. coli and other bacteria from shrimps was 5-20% and in marine fish it was 4.12% and this amount in the frozen marine food reaches 27% (Byamukama et al,2000). So Hamoure fish from Mahshahr and Hendijan could be the host of

Salmonella and Listeria and transfer these two bacteria to consumers and cause to illness. Frequency of contamination in the gills of this fish with Salmonella bacteria was 0.13 and Listeria was 0.05, therefore there is no significant difference between them ($P > 0.05$) and it cannot be concluded that gills contamination with Salmonella is greater than Listeria. Intestine contamination with Salmonella was 0.17 but no Listeria was seen in the intestine. It can be said that intestine contamination with Salmonella was high and the host of Salmonella is in the intestines of animals, birds, reptiles and animals such as insects (Feldhusen, 2000). It is believed that internal tissues of the fish and its liver are sterile and it has been demonstrated that bacteria present in three locations of external section, the respiratory system (gills) and the intestine (Hitchener, 1982). The salmonella and Listeria bacteria in the intestines of the species (*Alosa kessleria*) were 2.6 and 27% (Antimicrobial-resistant Salmonella serovars isolation from imported foods). Ebrahimiyan (2006) reported the contamination of the different species in the intestines and gills by Listeria 28 and 2% while, this contamination in the intestine and gills of carp was 8% and 8%. The physiologic conditions of the fish intestine flora in selection of intestinal bacteria cause to possibility of bacteria causing pathogenesis with the origin of soil. Meanwhile, the fish contact time with contaminated waters containing these bacteria is important. It has been shown that the fish living long time in the water contaminated with bacteria have contaminated organs like the gills, skin, etc. which can be as the host for the bacteria (Baron, 1991). According to the results of the research, it can be said that the gills and intestine of this fish due to long time contact with the contaminated water could provide the conditions for the growth of the bacteria such as salmonella and Listeria and transmission of these bacteria to other parts of the body. The amount of Salmonella contamination in the gills and intestines in Mahshahr fish was 0.30 and in Hendijan was 0.05 respectively and there is a significant difference between the two ratios ($P < 0.05$). Contamination with Salmonella was high in Mahshahr, and the resources are withdrawal of sewage, agricultural waters, contamination caused by waste in the animals and livestock, and food products (Gonzales et al, 1993, Souza et al, 2008). High temperature and organic and nitrogen substances increase the

bacteria Salmonella in water (Gonzalez-Rodriguez et al, 2002). One of the other factors of the growth of Salmonella is transfer by the birds and poultries feeding marine food through the mouth (Vanderant et al, 1992). Non-biologic factors, ecological distribution and function of the affected communities are important. Flora of bacteria in different stations, amount of water leaving the organic and non-organic compounds, pH, turbidity, salinity and temperature depends on these factors which are under the influence of the low density of bacteria (15FAO, 1999). Ramteke (1995) studied the relationship between some of the physical and chemical factors with a number of parameters. Onofrey and Macleod investigated the influence of salinity on the growth of bacteria also pursuant to a general rule declared the bacteria need to at least the amount of sodium and chlorine respectively. Petrochemical industries and placing commercial port of Mahshahr near Khor Musa has converted it to one of very polluted and susceptible ecosystem. The resulting effluent from Petrochemical industries, the fuel from different vessels and discharge of organic materials and of the food and other materials have caused to a lot of damages. Most organic materials and waste resulting from food and entry to these areas likely increase bacteria growth of bacteria such as Salmonella and Listeria is in this area. Absence of petrochemical industries and low marine traffic in the coast of Hendijan has cause to less contamination of the region. It can be concluded that in Hendijan, low contamination is due to less contamination and contamination with Listeria in Mahshahr fish is 0.05 and it is zero in Hendijan and the difference is 0.05 and since confidence level in Mahshahr is zero it can be concluded that Listeria contamination is high in Mahshahr and according to the results in both Listeria contamination was low.

Autio and colleagues (1999) report Listeria contamination about 0-1% in the raw and fresh fish. Listeria bacterium is psychrophilic pathogen that lives in the hot waters and provides conditions for the growth of the bacteria. *L. monocytogenes* And *L. innocua* species can be an ecological factor for contamination (Berg et al, 1972, Parihar et al, 2008). It was reported that Listeria can be seen in the coastal waters as host (Davies et al, 2001, Huntsman et al, 1993). In examining the industry beaches, contamination with Listeria particularly dangerous type *L. monocytogenes* can be reported (Doris, 1995).

Salinity of the water is the cause of reduce the growth of the bacteria and cold (lower temperature) is the main cause to the growth of *Listeria*. Factors causing the contamination with *Listeria* include manner of fishing, placing the fish in the ice in the refrigerator which transfer one bacterium (Huntsman et al,1999). According to the findings low count of *Listeria* in Hamoure fish is due to placing Persian Gulf (Coast of Hendijan and Mahshahr) in the hot area and increase of the temperature of the water in most days of the year. Also, the geographical conditions and high salinity (ppt 40) cause to reduce of growth of this bacterium. The marine species are decomposed after death through different mechanisms although a reduction of quality directly is related to the nature of fish, season and fishing area and size and manipulation but preserving and transpiration methods are influential (Laciar ,2002, Souza et al,2008) . However, it cannot be said the time for bacteria to penetrate the skin and muscle but one of the possible duration is 3 to 4 days (2). In the fish that their inner organs have not been emptied pathogenic bacteria damage the intestinal wall and penetrate inside the belly and cause to contamination of the muscles and on the other hand, presence of bacteria that cause spoilage in the abdominal area and reduced storage period. Thus, cleaning of the fish and emptying stomachs is useful and essential (Flores-Abuxapqui et al,2003).

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