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Full Length Research Paper

Prevalence of *Listeria* spp. in fish obtained from Urmia fish markets

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The present study was conducted to determine the prevalence of *Listeria* spp. in fish obtained from Urmia fish markets. A number of 194 fish comprising *Oncorhynchus mykiss* (n=42), *Sander lucioperca* (n=38), *Cyprinus carpio* (n=30), *Hypophthalmiachthys molitrix* (n=48), *Abramis brama* (n=12), *Astacus leptodactylus* (n=12) and *Silurus glanis* (n=12) were obtained from different fish markets of Urmia from June 2009 to February 2010. *Listeria* isolation was performed in two stages including enrichment in cold and selective plating. After colony formation, in order to confirming the genus of the Listeria, a fragment of *Prs* gene using *Listeria* genus specific primers was amplified from isolated bacteria using polymerase chain reaction (PCR). Results revealed that *Listeria* was isolated from 24 fish (12.37%). The highest prevalence of *Listeria* was observed in both *A. brama* and *A. leptodactylus* with 25%, while the lowest prevalence of *Listeria* was seen in *S. lucioperca* (9.7%). From the total of 24 Listeria isolates, five isolates (21%) were confirmed to be *L. monocytogenes;* seven isolates were *L. ivonoi* (29%) while *L. Seeligeri* was not isolated from any examined fish. The study showed that *L. monocytogenes* and other *Listeria* species are common contaminant of fish obtained from Urmia fish markets, and this may pose serious public health implications.

Key words: *Listeria, Listeria monocytogenes*, fish, PCR, Urmia.

INTRODUCTION

Listeria monocytogenes is a Gram-positive, facultative intracellular bacterium that is distributed in a diversity of environments. Given its capacity to withstand a wide range of pH, osmolarity and temperature conditions (Liu et al., 2005; Sleator et al., 2003), which underline many food manufacturing processes, *L. monocytogenes* has emerged as a significant foodborne pathogen in recent decades, along with a trend toward increasing consumption of ready-to-eat and heat-and-eat convenient food products. *Listeria* spp. are widespread and commonly found in soil, sewage, dust and water. One particular *Listeria* specie *monocytogenes*, can cause a serious foodborne illness called listeriosis, an atypical foodborne disease with a high fatality rate, ranging from 25 to 30% in susceptible populations (Laer et al., 2009). *L. monocytogenes* is carried within the intestinal tract of seemingly healthy animals (Kendall, 2004; Southwick and Purich, 2004).

L. monocytogenes and other *Listeria* species have been also isolated from a variety of raw and processed foods (Gudbjörnsdóttir et al., 2004). Since 1975, foodborne listeriosis outbreaks have been reported in industrialized countries in Europe, North America and

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Oceania with few or no reports from Africa, Asia and Latin America (Farber, 2000; Laciar and de Centorbi, 2002). While several reports indicate that fish and fishery products can be frequently contaminated with *L. monocytogenes*, no major outbreaks associated with these products have been reported (Farber, 2000). The involvement of seafood in the transmission of listeriosis was suggested by Lennon et al. (1984) who, based on epidemiological evidence, proposed that consumption of shellfish and raw fish was responsible for an epidemic of prenatal listeriosis in New Zealand in 1980. Subsequently, sporadic cases of foodborne listeriosis have been reported. Contaminated seafood, such as cold smoked salmon, smoked shrimp, mussels and undercooked fish have been assumed to be sources for sporadic cases of listeriosis (Brett et al., 1998; Cruz et al., 2008; Ericsson et al., 1997; Facinelli et al., 1989; Riedo et al., 1994). Since fish and fishery products may be a vehicle for *L. monocytogenes* it is important to have information on the incidence of this pathogen.

The present study was undertaken to investigate the prevalence of *Listeria* spp. in different fish from ten retail outlets in the city of Urmia, Iran.

MATERIALS AND METHODS

Fish samples

A number of 194 fish, including *Oncorhynchus mykiss* (n=42), *Sander lucioperca* (n=38), *Cyprinus carpio* (n=30), *Hypophthalmiachthys molitrix* (n=48), *Abramis brama* (n=12), *Astacus leptodactylus* (n=12) and *Silurus glanis* (n=12) were purchased from 10 retail outlets (5 fish shops and 5 supermarkets) of Urmia from June 2009 to February 2010. Fish were transported on ice and in sterile condition to Artemia and Aquatic Animals Research Institute (AAARI) of Urmia University for microbiological analysis within 10 h of purchase.

Listeria Isolation

Isolation was carried out according to the methodology described by Pelissier et al. (2001). Briefly, skins of fish were cleaned and disinfected using 70% ethanol and Povidone-iodine. Using a sterile scalpel blade the skin was removed from muscle and the muscle was packaged in sterile plastic sample bags. Buffered peptone water (BPW) (300 ml) was added to each bag and shaken fifty times to wash the muscle evenly.

Conventional culture method of isolating *Listeria* species was modified by the introduction of secondary enrichment step prior to plating. A 25 ml aliquot of BPW was added to 225 ml of Listeria Enrichment Broth–LEB (Bacton, France). The samples were incubated for 4 h at 30ºC, then selective supplements (SR 140E, Oxoid, UK) were added, and samples were incubated a further 20 h. This step helps the recovery of stressed *Listeria* cells from the food samples (Pelissier et al., 2001). Following incubation, 0.1 ml of LEB was transferred to 10 ml Fraser Broth (FB) and incubated at 30ºC for 24-40 h. A loop full of the broth was plated on PALCAM agar (Oxoid, UK) and incubated at 35ºC for up to 48 h. Three typical single colonies were streaked onto Tryptone Soya Yeast Extract Agar (TSYEA) (Merck, Germany), incubated at 35ºC for 24 h, and submitted to biochemical identification to *Listeria* species.

Listeria species identification

For species identification, conventional methods were used including Gram staining, synergistic lyses of erythrocytes (CAMP reaction), typical umbrella motility, biochemical tests (catalase, oxidase, nitrate, Oxidation and Fermentation, esculin hydrolysis) and fermentation of mannitol, rhamnose and xylose (Seeliger and Jones, 1986).

DNA extraction, primers and PCR

In order to confirming the genus of the *Listeria*, a 370 bp fragment of *Prs* gene was amplified using polymerase chain reaction (PCR). DNA extraction was performed using boiling method described by Bansal et al. (1996). Briefly, a single colony of bacteria was cultured in 5-6 ml *Listeria* enrichment broth for 24-48 h. Three milliliters of grown bacteria was centrifuged for 5 min at 10000 g. Bacterial pellets were washed once with 1 ml phosphate buffered saline (PBS), pH 7.4, resuspended in a same volume of cold water and incubated in a boiling water bath for 10 min. The clear supernatants obtained after a 5 min centrifugation at 12000 g were used for PCR reaction.

For amplification of the *Prs* gene two primers (For: GCTGAAGAGATTGCGAAAGAAG and Rev: CAAAGAAACCTTGGATTTGCGG) targeting a fragment of 370 bp was employed as described by Doumith et al. (2004). Amplification reactions were performed in a final volume of 25 µl containing 2.5 units Taq DNA Polymerase in reaction buffer, 4 mM $MgCl_2$, 50 µM each of dATP, dCTP, dGTP and dTTP, 0.5 µM of each primer and about 100 ng of extracted DNA as template. Amplification was performed in Mastercycler (Eppendorf, Germany) with an initial denaturation step at 94°C for 3 min; 35 cycles of 94°C for 40 s, 53°C for 75 s, and 72°C for 75 s; and one final cycle of 72°C for 7 min. Five microliters of the reaction mixture was mixed with 2 µl of loading buffer and separated on a 2% agarose gel in a TBE buffer. The PCR product was visualized by ethidium bromide staining.

RESULTS

Prevalence of *Listeria* spp. in examined fish

Results from biochemical tests showed that from 194 fish samples a number of 24 gram-positive and catalase-positive rod-shape bacteria were isolated and all of these isolates were confirmed to be belonged to *Listeria* genus by amplifying a 370 bp fragment of *Prs* gene. Based on the obtained result the prevalence of *Listeria* in examined fish was 12.37%. The highest prevalence of *Listeria* (25%) was observed in both *A. brama* and *A. leptodactylus*, while the lowest prevalence of *Listeria* was seen in *S. lucioperca* (9.7%). The contamination level of different examined fish has been shown in Table 1.

Differentiation of isolated *Listeria* spp. from examined fish

Using additional chemical tests including sugar fermentation, hemolytic reaction (CAMP test) and chromogenic agar, *Listeria* isolates were further characterized. From total of 24 isolates, five isolates

Table 1. Number and percentage of different fish samples with cultures positive for *Listeria* spp.

Fish	Number of samples	Number and percentage of *Listeria* spp. positive samples (%)
Oncorhynchus mykiss	42	4 (9.5)
Sander lucioperca	38	3 (7.9)
Cyprinus carpio	30	4 (13.3)
Hypophthalmiachthys molitrix	48	6 (12.5)
Abramis brama	12	3 (25)
Astacus leptodactylus	12	3 (25)
Silurus glanis	12	1 (1)

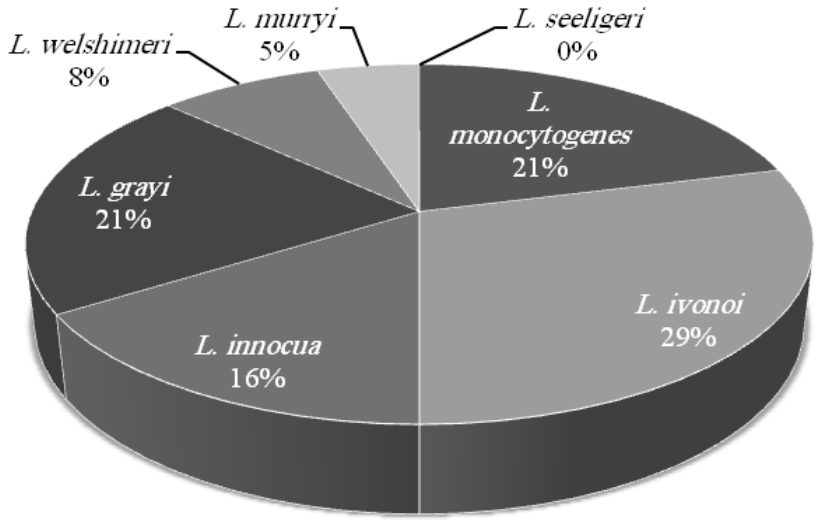

Figure 1. The frequency of different species of *Listeria* isolated from fish.

(21%) confirmed to be *L. monocytogenes,* seven isolates (29%) were *L. ivonoi*, four isolates (16%) were *L. innocua*, five isolates (21%) were *L. grayi*, two (8%) isolates were *L. welshimeri*, and one (5%) isolate was *L. murryi* (Figure 1). It should be noted that *L. seeligeri* was not isolated from any examined fish.

DISCUSSION

In the present study, different species of *Listeria* was isolated from examined fish, indicating that fish can be contaminated with *Listeria*. There are two sources of fish contamination with *Listeria*, which includes;

1) The attack of *Listeria* from intestinal contents to other fish tissues (for example, muscles) especially when the time from fish death till removing viscera is more than a few hours (Ertas and Seker, 2005),
2) Cross contamination (fish manipulation, using contaminated equipments and inappropriate transport) (Gudbjörnsdóttir et al., 2004; Souza et al., 2008).

In temperate regions, *L. monocytogenes* and other *Listeria* species have been isolated from fishery products on a regular basis since the late 1980's. Ben (1994) reviewed the incidence of *Listeria* in seafood worldwide and found that the prevalence of *L. monocytogenes* varied from 4 to 12% in surveys from temperate areas. Farber (1991) reported the presence of *L. monocytogenes* in salmon from the United States, Chile, Norway, and Canada. Other studies have found that the prevalence of *L. monocytogenes* in raw fish is quite low, ranging from 0 to 1% (Autio et al., 1999; Johansson et al., 1999) to 10% (Jemmi and Keusch, 1994). Hartemink and Georgesson (1991) stated that in Iceland 56% of fresh fish on sale were contaminated with *L. monocytogenes* and other *Listeria* species. An overall prevalence 3% of *L. monocytogenes* was observed in European fish (Davies et al., 2001).

It was reported that fish can be contaminated with *Listeria* spp. during farming. Miettinen and Wirtanen (2005) reported that the prevalence of *Listeria* spp. and *L. monocytogenes* in pooled unprocessed fresh rainbow trout was on average of 35.0 and 14.6%, respectively. In

a study by Soultos et al. (2007) on the incidence of *Listeria* spp. in fish and environment of fish markets in Northern Greece, *L. innocua* was more common specie that was detected in four fish samples and 13 environmental samples. They reported that the level of contamination of the environment of fish markets was higher than fish. In Turkey, the incidence of *Listeria* spp. was 30% in freshwater samples and 10.4% in marine fish samples. *L. monocytogenes* (44.5%) and *L. murrayi* (83.5%) was the most commonly isolated species from freshwater fish and marine fish samples, respectively (Yucel and Balci, 2010).

Conclusion

This study revealed an overall prevalence of *Listeria* spp. in different fish obtained from fish shops and super-markets in Urmia. Therefore, for public health matter, it was suggested to eviscerate fish immediately after harvesting to avoid bacteria attacking to other tissues. Furthermore, since cross contamination has been considered as a major cause of fish contamination with *Listeria* spp., transportation, handling and processing of fish and fish products should be performed in an extreme hygienic condition.

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