Ann Microbiol (2012) 62:37–40
DOI 10.1007/s13213-011-0222-9

ORIGINAL ARTICLE

Prevalence of *Listeria* species in fresh and frozen fish and shrimp in Iran

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Received: 20 May 2010 / Accepted: 1 February 2011 / Published online: 18 February 2011

Abstract The objective of this study was to determine the prevalence of *Listeria* spp. in sea food samples in Isfahan and Shahrekord, Iran. From April 2009 to January 2010, a total of 264 samples of fresh and frozen fish and shrimp samples were obtained from randomly selected retail stores in Isfahan and Shahrekord, Iran. The samples were tested for the presence of *Listeria* spp. using a selective enrichment and isolation protocol recommended by the United States Department of Agriculture. Only *Listeria monocytogenes* isolates identified by bacteriological methods were tested by polymerase chain reaction (PCR). Using conventional bacteriologic methods, out of the total of 264 samples examined, 20 (7.6%) were found to be positive for *Listeria*. *Listeria* species were isolated in 7.5%, 4.2%, 11.7% and 6.6% of fresh fish, frozen fish, fresh shrimp and frozen shrimp samples, respectively. *Listeria monocytogenes* and *L. innocua* were detected in 1.9% and 5.7% of the samples analyzed, respectively. All five *Listeria* strains identified as *L. monocytogenes* were also positive using PCR. Consumption of these sea foods, either raw or undercooked, may contribute to food-borne illness in Iran. Also, *L. monocytotenes* in raw seafood may pose a health risk in kitchens if contaminating ready-to-eat food.

Keywords Fish · Shrimp · *Listeria* · Prevalence · Iran

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Introduction

The bacteria *Listeria* spp. have become a significant subject in biomedical research due to its central role in medical microbiology and food microbiology. The genus *Listeria* comprises six species: *L. monocytogens*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*. In humans, *L. monocytogenes* is the major pathogen, although very rare cases of infection due to *L. ivanovii* and *L. seeligeri* have been described (Mclauchlin 1997). The presence of any *Listeria* species in food may be an indicator of poor hygiene. However, since *L. monocytogenes* is the major human pathogen, there is widespread agreement that the goal should be to exclude this organism from the food chain wherever possible, and to maintain conditions that will inhibit its multiplication in foods in which this bacterium can grow (Rocurt et al. 2000; Wyller et al. 1999).

Listeriosis is a severe and often fatal illness with clinical manifestations resembling sepsis or meningitis in immuno-compromised patients and neonatal babies, and flu-like illness or abortion during pregnancy in women (Delgado 2008). The case-fatality rate from listeriosis is generally about 20–30% (Farber and Peterkin 1991).

Major outbreaks of listeriosis have been associated with the consumption of foods of animal origin (Rocourt et al. 2000; Iida et al. 1998), especially sea foods such as shrimp, mussels and undercooked fish (Wan Norhana et al. 2010; Brett et al. 1998). Since fish and fishery products may be a vehicle for *L. monocytogenes*, it is important to have

information on the incidence of this pathogen. This organism has been isolated frequently from fish and fish products from different parts of the world (Wan Norhana et al. 2010; Parihar et al. 2008; Hassan et al. 2001; Basti et al. 2006; Mena et al. 2004).

In Iran, few surveys have been conducted to assess the presence of *Listeria* spp. in seafood. Thus, the objectives of this study were to determine the incidence of *Listeria* species and *Listeria monocytogenes* isolated from fresh and frozen fish and shrimp obtained from supermarkets and wet markets in Isfahan and Shahekord, Iran.

Materials and methods

Sample collection

A total of 264 sea food samples were collected from April 2009 to January 2010 from supermarket and retail outlets in Isfahan and Shahrekord, Iran. The sea food samples analysis comprised samples of fresh and frozen fish and shrimp (Table 1). The samples were transferred to the Food Microbiology Laboratory at the Islamic Azad University of Shahrekord Branch in portable insulated cold-boxes. Samples were analyzed on the day they were collected.

Isolation and identification of *Listeria*

The samples were tested for the presence of *Listeria* spp. using the selective enrichment and isolation protocol recommended by the United States Department of Agriculture (McClain and Lee 1988). Twenty-five grams of each sample were taken aseptically, blended for 2 min in 225 ml *Listeria* enrichment broth (UVM I) (Merck, Darmstadt, Germany) and incubated at 37°C for 24 h. A 1 ml sample of this primary enrichment was transferred to 9 ml UVM II (Frazer broth) (Merck) and incubated at 37°C for 24 h. Secondary enrichments were streaked onto Oxford agar (Merck) and Palcam agar (Merck) and incubated at 35°C for 48 h. The plates were examined for *Listeria* colonies (black colonies with black sunken center) and at least three suspected colonies were subcultured onto Tryptone Soy agar supplemented with 0.6% of yeast extract (TSAYE) (Merck) and incubated at 37°C for 24 h. All isolates were subjected to standard biochemical tests including Gram staining, catalase test, motility test at 25°C and 37°C, acid production from glucose, mannitol, rhamnose, xylose, α-methyl-D-mannoside, and nitrate reduction, hydrolysis of esculin, MR/VP test, β-hemolytic activity, and CAMP test (Aygun and Pehlivanlar 2006).

DNA extraction and PCR conditions

Only *L. monocytogenes* isolates identified by bacteriological methods were tested by PCR. The PCR procedures used in this study have been described previously (Zhou and Jiao 2005). Briefly, 1 ml pure culture of *L. monocytogenes* was centrifuged at 13,000 *g* for 5 min at room temperature. The DNA was then extracted using a genomic DNA purification kit (Fermentas, St. Leon-Rot, Germany, K0512) according to the manufacturer's protocol. Oligonucleotide primers for the PCR assay were selected based on the published nucleotide sequence of the *actA* gene (Cai et al. 2002). The pair of primers 01 (5′-GCTGATTTAAGAGATAGAGGAACA-3′) and 02 (5′-TTTATGTGGTTATTTGCTGTC-3′) were used to amplify an 827-bp DNA fragment that corresponds to the region of the 3′-end of the *actA* gene. A 25 μl aliquot of PCR buffer contained 22 μl PCR supermix (0.2 μl of each primer at 12.5 μM, 2.5 μl of 10 × PCR buffer, 1.0 μl of 25 mM $MgCl_2$, 1.0 μl of 1 mm of dNTPs mix, 0.1 μl of 3 U/ml *Taq* DNA polymerase in 17 μl of ddH_2O). A 3 μl aliquot of each supernatant was added to the PCR mix. Thermocycling conditions included an initial hold of 2 min at 94°C, then a denaturation step at 95°C for 10 s, annealing at 60°C for 30 s and a 30 s extension at 72°C for a total of 40 cycles. A final hold at 4°C followed a final extension at 72°C for 10 min. Amplification reactions were carried out in a DNA thermal cycler (Master Cycler Gradient, Eppendorf, Germany). PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with a 1% solution of ethidium bromide, and examined under UV illumination. In the present study, DNA extracted from *L. monocytogenes* (laboratory control strain) and DNase-free water were used as the positive and negative controls, respectively.

Table 1 Prevalence of *Listeria* spp. in fish and shrimp in Iran. Results expressed as the number of *Listeria*-positive samples/ number of samples analyzed (%)

Type of food sample	No. of samples	*Listeria* spp. positive, n (%)	*L. monocytogenes*, n (%)	*L. innocua*, n (%)
Fresh fish	80	6 (7.5)	2 (2.5)	4 (5.0)
Frozen fish	60	2 (3.3)	0 (0.0)	2 (3.3)
Fresh shrimp	60	7 (11.7)	2 (3.3)	5 (8.3)
Frozen shrimp	76	5 (6.6)	1 (1.3)	4 (5.3)
Total	264	20 (7.5)	5 (1.9)	15 (5.7)

Statistical analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft, Redmond, WA) for analysis. Using SPSS 16.0 statistical software (SPSS, Chicago, IL), a chi-square test and Fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $P<0.05$.

Results and discussion

Table 1 give a breakdown of the types of samples tested and the types of positive samples. Out of 264 samples tested, 20 samples (7.6%) were positive for *Listeria* spp. Out of these 20 isolates of *Listeria* spp., 5 isolates were hemolytic and CAMP positive, and were identified as *L. monocytogenes* (Table 1). All five *Listeria* strains identified as *L. monocytogenes* were also positive using PCR. The remaining 15 isolates were *L. innocua*. Other species of *Listeria* were not isolated in this study. *Listeria* spp. and *L. monocytogenes* were isolated from 7.5% and 4.2% of fresh and frozen fish samples, respectively, which is comparable to results reported from Japan (Ryu et al. 1992), Spain (de Simon and Ferrer 1998), Sweden (Parihar et al. 2008) and India (Dhanasheree et al. 2003). An overall prevalence 3% of *L. monocytogenes* was observed in European fish (Davies et al. 2001). Other studies have found that the prevalence of *L. monocytogenes* in raw fish is quite low, ranging from 0– 1% (Autio et al. 1999) to 10% (Jemmi and Keusch 1994). However, Hartemink and Georgasson (1991) stated that 56% of fresh fish on sale in Iceland was contaminated with *L. monocytogenes* and other *Listeria* species. Elsewhere, Mina et al. (2004) reported that 12% of fresh fish samples were contaminated with *L. monocytogenes*.

A total of 126 fresh and frozen shrimp samples were tested and 12 (9.5%) were found to be contaminated with *Listeria* spp. (Table 1). A wide range of prevalence of contaminated fresh and frozen shrimp have been reported elsewhere. Similarly, in a study in Brazil, Hofer and Ribeiro (1990) detected *Listeria* spp. in 8.8% of frozen shrimp (for export) samples. In study conducted in the United States, 27 of 74 frozen shrimp samples (20%) analyzed were positive for *Listeria* (Jinneman et al. 1999). In another US study, 25% of fresh and frozen shrimp samples were positive for *Listeria* (Buchanan et al. 1989). In France, Ravomanana et al. (1993) isolated *Listeria* in 23.5% of fresh shrimp samples. Manoj et al. (1991), Dhanashree et al. (2003), Moharem et al. (2007) and Parihar et al. (2008) detected *Listeria* spp. in 10.5%, 9.1%, 73.3% and 30.0% of fresh and frozen shrimp in India, respectively. The findings of the present study are in agreement with some previous studies. The incidence of *L. monocytogenes* in our study was found to be 2.2% in fresh and frozen shrimp samples. These results are in agreement with reports by Masuda et al. (1992) in Japan, Adesiyun et al. (1993) in Trinidad, McLauchlin and Nichols (1994) in England, Jinneman et al. (1999) in the United States, and Moharem et al. (2007) in India, where the bacteria were detected from 1.4%, 2.0%, 6.0%, 5.0% and 6.7% of fresh or frozen shrimp samples, respectively. However, higher contamination rates (10–53%) have also been reported (Jeyasekaran et al. 1996; Cordano and Rocourt 2001; Ellner et al. 1991; Minami et al. 2010; Wan Norhana et al. 2010).

Only a few studies on the prevalence of *Listeria* spp. in seafood in Iran have previously been conducted. A study by Jalali and Abedi (2008) on the prevalence of *Listeria* species in food products found that, of the 85 fresh and frozen fish and fresh shrimp samples studied, 2 (2.3%) were contaminated with *L. innocua* and 1 (1.6%) with *L. monocytogenes*. Also, another report from Iran indicated that, in agreement with our findings, 2.6% of fresh fish samples were found to be contaminated by *L. monocytogenes* (Basti et al. 2006). The real situation regarding listeriosis in Iran is unclear, and little information exists on the prevalence of *L. monocytogenes* in foods consumed in the country. It is also important to note that listeriosis is not a notifiable disease in the Iranian health system (Jalali and Abedi 2008).

The frequent presence of *L. innocua* in fish and shrimp in the present study is in agreement with other reports (Dhanashree et al. 2003; Lacier and de Centorbi 2002; Soultos et al. 2007; Jalali and Abedi 2008). As in other raw foods, fishery products more frequently contain *L. innocua* than *L. monocytogenes*. Since both species share ecological niches, the presence of *L. innocua* is considered an indicator of possible contamination with *L. monocytogenes* (Jinneman et al. 1999).

Isolation of *L. monocytogenes* from seafood suggests that there is a risk of acquiring listeriosis through seafoods in Iran. *Listeria monocytogenes* will be killed by cooking, and raw or semi-raw seafood are not consumed in Iran. However, *L. monocytogenes* in raw sea foods may pose a health risk in kitchens as it could contaminate cooked food or other ready-to-eat foods. Considering outbreaks of listeriosis associated with different foods, avoidance of consumption of insufficiently cooked seafoods by at-risk populations is recommended. Diligent enforcement of sanitary conditions of food contact surfaces and handling areas, and good personal hygiene practices should reduce the potential contamination of fishery products by *L. monocytogenes* at the retail level.

Acknowledgments The authors would like to thank Hassan Momtaz and Majed Riahi for their sincere help in performing the technical parts of this project. We are also grateful to Amir-Masoud Shahbazi, Reza Sohrabi, and Ali Drakhshesh for assistance with sampling. The present paper was developed with the cooperation and financial support of the Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

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