Journal of Food Safety and Food Quality 62, Heft 1 (2011), Seiten 1–32

Arch Lebensmittelhyg 62,
16–19 (2011)
DOI 10.2376/0003-925X-62-16

ISSN 0003-925X

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Detection of *Listeria* species in fresh fish and fish market environment by IMS technique in Turkey

Nachweis von Listerien bei Frischfisch und auf Fischmärkten der Türkei mittels immunmagnetischer Separation

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Summary

The incidence of *Listeria* spp. was investigated in fresh fish (n = 100) sold at retail markets and in the environmental and personnel samples (n = 100) obtained from several fish markets in Afyonkarahisar, Turkey by immunomagnetic separation technique. The fish samples analysed included anchovy, trout, carp and grey mullet (25 of each). Six (6 %) of the fish samples were found positive for *Listeria* spp. and the overall incidence of *Listeria* spp. was 10 % in the environmental and personnel samples. Three *Listeria* spp., *Listeria monocytogenes, L. ivanovii, L. grayi* were recovered from the samples examined. In addition, *L. seeligeri* from a fish sample and *L. innocua* from an environmental sample (box) were isolated. It was found that *L. monocytogenes* was only detected in fresh water fish with an incidence of 8 %. For the environmental samples, knives and refrigerators had the highest (20 %) and personnel samples had the least (5 %) levels of contamination. It can be concluded that the fish sold at retail markets are moderately contaminated with various species of *Listeria* including *L. monocytogenes,* which may pose a risk for human health. The presence of these bacteria in environmental and personnel samples of fish markets is also an important possible sources of cross-contamination of the fish and other seafood. The results of this study indicate the necessity of the implementation of good hygiene and sanitary practices in order to prevent and/or reduce the contamination of fishery products by *Listeria* spp. at the retail level.

Keywords: *Listeria* spp., *L. monocytogenes,* fish, immunomagnetic separation, Turkey

Zusammenfassung

Die Inzidenz von *Listeria* spp. wurde anhand von frischen Fischen (n = 100), Umwelt- und Personalproben (n = 100) aus Einzelhandelsmärkten der Provinz Afyonkarahisar (Türkei) mittels immunmagnetischer Separation untersucht. Es wurden jeweils 25 Sardellen, Forellen, Karpfen und Graue Meeräschen beprobt. In sechs (6 %) Fischproben wurde *Listeria* spp. nachgewiesen. Die durchschnittliche Inzidenz von *Listeria* spp. der Umweltproben (Messer, Tische, Kühlräume usw.) und Personalproben lag bei 10 %. Vor allem wurden *L. monocytogenes, L. ivanovii* und *L. grayi* isoliert. Zusätzlich wurden *L. seeligeri* in einer Fischprobe und *L. innocua* in einer Umweltprobe (Behälter) identifiziert. *L. monocytogenes* wurde nur in Süßwasserfischen mit einer Inzidenz von 8 % isoliert. Die Messer und Kühlräume der Umweltproben waren am stärksten (20 %) und die Personalproben am geringsten (5 %) kontaminiert. Es kann daraus geschlossen werden, dass die Fische, die im Einzelhandel verkauft werden, mit *Listeria* spp., einschließlich *L. monocytogenes,* das ein Risiko für die menschliche Gesundheit darstellen kann, im mäßigen Umfang kontaminiert werden. Das Vorhandensein dieser Bakterien im Umfeld der Handelsstätte und des Personals ist eine wichtige Kontaminationsquellen der Fische und Fischereiprodukte. Die Ergebnisse dieser Studie zeigen die Notwendigkeit der Implementierung der Guten Hygiene Praxis, um die Kontaminierung der Fischereiprodukte durch *Listeria* spp. zu verhindern und/oder zu verringern.

Schlüsselwörter: *Listeria* spp., *L. monocytogenes,* Frischfisch; immunmagnetische Separation, Türkei

Introduction

Listeria spp. are widespread in the environment and cause serious diseases in humans with a variety of symptoms such as spontaneous abortion, meningitis and bacteraemia (Miettinen et al., 1999; Rocourt et al., 2000; Lecuit, 2007). *L. monocytogenes* is carried asymptomatically in the feces of healthy people (Rocourt et al., 2000). The vast majority of the infections in humans are caused by *L. monocytogenes* (Lecuit, 2007). Listeriosis in humans occurs as sporadic cases in most situations but occasional outbreaks have also been reported from various geographical locations (Rocourt et al., 2000). *L. monocytogenes* has been isolated from several varieties of foods including meat and meat products, vegetables, milk and dairy products (Ben Embarek et al., 1994), all of which have been associated with outbreaks (Schlech et al., 1983; Fleming et al., 1985; Goulet et al., 1998;) and sporadic cases (Facinelli et al., 1989; Mitchell, 1991) indicating the importance of foodborne transmission of listeriosis. Ready-to-eat foods and foods that are kept under chilled conditions for long time carry high risk for foodborne *Listeria* infections since *Listeria* spp. are able to grow at lower temperatures (Rocourt et al., 2000). It is reported that fish and fish products are commonly contaminated by various species of *Listeria* (Farber, 1991; Hartemink and Georgsson, 1991; Laciar and De Centorbi, 2002; Soultos et al., 2007; Jallewar et al., 2007) and they have been implicated to be responsible for several human listeriosis cases by many researchers (Ericsson et al., 1997; Miettinen et al., 1999). Due to contamination of fish products by *L. monocytogenes* several types of fishery products were reported to be withdrawn from the market (Rocourt et al., 2000). *Listeria* spp. are naturally present on fresh water fish and not likely to be present on fish reared in clean seawater. It is suggested that contamination of live fish with *Listeria* spp. is probably as the result of contaminated surrounding environment (Ben Embarek, 1994).

This study was undertaken to investigate the incidence of *Listeria* spp. in fresh fish samples and from the samples obtained from the environment and personnel of several fish markets in Afyon, Turkey.

Materials and Methods

Samples

A total of 100 fresh fish samples consiting of anchovy *(Engraulis encrasicholus)*, trout *(Trutta faris)*, carp *(Cyprinus carpio)* and grey mullet *(Mugil cephalus)* (25 of each) were purchased from retail fish markets between April–July 2009 in Afyonkarahisar (Turkey). In addition, a total of 100 environmental and personnel samples were taken from the hands (20) and boots (20) of workers and from transport boxes (20), floors (10), cutting surfaces (10), knives (10) and refrigerators (10) at ten different retail fish markets using sterile swabs. The swab samples were put into 10 ml of University of Vermont listeria enrichment broth (UVM I; Oxoid, CM863 plus SR 142) and all the samples were immediately brought to laboratory under cooled conditions until bacteriological analysis.

Detection of *Listeria* spp. by IMS technique and identification of the isolates

The fish samples were enriched following the procedure recommended by United States Department of Agriculture (USDA) (Mcclain and Lee, 1988). Briefly, twenty-five grams of each fish sample (flesh and skin) was aseptically taken, blended in 225 ml of UVM I and incubated at 30 °C for 24 h. The swab samples present in 10 ml UVM I were also incubated at 30 °C for 24 h. All the samples enriched in UVM I were subjected to immunomagnetic separation (IMS) technique to concentrate *Listeria* spp. The IMS technique was performed according to manufacturer's instructions (Dynal, Norway). Briefly, one ml of the enriched culture and 20 µl of Dynabeads (Dynal, Norway) were mixed in an Eppendorf tube, and incubated with continuous mixing for 10 min at room temperature and rotated approximately 3 min using a vortex (Dynal MX1, Norway). A magnetic field was applied to the side of the tube for about 3 min, and the beads with any adherent *Listeria* spp. were drawn to the side of the tube. The supernatant was carefully removed by aspiration and the beads were resuspended in 1 ml phosphate-buffered-saline-Tween (PBS, Sigma) by mixing for a few min. This washing step was repeated twice. After the final washing, the beads were resuspended in 100 µl of washing diluents. Fifty µl of the suspended Dynabeads–Listeria complex was streaked onto Palcam (Oxoid, CM 877 and SR 150) and Oxford (Oxoid, CM 856 and SR 140) agars and the agars were incubated at 30 °C for 36–48 h. The plates were examined for typical *Listeria*-like colonies (black colonies with black sunken) and at least three suspected colonies were sub-cultured on Trypton Soy Agar supplemented with 0.6 % of yeast extract (TSAYE) and incubated at 37 °C for 24 h. All the isolates were subjected to standard biochemical characterization such as Gram staining, catalase test, motility at 25 °C and 37 °C, acid production from glucose, manitol, rhamnose, xylose, α-methyl-D-mamoside, and nitrate reduction, hydrolysis of esculin and MR/VP test for identification. In addition, ß-haemolytic activity and CAMP test were done according to the Bergey's Manual of Systematic Bacteriology for further characterization (Seeliger and Jones, 1986). Microbact TM 12L *Listeria* Identification System (Oxoid, MB1128) was used for final identification of the isolates according to the manufacturer's instructions.

Results and Discussion

Table 1 and Table 2 show the incidence and distribution of *Listeria* spp. in fresh fish samples and environmental samples obtained from several fish markets. Overall, six (6 %) of the fresh fish samples examined were found positive for *Listeria* spp. and four fish samples were positive for *L. monocytogenes.* Of the *L. monocytogenes* positive samples two were from trouts and two from carps. Three other species of *Listeria, L. ivanovii* (from trout), *L. grayi* (from anchovy) and *L. seeligeri* (from grey mullet) were also isolated from the fish samples. The incidence of *Listeria* spp. was determined to have ranged between 5 % (hands and boots of workers), 10 % (transport boxes, floors and cutting surfaces) and 20 % (refrigerators and knives) on the environmental and personnel samples of fish markets with an overall incidence of 10 %.

Listeria spp. was detected in nine out of the 10 fish markets examined and *L. monocytogenes* isolated from knife (on two occasions) and boot samples of a personnel (on one occasion) was found in three (30 %) of the markets. *L. innocua* (detected on a transport box) was also isolated from a fish market in addition to *L. ivanovii* and

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TABLE 1: *Incidence of Listeria spp. in fish samples.*

Fish	Number of samples	Number (%) of positive samples	*Listeria* spp. isolated
Trout (*Trutta faris*)	25	2 (8)	*L. monocytogenes* *L. ivanovii*
Anchovy (*Engraulis encrasicholus*)	25	1 (4)	*L. grayi*
Carp (*Cyprinus carpio*)	25	1 (4)	*L. seeligeri*
Grey mullet (*Mugil cephalus*)	25	2 (8)	*L. monocytogenes*
Total	**100**	**6 (6)**	

environments and lower level of secondary cross-contamination might have occurred until the fish reaches the retail markets. However, determination of a very high prevalence of *Listeria* spp., in particular that of *L. monocytogenes*, from majority of the fish markets analysed (9 of the 10 markets) suggests that these bacteria can be transmitted to the fish and other seafood and the fishery products can readily be contaminated from these contaminating areas even if they

TABLE 2: *Incidence and distribution of Listeria spp. in environmental and personnel samples taken from ten different retail fish markets.*

Samples examined	N	n	Retail Fish Markets A	B	C	D	E	F	G	H	I	K	Total Positive	%
Hands	20	2	–	–	–	*L. ivanovii*	–	–	–	–	–	–	1	%5
Boots	20	2	–	–	–	–	*L. monocytog.*	–	–	–	–	–	1	%5
Transport boxes	20	2	–	–	NE	*L. inocua*	–	–	–	–	–	–	2	%10
Floor	10	1	–	–	–	–	–	–	–	*L. ivanovii*	–	–	1	%10
Cutting board	10	1	–	–	–	–	–	*L. grayi*	–	–	–	–	1	%10
Refrigerators	10	1	*L. grayi*	*L. grayi*	–	–	–	–	–	–	–	–	2	%20
Knives	10	1	–	–	*L. monocytog.*	–	–	–	–	–	*L. monocytog.*	–	2	%20
Total	100	10	1	1	2	2	1	1	–	1	1	–	10	%10

N: Total number of samples examined. n: Total number of samples collected from each market environment and personnels. NE: Not examined.

L. grayi. The latter species was detected on the samples taken from refrigerators (on two occasions) and cutting surface (on one occasion) and *L. ivanovii* from the hands of a worker from a fish market. This relatively high prevalence of *Listeria* spp. detected in fish market environmental and personnel samples highlights the importance of secondary cross-contamination sources of the fish and other seafood, which necessitates the implementation of diligent sanitary practices of both contact surfaces and personnel working in these establishments. This is imperative for the prevention of infections caused by *Listeria* spp. and in particular that of *L. monocytogenes* for the protection of consumer health given their association with a variety of serious human diseases (Rocourt et al., 2000).

The incidence of *Listeria* spp. in fresh fish was reported to have ranged in various surveys conducted in different regions all over the world. Adesiyun (1993) isolated *Listeria* spp. in 14.8 % and Gohil et al. (1995) in 4.5 % of the fresh fish samples while 4 % to 62.5 % from fresh fish were determined by various researchers (Weagant et al., 1988; Adesiyun, 1993; Laciar and De Centorbi, 2002; Ertas and Seker, 2005; Jallewar et al., 2007; Soultos et al., 2007). *Listeria* spp. are isolated more commonly from fresh water samples and from polluted or contaminated water sources than the clean sea water (Ben Embarek, 1994). In the present study, the overall incidence of *Listeria* spp. in fresh fish was 6 % which is, although it lies between the results obtained by the other studies mentioned above, relatively lower than some other studies (Weagant et al., 1988; Fuchs and Surrandran, 1989). The reason for this discrepancy may be explained by the fact that the presence of *Listeria* spp. in fish is generally regarded as a reflection of the contamination of surrounding environment (Ben Embarek, 1994) and thus, the fish samples examined in the present study might have derived from less and/or uncontaminated water enter into the retail market free of *Listeria*. The rate of recovery of *Listeria* spp. (10 %) from the environmental and personnel samples of fish markets examined in this study is relatively lower than that of Soultos et al. (2007) who detected *Listeria* spp. in 19 % of the environmental and personnel samples taken from fish markets in Greece. However, more variety of *Listeria* spp. were determined from fish market samples in the current study.

It is noteworthy that the incidence of *Listeria* spp. was slightly higher on fresh water fish (trout and carp) than that of sea water fish (anchovy and grey mullet) samples and *L. monocytogenes* was detected only on fresh water fish but not on any of the sea water fish samples examined in this study. This is not surprising since it is considered that *Listeria* spp. occur naturally more likely on fresh water fish and its presence is less likely on clean sea water fish (Ben Embarek, 1994). *Listeria* spp. isolated from the fish samples in this study might have derived either from contaminated surrounding waters and/or a secondary cross-contamination might have occurred from various sources including the environments of fishing vessels and fish markets (Soultos et al., 2007) as also encountered in the present study.

Four species of *Listeria* were isolated from the fish samples and *L. monocytogenes* was only recovered from the fresh water fish with relatively high prevalence. Soultos et al. (2007) examined 120 sea water fish samples and recovered two different *Listeria* spp. (*L. monocytogenes* and *L. innocua*) in their survey with *L. monocytogenes* being isolated from only one fish sample. Jallewar et al. (2007) examined 200 samples of fresh water fish and found four species in their study but they were able detect *L. welshimeri* rather than *L. ivanovii* that was recovered in the current study. In addition, the recovery rate of *L. monocytogenes* was 13 % in their study. The prevalence of

L. monocytogenes in fish from temperate regions was reported to have ranged from 4 % to 12 % in several surveys (Ben Embarek, 1994). Determination of the 4 % prevalence rate (even 8 % when the fresh fish samples were considered) from fish samples in the present study agrees with the published data. The lower recovery of *L. monocytogenes* by Soultos et al. (2007) may be due to inclusion of the sea water fish samples in their study. This hypothesis is also supported by the findings of Jallewar et al. (2007) that they found 13 % prevalence rate from fresh water fish samples.

It can be concluded from the findings of this study that the fish, in particular fresh water fish, sold at retail markets in Afyon, Turkey are contaminated with various species of Listeria including *L. monocytogenes*, which may pose a risk for human health. The presence of these bacteria in environmental and personnel samples of fish markets indicates the existence of additional points for cross-contamination of the fish and other seafood. Enforcement of diligent hygiene practices for personnel and sanitary conditions of the fish markets will be very useful in reducing the contamination of the fish and fish products and thus for the protection of human health.

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