

# Occurrence of *Listeria monocytogenes* in fish and fishery products

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Original scientific paper

## SUMMARY

Listeriosis is an infection that is caused by eating food contaminated by bacteria *Listeria monocytogenes*, which represents one of the major public health issues and mostly occurs during the transport of food worldwide. It is primarily associated with the consumption of finished products. This paper outlines our findings of bacteria *L. monocytogenes* in analysed samples of fresh fish (sea bass) and fishery products (smoked and marinated fish, gilt-head bream and sea bass). We also examined swabs taken from work surfaces and hands of fish-processing plant employees. Bacteria *L. monocytogenes* were not present in samples of fresh, smoked and marinated fish. However, bacteria *L. innocua* were present in samples of marinated and smoked fish. Although bacteria *L. monocytogenes* were not present in samples of swabs, bacteria *L. innocua* were present in swabs taken from work surfaces during handling, grading and processing of fish, i.e. in areas designated for evisceration and the control of fish processing, as well as on the hands of employees who operated the process for the filleting of fish.

**Key words:** *L. monocytogenes*, *L. innocua*, fish, fishery products, plant hygiene

## INTRODUCTION

The persistent issue of food safety in practice includes potential public health problems related to medical conditions in humans that are caused by consuming foods contaminated by bacteria. According to World Health Organization data, in addition to bacteria of the genera *Salmonella*, *Listeria monocytogenes* is considered the most significant public health problem. According to the European Food Safety Agency (EFSA) report, European Union Member States have in 2013 reported 1,763 cases of listeriosis and a total of 191 deaths, with 64 deaths recorded in France alone (Anon., 2015). Such infection can either manifest as an epidemic or sporadically. It is primarily associated with the consumption of "ready to eat" (RTE) products whose shelf life is extended at refrigerator temperatures. The occurrence of the infection is usually associated with dairy and meat products in both fish and minimally

processed fishery products (smoked fish). Accordingly, 17 EU Member States have reported findings of *L. monocytogenes* in fishery products in 2013. *L. monocytogenes* was found in 1.6 % of the total of 1,649 samples of various fishery products. Just as in previous years, *L. monocytogenes* was more often determined in RTE fish (usually smoked fish).

*L. monocytogenes* is a microorganism that is widely distributed in nature. It can be found in plant tissue, soil and water, as well as in infected animals. Infected animals represent a major route of transmission to humans. It is mostly transmitted to humans and animals by consuming contaminated food or feed. It grows best in cold conditions and not only tolerates drought and higher levels of salinity, but is able to survive mild heat treatment (Helwich et al., 2012). *L. monocytogenes* can be present in different food products. Microbiological contamination of finished products is often associated with either the contamination of the product itself or

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irregularities in production process. In the case of *L. monocytogenes*, the high-risk group comprises of fresh, unprocessed foodstuffs such as milk, meat, cream cheeses, as well as fish and fishery products, including frozen fish, cold and hot smoked fish, marinated fish, fermented fish and fish salads (Kožačinski et al., 2000; Dominguez et al., 2001; González-Rodríguez et al., 2002; Gombas et al., 2003; Popović and Đurđević-Milošević, 2008; Papadopoulos et al., 2010. Tocmo et al., 2014; Kuzmanović et al., 2011).

Fishery products can be easily polluted (contaminated) by *L. monocytogenes* during production. Raw fish can be an important source of pollution on equipment and in facilities that will result in the contamination of products (Miettinen and Wirtanen, 2005). Both evisceration and cleaning of fish before retail can cause the spread of bacteria in the production facility that will, in turn, result in cross-contamination of fish, equipment, employees and the environment in general (Papadopoulos et al., 2010).

This paper will therefore present the results of examination for the presence of *L. monocytogenes* that was conducted on samples of fresh fish (sea bass) and fishery products (smoked sea bass and gilt-head bream). Since these bacteria are ubiquitous, we shall analyse the results of their findings in fish processing facilities and try to compare the incidence of reported bacteria findings in fresh fish and nature to the incidence of reported bacteria findings in fishery products. This paper shall also present a part of results obtained within the framework of the project FP7 Selection and Improving of Fit - For - Purpose Sampling Procedures for Specific Foods and Risks – BASELINE Grant Agreement number 222738; 2009 – 2013 (Faculty of Veterinary Medicine Team Leader Ph. D. Lidija Kožačinski, Professor).

## MATERIALS AND METHODS

This paper outlines our findings of bacteria *L. monocytogenes* in analysed samples of fresh fish and fishery products. We examined a total of 21 samples, namely nine samples of fresh fish (sea bass), six samples of marinated fish (three samples of marinated sea bream and three samples of marinated sea bass) and six samples of smoked fish (three samples of smoked sea bream and three samples of smoked sea bass). Our study has also investigated a part of the results obtained by bacteriological examination of fish, namely sea bass (n = 39) obtained within the framework of the project BASELINE, and the results of the evaluation of microbiological purity of fish processing facilities related to findings of *L. monocytogenes* (n = 40).

Provided samples of fish and fishery products were

delivered to the Department of Hygiene, Technology and Food Safety after due production process, at the moment they were ready to be distributed on the market. Samples were delivered in a portable freezer without damaging the packaging. Fresh fish (sea bass) weighed about 300 g and was packaged in modified atmosphere. Marinated sea bass and gilt-head bream were packaged in containers; each weighing 100 g. Marinated fish was filleted. Smoked sea bass and gilt-head bream fillets were also packaged in packs weighing about 100 g each.

Activities within the framework of the project BASELINE included two deliveries of fresh sea bass crates. Samples were collected after fishing and after primary handling (evisceration) in fish processing facility, prior to packaging for retail. In the second sampling, samples of sea bass were collected on the fish market. During the collection of fish samples in processing facilities, we also took swabs from work surfaces and hands of fish-processing plant employees. Both fish samples and swabs were examined only for the presence of *L. monocytogenes*.

In accordance with the standard HRN EN ISO 11290-2:1999 (Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Enumeration method) (HRN EN ISO 11290-1:1999 + HRN EN ISO 11290-1:1999/A1:2008), all samples of fish and products were screened for the presence of *L. monocytogenes* per 25 g. *L. monocytogenes* was identified using a commercially available API Listeria test kit, as prescribed by the manufacturer (Biomérieux).

## RESULTS AND DISCUSSION

The results of bacteriological examination of fish and products, as well as the results of *L. monocytogenes* findings among analysed samples of fish and products are presented in Table 1. *L. monocytogenes* was not identified in any of the examined samples of fish and fishery products. However, during the biochemical identification, bacteria *L. innocua* were confirmed in all analysed samples of fishery products (n = 12).

**Table 1:** Results of examination for the presence of *L. monocytogenes* in fish and fishery products

Sample	n	<i>L. monocytogenes</i> / 25 g	Note
Fresh sea bass	9	Neg.	-
Marinated gilt-head	3	Neg.	<i>Listeria innocua</i> / 25 g = Pos.
Marinated sea bass	3	Neg.	<i>Listeria innocua</i> / 25 g = Pos.
Breamed gilt-head	3	Neg.	<i>Listeria innocua</i> / 25 g = Pos.
Smoked sea bass	3	Neg.	<i>Listeria innocua</i> / 25 g = Pos.

**Table 2:** Rezultati pretrage svježe ribe (brancin) na prisutnost bakterije *L. monocytogenes*

Uzorkovanje		<i>L. monocytogenes</i> /25 g
Pogon prerade ribe	Maloprodaja	
8	31	neg.

Table 2 presents the results of microbiological analysis of fresh cultivated fish (sea bass) samples collected in dispatch centres and retail facilities. Bacteria *L. monocytogenes* were not identified in any of the examined samples.

*L. monocytogenes* was not identified in any of the examined swabs taken from work surfaces (Table 3) and hands of employees. However, *L. innocua* was identified in three instances of both samplings, namely in swabs taken from work surfaces designated for handling, gra-

**Table 3:** Results of microbiological examination of work surfaces and hands of employees in fish processing facilities

Sampling site	Sampling 1			Sampling 2		
	n	Lm	Li	n	Lm	Li
Work surface - evisceration	2	Neg.	Neg.	2	Neg.	Pos.
Work surface - fish handling	2	Neg.	Pos.	2	Neg.	Neg.
Work surface - fish grading	2	Neg.	Pos.	2	Neg.	Neg.
Work surface - fish filleting	2	Neg.	Pos.	2	Neg.	Neg.
Work surface - fish salting	2	Neg.	Neg.	2	Neg.	Neg.
Work surface - control of fish processing	2	Neg.	Neg.	2	Neg.	Pos.
Hands of workers - evisceration	2	Neg.	Neg.	2	Neg.	Neg.
Hands of workers - filleting	2	Neg.	Neg.	2	Neg.	Pos.
Hands of workers - grading	2	Neg.	Neg.	2	Neg.	Neg.
Hands of workers - control	2	Neg.	Neg.	2	Neg.	Neg.
Total	20	0	3	20	0	3

Lm = *Listeria monocytogenes*; Li = *Listeria innocua*

ding and processing of fish, i.e. areas designated for evisceration, control of fish processing, and hands of employees who operated the process for the filleting of sea bass in second sampling.

Research results presented in Table 1 and 2 demonstrate that bacteria *L. monocytogenes* were not identified in analysed samples of fresh fish that were sampled in fish processing facility prior to placement on the market, or in fish samples collected on the fish market, or in samples of smoked and marinated fish. Although other authors identified *L. monocytogenes* in fresh fish and fishery products - smoked and marinated fish (Jeyasekaran et al., 1996; Dominguez et al., 2001; González-Rodríguez et al., 2002; Papadopoulos et al., 2010; Dimitijevic et al., 2011; Tocmo et al., 2014; Lakičević et al., 2015), samples in our research tested negative. However, bacteria *L. innocua* were present in sam-

ples of marinated and smoked fish (Table 1). Obtained results reflect the research conducted by other authors that identified bacteria *L. innocua* in fishery products (Jeyasekaran et al., 1996; Dominguez et al., 2001). Moreover, these bacteria are often present along with *L. monocytogenes* and have very similar atypical haemolytic strains (Petran and Swanson, 1993; Johnson et al., 2004; Moreno et al., 2012). Findings of *L. innocua* in our research reflect the research conducted by Kuzmanović et al. (2011) that identified *L. innocua* in 8.51% of examined fish samples. Our research results (Table 1) confirmed the occurrence of subsequent contamination in fish processing facilities that can according to Papandopoulos et al. (2010) be considered a by-product of fish processing (evisceration, removal of gills, scaling, filleting). Above all, such findings reveal the need for additional caution, primarily because the transfer of *L. innocua* in production facilities resembles the transfer of *L. monocytogenes*. Since these bacteria are ubiquitous, they can survive in extreme conditions such as high pH environments, both high and low temperatures, as well as high levels of salinity (Moreno et al., 2012). Although they are very similar to *L. monocytogenes*, they are not considered a pathogenic bacterial species. Further research on the source and manner of bacterial contamination in fish processing facilities is needed. Upon considering the research conducted by Vitt et al. (2008) and Sabanadesan et al. (2000) on the effects of smoke, i.e. type of liquid smoke preparations and the duration of smoking, we concluded that additional attention should also be paid to parameters of processing fish fillets with smoke.

*L. monocytogenes* was not identified in samples of the examined swabs taken from work surfaces and hands of employees (n = 40) (Table 3). Bacteria *L. innocua* were identified in three instances of both samplings conducted on work surfaces during handling, grading and processing of fish, i.e. in areas designated for evisceration and the control of fish processing, as well as on the hands of employees who operated the process for the filleting of sea bass (15 % of examined swabs). If we compare the occurrence of *L. innocua* on work surfaces in fish processing facilities (Table 3) to conclusions reached by Papandopoulos et al. (2010), we can consider the contamination of marinated and smoked fish by *L. innocua* a result of the occurrence of such bacteria in the environment. Jørgensen et al. (1998) reported that all examined samples of smoked fish in processing facilities involved in their study tested positive for *L. monocytogenes*. The fact that *L. monocytogenes* has not been identified in either fish or products involved in our study does not eliminate a possibility of subsequent contamination in any latter production stage

pertaining to handling, salting, filleting or smoking of fish. Furthermore, Miettinen and Wirtanen (2005) claim that raw fish can be an important source of pollution on equipment and machines, which will result in the contamination of products. In addition, Lakicevic et al. (2015) found that 2.3 % of swabs obtained from production facility samples tested positive for *L. monocytogenes*. Unlike our study, the study conducted by Dimitrijevic et al. (2011) identified *L. monocytogenes* in 7 out of 99 examined swabs (7.0 %) obtained from work surfaces and the hands of employees in fish processing facilities. Authors have identified *L. monocytogenes* in 3 out of 66 examined swabs (4.5 %) in the second production facility.

Findings of any bacterial species and, in particular, *L. monocytogenes*, as well as findings of *L. innocua* (Table 3) during handling, control of fish processing and on hands of employees therefore indicate a strong need for the monitoring and control of hygiene in due facilities. According to the provisions of the Ordinance on the Frequency of Controls and Standards for Microbiological Purity in Facilities under Sanitary Supervision (Official Gazette No. 137/09), assessments of microbiological purity of the facility for the production of food must be undertaken. The number of required samples depends on the type and purpose of such facility, and microbiological purity is assessed based on the number of aerobic mesophilic bacteria and enterobacteria revealed in results of due bacteriological examinations. The results of our study confirm that cleaning, washing and disinfection should be increased, while bacteriological examinations of not only finished products but also swabs that were obtained within the framework of internal control activities should be introduced, all in line with due recommendations for the reduction of *Listeria* bacteria findings in production facilities (Gram, 2004). Since *Listeria* bacteria are ubiquitous, they can survive in different environmental conditions and can hide in brine, cutting machines, areas designated for filleting of fish, work surfaces, floors, etc. We therefore emphasize authors' notes that point out that in order to eliminate bacteria that come in contact with food from production facilities and work surfaces, implemented programmes of good production and hygienic practices must be based on the specific sampling of due environments and obtained evidence of bacteria found in facilities (Rorvik et al., 1995; Autio et al., 1999; Bagge Ravn et al., 2003; Dimitrijevic et al., 2011).

## CONCLUSION

The results of bacteriological examination revealed that samples of fresh fish (sea bass), smoked (gilt-head

and sea bass) and marinated fish (gilt-head bream and sea bass) did not test positive for *L. monocytogenes*. According to the provisions of the regulations on microbiological criteria for foodstuffs (Regulation No. 2073/2005), examined samples of fish and fishery products can be considered satisfactory and microbiologically safe. However, bacteria *L. innocua* were present in samples of fishery products. Furthermore, *L. innocua* was also identified in 6 sampled swabs obtained from fish processing facilities. The results of microbiological analysis of swabs collected from fish processing facilities suggest the violation of hygiene standards and good hygienic practices in such facilities. Recommendations aiming at reducing the number of *Listeria* occurrences indubitably include extensive cleaning, washing and disinfection of facilities, as well as effective implementation of bacteriological examinations intended for not only finished products but also swabs obtained within the framework of internal control activities.

**Acknowledgements:** *This paper also presented a part of results obtained within the framework of the project FP7 Selection and Improving of Fit - for - Purpose Sampling Procedures for Specific Foods and Risks – BASELINE Grant Agreement number 222738; 2009 – 2013.*

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Received: 10.3.2016.

Accepted: 31.3.2016.

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