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Occurrence of *Listeria monocytogenes* in a Serbian salmon and seafood processing line during 2013

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Abstract

The objective of this study was to examine the occurrence of *L. monocytogenes* in a selected fish and seafood processing line. Results showed that during 2013, 12.4 %, 8.3 % and 2.3 % of fish, seafood salads and environmental swabs were positive for *L. monocytogenes*. All positive food samples showed a contamination level below 100 CFU/g. Environmental swabs from surface of slicing and trimming tables, slicing machines, fish filleting and trimming knives, belt glazer and working table were positive for *L. monocytogenes*. Therefore, strict attention must be paid to cleaning and disinfection to control the level of *L. monocytogenes*.

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Keywords: L. monocytogenes; salmon; seafood; swabs; plant

1. Introduction

Listeria monocytogenes, an intracellular pathogen, is a cause of concern to food industries[1], mainly for those producing ready-to-eat (RTE) products. This bacterium, classified in Risk Group 2 for human infection, can differ in

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many aspects from other foodborne pathogens[2]. Namely, it tolerates well salt and nitrite and grows under low oxygen conditions[3]. Seafood is known as a vehicle for the microorganism and has been linked to episodes of listeriosis[4]. Cold smoked salmon, a raw ready-to-eat food, poses a risk to human health if it is contaminated with pathogens along the food chain[5].

Taking into consideration the fact that studies on the prevalence of *L. monocytogenes* in food processing establishments in Serbia are lacking, the objective of the present study was to examine the occurrence of *L. monocytogenes* in a selected fish and seafood processing line during 2013.

2. Materials and methods

2.1. Samples

A total of 811 samples were tested over 1-year period (2013). Food samples consisted of 5 sample units collected from a production batch, which included fresh, hot and cold smoked salmon and seafood salads (squid, octopus and prawns). Samples from the processing environment (swabs from surfaces and drains) were also tested.

2.2. Microbiological method

Isolation and identification of *L. monocytogenes* was performed according to ISO 11290-1[6a.] while enumeration of *L. monocytogenes* was performed following the ISO 11290-2 method[6b].

2.3. Enzyme linked fluorescent assay

The procedure of detecting *L. monocytogenes* by compact automated miniVidas requires an enrichment step as indicated by the VIDAS LMX kit producer (bioMérieux, France). All positive results obtained by miniVIDAS were further confirmed by the ISO 11290-1[6a.] method (culture dependent).

2.4. PCR identification

The total genomic DNA from isolates was extracted using the PrepMan Ultra Sample Preparation reagent (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. PCR was performed in a final volume of 50 µl and thermal cycler 2720 (Applied Biosystems) was used with temperatures set at initial denaturation at 94°C for 5 min, followed by 35 cycles, each with a denaturation phase at 94°C for 30 sec, an annealing phase at 50°C for 45 sec and an extension phase at 72°C for 45 sec, followed by a final extension phase at 72°C for 5 min. Primers LM1 / LM2 were synthesized by Metabion GmbH (Martinsried, Germany) were for the listeriolysin O encoding gene (*hlyA*). The target gene specific for *L. monocytogenes* produced PCR products of 702 bp in size.

3. Results and Discussion

The overview of analyzed samples and occurrence of *L. monocytogenes* in the selected fish and seafood processing line in Serbia during 2013 is presented in Table 1.

Table 1. Overview of analyzed samples and occurence of *L. monocytogenes* in a selected fish and seafood processing line.

Food group	Product type	No of samples	No (%) positive for *L. monocytogenes*
Fish products	Fresh, hot and cold smoked salmon (with / without herbs)	218	27 (12.4 %)
Seafood products	Seafood salads	108	9 (8.3 %)
Environmental samples	Swabs	485	11 (2.3 %)
Total		811	47 (5.8 %)

Namely, *L. monocytogenes* was isolated from 12.4 %, 8.3 % and 2.3 % of fish, seafood salads and environmental swabs, respectively. All *L. monocytogenes* positive food samples (36 out of 326) showed a contamination level below 100 CFU/g. This is comparable with data provided by Uyttendaele et al.[7]. The product with the highest prevalence of *L. monocytogenes* was smoked salmon (Table 1). This high prevalence could be due to the low smoking temperature involved during the cold-salmon processing; as these conditions would be ideal for proliferation of *L. monocytogenes* if the raw salmon harboured the pathogen or acquired the pathogen from the processing environment[8]. In addition to smoked salmon, a relatively high prevalence was observed in seafood salads. Namely, out of 108 seafood salads examined, 8.3 % were positive for *L. monocytogenes*.

Fig. 1. shows PCR results, for 12 *L. monocytogenes* isolates, proved to be positive for *hlyA*.

The second part of our study was related to the detection of *L. monocytogenes* in environmental samples from the fish and seafood processing line (surfaces and drains). Out of 485 environmental samples analyzed, 11 (2.3 %) samples were *L. monocytogenes* positive. Our results demonstrated that environmental swabs from the surface of slicing and trimming tables, slicing machines, fish filleting and trimming knives, belt glazer and work table were positive for *L. monocytogenes*.

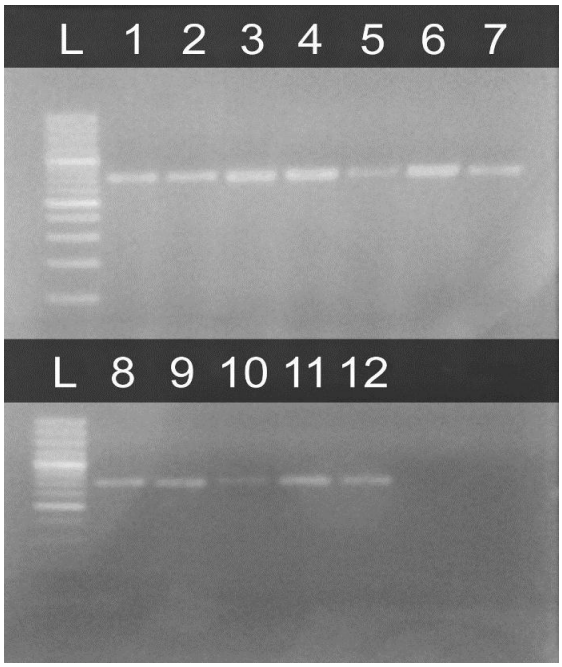

Fig. 1. Agarose gel electrophoresis of the PCR products obtained by using LM1/LM2 primers from 11 positive samples for *L. monocytogenes.*

L-Ladder 100 bp, Lane 1-11 *L. monocytogenes* food isolates, Lane 12 ATCC 19115, Lane 13 Negative control.

4. Conclusion

Listeria monocytogenes should be considered a serious hazard in relatively closed environments such as food processing plants. This study demonstrated that *L. monocytogenes* was present in ready to eat salmon and seafood on a processing line in Serbia, although at low concentrations. Nevertheless, considering that the bacterium has the potential to grow well at refrigerated temperatures and in high salt matrixes such as seafood, its presence in these products should not be overlooked. To minimize the potential for *L. monocytogenes* contamination of finished products, it is necessary to have sanitation controls measures that prevent contamination of product contact surfaces and eliminate niches where *L. monocytogenes* can establish itself, grow, and persist.

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