



The occurrence, transmission, virulence and antibiotic resistance of *Listeria monocytogenes* in fish processing plant

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ABSTRACT

The aim of this research was to investigate the occurrence of *Listeria monocytogenes* in fish and fish processing plant and to determine their transmission, virulence and antibiotic resistance.

L. monocytogenes was isolated according to the ISO 11290–1. The identification of *L. monocytogenes* was confirmed by multiplex PCR method. Genetic similarity of *L. monocytogenes* strains was determined with the Pulsed-Field Gene Electrophoresis (PFGE) method. The multiplex PCR was used for identification of *L. monocytogenes* serogroups and detection of selected virulence genes (*actA*, *fbpA*, *hlyA*, *iap*, *inlA*, *inlB*, *mpl*, *plcA*, *plcB*, *prfA*). The *L. monocytogenes* isolates susceptibility to penicillin, ampicillin, meropenem, erythromycin, trimethoprim/sulfamethoxazole was evaluated with disc diffusion method according to EUCAST v. 7.1.

The presence of 237 *L. monocytogenes* isolates (before genetic similarity assessment) in 614 examined samples was confirmed. After strain differentiation by PFGE techniques the presence of 161 genetically different strains were confirmed. The genetic similarity of the examined isolates suggested that the source of the *L. monocytogenes* strains were fishes originating from farms. All tested strains possessed all detected virulence genes. Among examined strains, the most (26, 38.6%) belonged to the group 1/2a–3a. The most of tested strains were resistant to erythromycin (47.1%) and trimethoprim/sulfamethoxazole (47.1%).

1. Introduction

Since the beginning of the 1990s, the consumption of fish, and especially salmon, in the European Union member countries has increased dramatically (ReportLinker, 2016). With the increase in demand for fish products, the fish processing industry has also developed in Poland. A significant threat to product safety is microbial contamination in the farm environment, which can consequently lead to continuous fish infection (FAO Fisheries Report, 1999). Farms producing and exporting fish pose a direct contamination threat to the target processing plant environment and indirect threat to the health of the consumer (FAO Fisheries Report, 1999). Introducing microbiologically contaminated fish to the processing plant affects the whole production cycle. In subsequent stages of the technological process, the transfer of the microorganism to healthy fish may lead to the contamination of intermediate and ready-to-eat (RTE) products (FAO Fisheries Report,

1999).

Listeria spp. are Gram-positive, rod-shaped, facultatively anaerobes, and do not produce endospores. They have the ability to grow over a wide temperature range (0.5–45 °C), pH (4.7–9.2) and osmotic pressures. These characteristics allow *Listeria* spp. for survival in adverse environmental conditions (Dongyou, 2008; Vázquez-Boland et al., 2001). Among 17 known *Listeria* species, two - *L. monocytogenes* and *L. ivanovii* - are pathogenic for humans (Vos et al., 2009). *L. monocytogenes*, as well as non-pathogenic strains of *L. innocua*, are capable of colonizing the human gastrointestinal tract (Pappelbaum, 2007).

The main source of human listeriosis is contaminated food. Direct infections from human to human are relatively rare (Kołakowska and Madajczak, 2011). *L. monocytogenes* may be present in RTE products with a long shelf life (EFSA, 2017). According to the report of the European Food Safety Authority (EFSA), in 2016 the presence of *L.*

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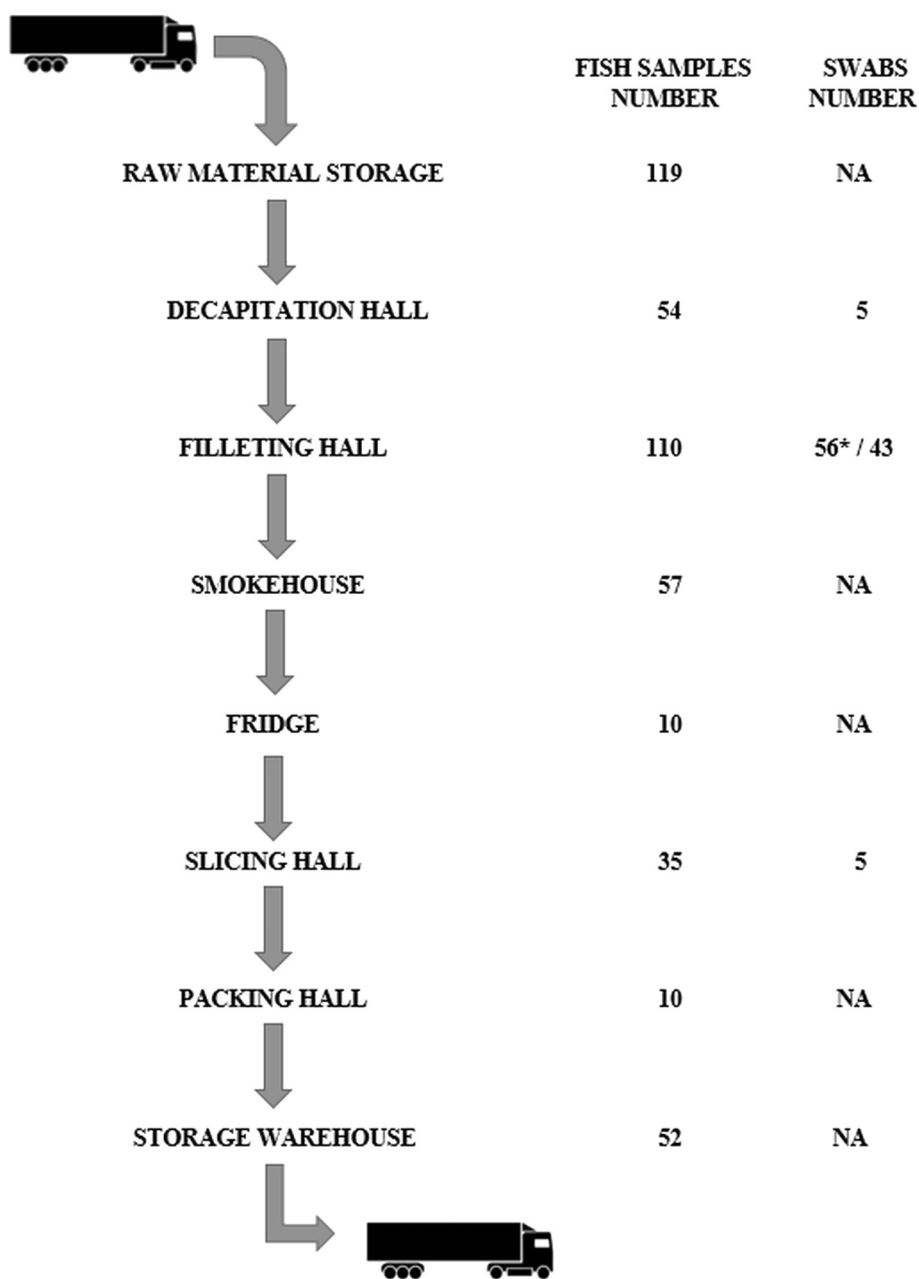


Fig. 1. Scheme of sampling.

monocytogenes has been detected in samples of fishes, poultry, beef and pork, fruits and vegetables, bread, delicatessen products and cheeses (EFSA, 2017). In 2016, EFSA reported 2536 confirmed human cases of listeriosis. (0.47 cases per 100,000 population), which was more than in 2015 (EFSA, 2017). It is estimated that in the USA *L. monocytogenes* causes almost 1600 cases each year, of which 1400 require hospitalization, and 250 of them result in death (Scallan et al., 2011).

A study by EFSA (2017) found that in 2016 fish and fishery products, represented the highest rate of non-compliance with EU standards and stood at 5.6% for fishery products and 4.7% for fish.

Listeria spp. may be a component of water microbiota, especially when contaminated with sewage or animal excrements. *L. monocytogenes* is present on the outer surface of fish swimming in contaminated water (Miettinen and Wirtanen, 2005). The presence of these bacteria is also observed in the gastrointestinal mucosa and gills (Jami et al., 2014; Pizarro-Cerdá and Cossart, 2006). There are two pathways of potential fish contamination with *L. monocytogenes* in fish processing plants: (Autio et al., 1999) the spread of intestinal bile to other tissues (including muscles), especially when the time between death and removal of the bowel is longer than several hours;

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