



Phenotypic and genotypic evaluation of *Listeria monocytogenes* strains isolated from fish and fish processing plants

Krzysztof Skowron¹ · Natalia Wiktorczyk¹ · Katarzyna Grudlewska¹ · Ewa Wałęcka-Zacharska² · Zbigniew Paluszak³ · Stefan Kruszewski⁴ · Eugenia Gospodarek-Komkowska¹

Received: 12 September 2018 / Accepted: 28 December 2018 / Published online: 6 January 2019
© The Author(s) 2019

Abstract

The aim of this study was to perform the phenotypic and genotypic evaluation of *Listeria monocytogenes* strains isolated from fish and equipment used in fish processing plants. The prevalence of selected gene-encoding virulence factors among *L. monocytogenes* strains was assessed by multiplex PCR. The genetic (PFGE method) and protein similarities (MALDI-TOF MS technique) of isolates were determined. Their drug resistance (disk-diffusion method and MIC values), serogroup classification (multiplex-PCR), and the ability to co-aggregate with *Salmonella enteritidis* were also evaluated. Among 37 *L. monocytogenes* isolates, 36 strains were found, one of which included two genetically identical isolates (PFGE method). In all examined strains, the following genes were found: *hlyA*, *plcB*, *plcA*, *inlA*, *inlB*, *prfA*, *iap*, and *actA*. The presence of virulence genes, *mpl*, and *fbpA* was confirmed in 32 (88.9%) strains. It was reported that 30 (83.3%) of the strains belonged to serogroup 1/2a-3a. It was also found that the rate of coaggregation with *S. enteritidis* bacilli was 16.5–36.3%. Among the investigated *L. monocytogenes* strains, 25 (69.4%) were sensitive to all antibiotics used. Resistance to penicillin was reported most often among strains ($n = 6$, 16.7%). The assessment of *L. monocytogenes* virulence level is an important aspect for the protection of public health. It was reported that strains isolated from fish contain genes coding for virulence factors and some of them are antibiotic-resistant. In our study, it was found that strains with a high degree of genetic similarity also showed a high degree of similarity at the level of protein profiles.

Keywords *Listeria monocytogenes* · Virulence genes · Drug resistance · Serogroups · Coaggregation · Fish

Introduction

Listeria monocytogenes is a Gram-positive, rod-shaped, non-spore-forming facultative anaerobes, widespread in the

environment, e.g., soil, water, and sewage. (Camejo et al. 2011; Muskalska and Szymczak 2015). *L. monocytogenes* is an etiological agent of a dangerous disease—listeriosis, which occurs mainly in immunocompromised people, the elderly, pregnant women, and newborns. In recent years, an increasing number of recognized listeriosis cases and resistance to antibiotics has become a serious problem (Gahan and Hill 2005; Schuppler and Loessner 2010).

The main source of *L. monocytogenes* is food products, including dairy, meat (mainly raw), fish (smoked and raw), as well as fresh fruit and vegetables (Gandhi and Chikindas 2007; Jami et al. 2014). According to the EFSA (European Food Safety Authority) data, in 2016, the most frequently implicated in *L. monocytogenes* infection were fish and fishery products. The presence of *L. monocytogenes* was confirmed in 5.6% of fish products and 4.7% of ready-to-eat (RTE) fish products (EFSA 2017). There are two ways of potential fish contamination with *L. monocytogenes* in fish processing plants: (1) the spread of bacilli from the intestinal content to other tissues (including muscles), especially when the time between death

✉ Krzysztof Skowron
skowron238@wp.pl

¹ Department of Microbiology, Ludwik Rydygier Collegium Medicum, Nicolaus Copernicus University in Toruń, 9 M. Skłodowskiej-Curie Street, 85-094 Bydgoszcz, Poland

² Department of Food Hygiene and Consumer Health, Wrocław University of Environmental and Life Sciences, 31 C.K. Norwida St., 50-375 Wrocław, Poland

³ Department of Microbiology and Food Technology, UTP University of Science and Technology, 6 Bernardyńska St., 85-029 Bydgoszcz, Poland

⁴ Biophysics Department, Faculty of Pharmacy, Collegium Medicum of Nicolaus Copernicus University, Jagiellońska 13-15 St., 85-067 Bydgoszcz, Poland

and removal of viscera is longer than a few hours, and (2) cross-contamination due to improper transport conditions or use of contaminated fish processing equipment (Jami et al. 2014). The contamination of fish may also occur during processing, such as filleting, rinsing, and salting (Gambarin et al. 2012; Jami et al. 2014). Secondary food contamination in processing plants may be the result of biofilm formation by *L. monocytogenes* on abiotic surfaces (De Oliveira et al. 2010).

L. monocytogenes bacilli isolated from fish and fish products most often include serotype 1/2a and less often 4b, while strains colonizing devices and surfaces in fish processing plants mainly include serotypes: 1/2a, 1/2b, and 4b (Gudmundsdóttir et al. 2005; Jami et al. 2014).

In 2016, 28 European countries reported a total of 2536 listeriosis incidences among humans (an upward trend as compared to 2008–2015) (EFSA 2017). In 19 countries, 247 deaths were reported due to invasive listeriosis (EFSA 2017). Moreover, between January 1, 2017, and April 27, 2018, 1024 cases of listeriosis were diagnosed in the Republic of South Africa, of which 200 were fatal (The Health Department of Republic of South Africa 2018). Ready-to-eat processed meat products were the source of pathogenic bacilli (The Health Department of Republic of South Africa 2018). In mid-October 2018, data on 12 listeriosis cases related to the consumption of salmon products in three EU countries (Denmark (six cases), Germany (five cases), France (one case)) were published. Four patients died from infection with *L. monocytogenes* (ECDC 2018).

The aim of the study was to evaluate the prevalence of selected genes encoding virulence factors among *L. monocytogenes* strains isolated from fish and equipment colonized by the bacilli in fish processing plants. Additionally, their drug resistance, genetic and proteomic similarity, and the level of coaggregation with *Salmonella enteritidis* were evaluated.

Material and methods

Material

During the research, 283 samples were taken—197 samples of fish and 86 swabs from devices used in fish processing plants. The biological material consisted of 37 isolates of *L. monocytogenes*, including 26 (13.2% positive samples) isolates obtained from fish, i.e., fresh fillet (12 isolates from 94 samples—12.8%), whole salmon (raw material) (ten isolates from 64 samples—15.6%), smoked fillet (four isolates from 39 samples—10.3%), and 11 (12.8% positive samples) isolates from devices used in fish processing plants, i.e., skinning machine (three isolates from 11 swabs—27.3%), brine (two isolates from 11 swabs—18.2%), guillotine (one isolate from 11 swabs—9.1%), slicer conveyor rollers (one

isolate from 12 swabs—8.3%), slicer control panel (one isolate from 10 swabs—10.0%), slicer conveyor tape (one isolate from 10 swabs—10.0%), rolls of fish bone remover (one isolate from 11 swabs—9.1%), injector needles (one isolate from 10 swabs—10.0%); located in northwestern Poland. These isolates were collected in 2015. The reference strain *Salmonella enteritidis* ATCC 13076 and five various reference strains of *L. monocytogenes* were included in the study, depending on the application described below.

Isolation of *L. monocytogenes* strains

Analysis of the intermediate and finished product samples was carried out in accordance with the ISO 11290-1 (ISO 11290-1 2017). Samples of fish meat (25 g) were homogenized with 225 ml of half-Fraser broth (Merck). In the case of swabs from devices used in fish processing plants, the gauze was immersed in 100 ml of half-Fraser broth (Merck). Samples were incubated at 30 °C for 24 h. Then 0.1 ml of the culture was introduced into 10 ml of Fraser broth (Merck) and the secondary selective enrichment was performed at 37 °C for 48 h. After incubation, a reductive inoculation of material from the culture was performed on the agar plate according to Ottaviani and Agosti (ChromoCult® *Listeria* Selective Agar, ALOA®, Merck). Cultures were incubated for 24 h at 37 °C. Selected colonies, initially identified according to the manufacturer's recommendations as *Listeria* spp. were transferred to Columbia Agar with 5% sheep blood (bioMérieux). Then, the hemolysis type was assessed and finally, identification was performed using the PCR method. Next, the obtained and identified isolates were frozen and stored in brain-heart infusion broth (BHI, Merck) with 15% glycerol (Avantor) at –80 °C.

Isolation of genomic DNA

Isolation of genomic DNA was performed using the Genomic Mini AX Bacteria Spin Kit (A&A Biotechnology) according to the manufacturer procedure.

Species identification of the isolates tested

Identification of examined isolates was made on the basis of the multiplex PCR reaction. Two pairs of L1 (5'-CAGC AGCCGCGGTAATAC-3') and L2 primers (5'-CTCC ATAAAGGTGACCT-3'), target gene: *rrs* (product size 938 bp) (Border et al. 1990), as well as LM1 (5'-CCTA AGACGCCAATCGAA-3') and LM2 (5'-AAGC ACTTGCAACTGCTC-3'), target gene: *hlyA* (product size 750 bp) were applied (Bansal et al. 1996). The following reaction mixture (25 µl) was used: 1.5 × PCR buffer (Promega), 2 mM MgCl₂ (Promega), 1.25 mM dNTPs (Promega), 0.5 µM of each primer (Oligo.pl), 1 U GoTaq DNA polymerase

Table 1 Primer sequences and amplification conditions

Reaction	Virulence gene	Primer sequences (5'-3')	Amplicon size [bp]	Reference	Amplification conditions
MIX I	<i>fbpA</i>	TTATTTCCCTCGCATCCTAGC TATCAAITTCGACCTGCTGAG	435	This study	Initial denaturation—94 °C/2 min. 35 cycles: denaturation—94 °C/15 s, annealing—48.5 °C/30 s, elongation—72 °C/50 s final elongation—72 °C/1 min
	<i>plcA</i>	ACACGAGCAATAAAATCCCT ATACTGACGAGGTGTGAATG	278	Suo et al. (2010)	
	<i>hlyA</i>	TTTTCGAITGGCGTCTTAGGA ACTGAAGCAAAAGGATGCATC TG	101	This study	
MIX II	<i>plcB</i>	GCAAGTGTCTAGTCTTTCCGG ACCTGCCAAAAGTTTGCTGTGA	794	Franciosa et al. (2005)	Initial denaturation—94 °C/2 min 16 cycles: denaturation—94 °C/30 s, annealing—57 °C/45 s, elongation—72 °C/45 s
	<i>inlB</i>	TCCGACTAAACAAGGCTATG TGTACCATAAATTTCCGCCA	302	this study	19 cycles: denaturation—94 °C/30 s, annealing—49 °C/45 s, elongation—72 °C/45 s
	<i>actA</i>	ACGAAACAAAAGCAGACCTAAT TGTACCATAAATTTCCGCCA	231	this study	Final elongation—72 °C/1 min
	<i>iap</i>	ACAAAGCTGCACCTGTTGCAG TGACAGCGTGTAGTAGCA	131	Rawool et al. (2007)	
	<i>inlA</i>	CAGGCAGCTACAATTACACA ATATAGTCCGAAAACCCACATCT	2341	Franciosa et al. (2005)	
MIX III	<i>mpl</i>	TATGACGGTAAAAGCAGATT TTCCCAAGCTTCAGCAACTT	1458		
	<i>prtA</i>	CATGAACGCTCAAGCAGAAG AATTTTCCCAAGTAGCAGGA	706		

(Promega), ultrapure water (Sigma Aldrich), and previously isolated genomic DNA. Amplification conditions included initial denaturation at 94 °C (2 min.); 30 cycles of DNA denaturation at 94 °C (30 s), primer annealing at 50 °C (30 s), and primer elongation at 72 °C (1 min); final elongation of the primers at 72 °C (5 min). The obtained amplification products were separated in 1.5% agarose gel (Sigma Aldrich) stained with Midori Green (NIPPON Genetics EUROPE GmbH) in 1 × TBE buffer (BioRad), in the presence of a DNA size standard (GeneRuler™1000 bp DNA Ladder) (Fermentas) (90 V, 1 h).

Evaluation of genetic similarity (PFGE)

After confirming the species identity, the genetic similarity of the selected *L. monocytogenes* strains was determined with the pulsed-field gel electrophoresis (PFGE). The procedure for genotyping was performed in accordance with the standard operating procedure for PulseNet PFGE of *Listeria monocytogenes* (PNL04, April 2013). To determine the degree of genetic similarity between isolates, a phylogenetic dendrogram was drawn in the CLIQS 1D Pro program (TotalLab). Clustering analysis was performed using hierarchical clustering with the UPGMA technique and Dice's coefficient. The PFGE technique was the main method of evaluating the genetic similarity of the isolates tested.

Determination of protein similarity

Protein similarity of isolates was determined by using the MALDI—TOF MS method (matrix-assisted laser desorption ionization time of flight, mass spectrometry) in accordance with the manufacturer procedure with MALDI Biotyper (Bruker). To evaluate the level of similarity between the isolates tested, a dendrogram was drawn using the software of the equipment producer—ClinProt-Tools. The analysis of clustering was prepared based on the principal component analysis (PCA).

Molecular serotyping of *L. monocytogenes* strains

Multiplex PCR for the identification of the main *L. monocytogenes* serogroups (1/2a-3a, 1/2b-3b, 1/2c-3c, and 4b-4d-4e) was performed as described by Doumith et al. (2004). The four selected *L. monocytogenes* strains kindly provided by Walecka-Zacharska et al. (2013) were used as control strains for serogroups identification.

Detection of selected virulence genes

The multiplex PCR technique was used to determine the frequency of ten selected virulence genes occurrence among *L. monocytogenes*. Three separate PCR reactions were prepared: MIX I (genes *fbpA*, *plcA*, *hlyA*), MIX II (genes *plcB*, *inlB*, *actA*, *iap*), and MIX III (genes *inlA*, *mpl*, *prfA*) (Table 1). The multiplex PCR reactions were set using the previously isolated genomic DNA. The *L. monocytogenes* IW 41 strain, possessing all detected genes, was used as for the reference.

The reaction mixture (25 µl) contained 1 × PCR buffer (Promega), 25 mM MgCl₂ (ABO), 10 mM dNTPs (Promega), 10 µM of each primer (Oligo.pl), 1 U Taq DNA polymerase (Promega), ultrapure water (Sigma Aldrich), and 2 µl DNA. The amplification conditions and primer sequences are presented in Table 1. The separation of PCR products was performed in 1.5% agarose gel (Sigma Aldrich) with an addition of the intercalating dye Midori Green (NIPPON Genetics EUROPE GmbH).

Evaluation of drug resistance

The evaluation was performed using the disk-diffusion method. Briefly, 24-h bacterial cultures were diluted in 0.9% saline solution (Avantor) OD of 0.5 Mac Farland standard. The prepared suspensions were plated on MHF medium (Mueller Hinton Agar with 5.0% horse blood and 20 mg/L β-NAD, bioMérieux) and then antibiotic discs were added. The

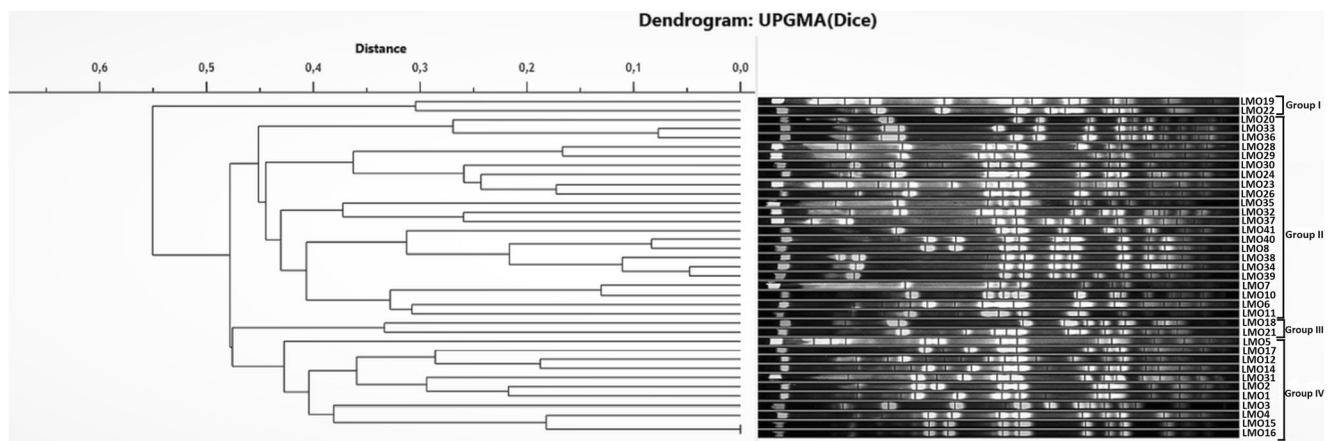


Fig. 1 Phylogenetic dendrogram of the isolates tested

Table 2 Phenotypic and genotypic characterization of *L. monocytogenes* strains tested (S-sensitive, R-resistant, P-penicillin, AM-ampicillin, MEM-meropenem, E-erythromycin, SXT-cotrimoxazole)

Strain number	Origin	Serogroup	Virulence genes	Drug resistance	Coaggregation level with <i>S. enteritidis</i>
1	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	32.2
2	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	31.0
3	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	27.6
4	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	36.3
5	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	29.8
6	Smoked fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	32.7
7	Smoked fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	35.1
8	Whole salmon; raw material	1/2b-3b	<i>hlyA, plcA, iap, plcB, inlB, actA, inlA, prfA</i>	R: -; S: P, AM, MEM, E, SXT	35.7
11	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	35.1
14	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	32.8
15	Fresh fillet	4b-4d-4e	<i>hlyA, plcA, iap, plcB, inlB, actA, inlA, prfA</i>	R: -; S: P, AM, MEM, E, SXT	35.7
17	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	32.8
19	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	32.8
20	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, iap, plcB, inlB, actA, inlA, prfA</i>	R: -; S: P, AM, MEM, E, SXT	33.9
21	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: P; S: AM, MEM, E, SXT	32.8
24	Fresh fillet	4b-4d-4e	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: E; S: P, AM, MEM, SXT	24.6
26	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	26.3
28	Whole salmon; raw material	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	19.3
29	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: P; S: AM, MEM, E, SXT	29.8
33	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	32.8
34	Smoked fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: MEM, SXT; S: P, AM, E	28.1
35	Smoked fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: MEM, E; S: P, AM, SXT	32.2
37	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	33.9
38	Fresh fillet	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	28.1
40	Whole salmon; raw material	4b-4d-4e	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: P, AM, E; S: MEM, SXT	35.1
41	Fresh fillet	4b-4d-4e	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: P; S: AM, MEM, E, SXT	35.1
10	Skimming machine	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	33.9
12	Guillotine	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	16.5
16	Brine	4b-4d-4e	<i>hlyA, plcA, iap, plcB, inlB, actA, inlA, prfA</i>	R: -; S: P, AM, MEM, E, SXT	35.7
18	Slicer conveyor rollers	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	29.2
22	Rolls of fish-bone remover	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: E; S: AM, MEM, E, SXT	33.9
23	Slicer conveyor tape	4b-4d-4e	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	22.8
30	Skimming machine	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: -; S: P, AM, MEM, E, SXT	29.8
31	Skimming machine	1/2a-3a	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	R: P, AM, E; S: MEM, SXT	25.9

Table 2 (continued)

Strain number	Origin	Serogroup	Virulence genes	Drug resistance	Coaggregation level with <i>S. enteritidis</i>
32	Rolls of fish-bone remover	1/2a–3a	<i>hlyA</i> , <i>plcA</i> , <i>fbpA</i> , <i>iap</i> , <i>plcB</i> , <i>inlB</i> , <i>actA</i> , <i>inlA</i> , <i>mpl</i> , <i>prfA</i>	R: P, S; AM, MEM, E, SXT	28.1
36	Injector needles	1/2a–3a	<i>hlyA</i> , <i>plcA</i> , <i>fbpA</i> , <i>iap</i> , <i>plcB</i> , <i>inlB</i> , <i>actA</i> , <i>inlA</i> , <i>mpl</i> , <i>prfA</i>	R: -, S; P, AM, MEM, E, SXT	35.1
39	Brine	1/2a–3a	<i>hlyA</i> , <i>plcA</i> , <i>fbpA</i> , <i>iap</i> , <i>plcB</i> , <i>inlB</i> , <i>actA</i> , <i>inlA</i> , <i>mpl</i> , <i>prfA</i>	R: SXT; S; P, AM, MEM, E	35.1

susceptibility of isolates to penicillin (1 IU), ampicillin (2 µg), meropenem (10 µg), erythromycin (15 µg), and cotrimoxazole (1.25–23.75 µg) was evaluated. The prepared antibiograms were incubated at 35 °C for 20 h. After the incubation period, growth inhibition zones around the antibiotic discs were measured. In order to confirm the results obtained by the disk-diffusion method, the MIC (minimum inhibitory concentration) values were determined by the broth microdilution method in the titration plate according to Wiegand et al. (2008). The results were analyzed in accordance with the EUCAST (The European Committee on Antimicrobial Susceptibility Testing) v. 8.0 recommendations (EUCAST 2018).

Assessment of coaggregation ability between *L. monocytogenes* isolates and *S. enteritidis*

Assessment of coaggregation ability was performed with quantitative assays of bacterial coaggregation in suspension with spectrophotometric measurements described by Kinder and Holt (1994).

Statistical analysis

The statistical analysis was performed in Excel (Microsoft) and Statistica 12.0 PL (StatSoft) software. To check the dependencies between categorical variables, the chi-square homogeneity test, chi-square independence test, and Fisher's exact test were used. The differences were considered statistically significant at the probability level $p < 0.05$.

Results

Species identification within the tested isolates

The presence of *rrs* and *hlyA* genes was demonstrated in all 37 examined isolates, which confirmed that they belonged to the *L. monocytogenes* species.

Evaluation of genetic (PFGE) and protein (MALDI—TOF MS) similarity levels

The analysis of genetic similarity (PFGE) between 37 *L. monocytogenes* strains revealed 36 genotypes (Fig. 1). Strains 15 and 16 isolated from the fresh fillet and brine, respectively, had the same PFGE profile (Table 2). Four main phylogenetic groups of *L. monocytogenes* were distinguished (Fig. 1). Group I included two (5.6%) strains, group II—22 (69.4%) strains, group III—two strains (5.6%), and group IV—11 (30.6%) strains (Fig. 1). The genetic similarity of strains belonging to group I was approx. 69%, group II—approx. 55–92%, group III—approx. 66%, and to group

IV—approx. 58 to 100% (Fig. 1). The highest level of genetic similarity (95%) among the investigated bacilli was recorded for strains with the numbers 34 and 39 (Fig. 1). Among the isolates obtained from devices used in fish processing, most strains were classified into the II ($n = 6$; 60.0%), IV ($n = 3$; 30.0%) phylogenetic groups, and less to groups I ($n = 1$; 10.0%) and III ($n = 1$; 10.0%) (Fig. 1). Strains isolated from fish usually represented the III ($n = 16$; 61.545) and IV ($n = 8$; 30.8%) phylogenetic groups. Phylogenetic groups I and III contained only one strain isolated from fish, each (Fig. 1).

Based on MALDI-TOF MS profiles, three groups of similarity were distinguished (Fig. 2). The first cluster ($n = 16$, 44.44%) mostly included bacilli isolated from fish ($n = 14$, 53.9%). The second cluster included 13 strains (36.11%), of which nine (34.62%) were obtained from fish and four (40.0%) from processing equipment (Fig. 2). A pair of strains numbered 34 and 39 was characterized by the highest level of protein similarity (distance around 0.07) as compared to the other strains of the II cluster. Cluster III was represented by seven strains (19.44%), with the majority of bacilli isolated from production equipment ($n = 4$, 40.0%) as compared to fish-isolated strains ($n = 3$; 11.54%). A high level of protein similarity within cluster III was demonstrated for strains numbered 23 and 26 (distance of approx. 0.1) (Fig. 2). Among the entire population studied, the highest level of protein similarity was demonstrated for isolates numbered 15 and 16

(distance around 0.05). On the other hand, the pair of isolates numbered 15 and 16 showed the lowest protein similarity level (distance around 1.2) with strains numbered 34 and 39 (Fig. 2). The highest similarity at the level of protein profiles, among the strains obtained from fish, was found for strains numbered 34 and 38 (distance of about 0.17). However, strains numbered 20 and 24 were the pair with the lowest similarity (distance around 1.1) (Fig. 2). As for the population of isolates from the processing plant equipment, it was reported that the pair with the highest protein similarity level were strains 23 and 30 (distance around 0.3). The lowest degree of protein similarity (distance of about 1.1) was found for strains numbered 12 and 32 (Fig. 2).

The application of PFGE and MALDI-TOF MS methods, determining the similarity level between strains, allowed to distinguish four and three main monophyletic clusters, respectively (Figs 1 and 2). The comparison of genetic profiles allowed to detect two genetically identical isolates numbered 15 and 16 (genetic similarity rate 100.0%). This pair of isolates was also characterized by the highest protein similarity level among the representatives of the protein cluster I and the entire population studied (distance about 0.05) (Figs 1 and 2). Strains numbered 34 and 39 were the pair with the highest level of protein profile similarity among the representatives of the II protein cluster (distance around 0.07) (Fig. 2). The genetic similarity rate of these strains was also high reaching

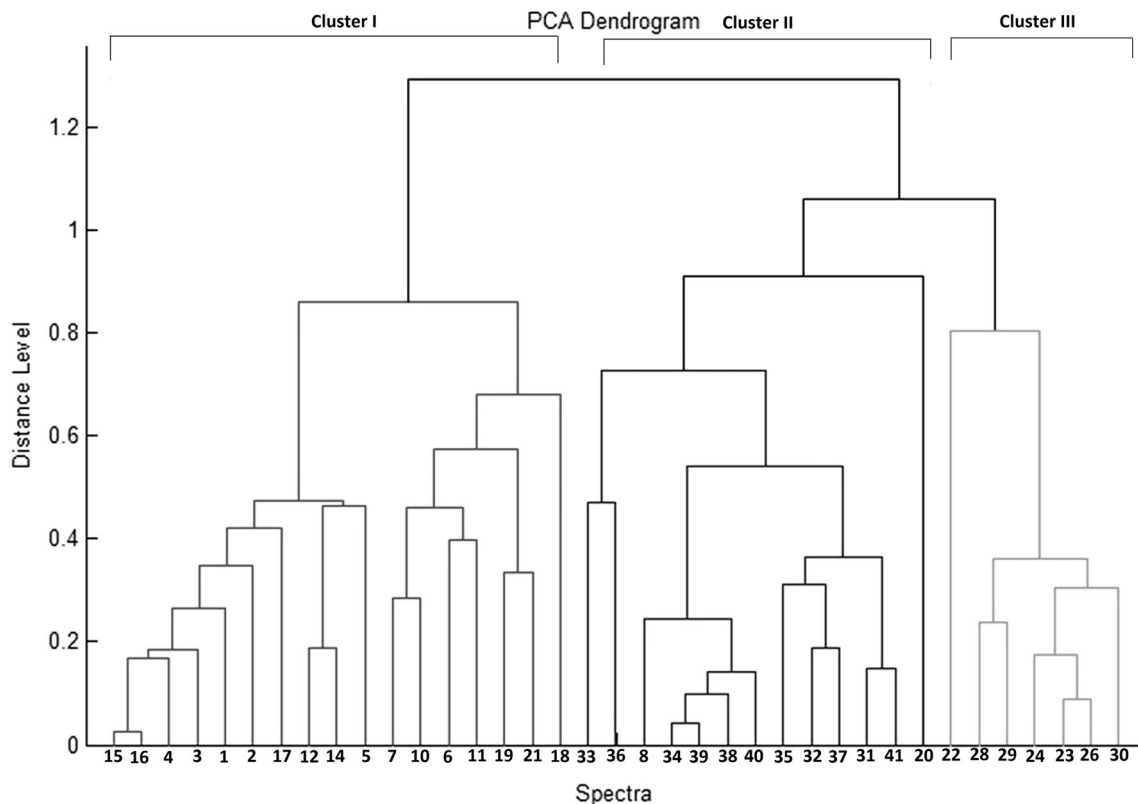


Fig. 2 Dendrogram of the protein similarity of *L. monocytogenes* bacilli tested

Table 3 Serogroup affiliation of the *L. monocytogenes* strains studied

Serological group	Number of strains depending on the origin [<i>n</i> (%)]			Total <i>n</i> = 36
	Fish <i>n</i> = 26	Equipment at the fish processing plant <i>n</i> = 10	<i>p</i> value	
1/2a–3a	21 (80.8%)	9 (90.0%)	<i>0.048</i>	30 (83.33%)
1/2b–3b	1 (3.85%)	0 (0.0%)	<i>0.048</i>	1 (2.78%)
4b–4d–4e	4 (15.4%)	1 (10.0%)	0.251	5 (13.89%)

p value indicating the significance of differences is set in italics

approx. 95% (Fig. 1). In contrast, the lowest similarity at the level of protein profiles (distance of about 1.2) was reported for strains in the arrangement: a pair of isolates numbered 15 and 16 and a pair of strains numbered 34 and 39 (Fig. 2). The level of protein similarity for strains 19 and 21 was at around 0.38, while for strains numbered 22 and 29, at around 0.83 (Fig. 2).

Evaluation of *L. monocytogenes* strains serogroup affiliation

It was reported that 30 (83.3%) *L. monocytogenes* strains belonged to serogroup 1/2a–3a, i.e., 21 (80.8%) strains obtained from fish and nine (90.0%) from equipment used in fish processing (Table 3). A significantly higher percentage of strains belonging to serogroup 1/2a–3a was found among strains isolated from processing equipment than from fish (Table 3). Affiliation in the 1/2b–3b group was confirmed in one strain (2.8%), isolated from whole salmon; raw material (Tables 2 and 3). Five (13.9%) studied strains showed serogroup 4b–4d–4e affiliation, i.e., four fish-originating strains (15.4%) and one (10.0%) from the equipment used in the fish processing plant (Tables 2 and 3).

Detection of selected virulence genes

The *hlyA*, *plcA*, *iap*, *plcB*, *inlB*, *actA*, *inlA*, and *prfA* genes were found in all 36 strains tested (Table 4). There were no significant differences in the frequency of genes encoding virulence factors occurrence between fish- and processing equipment-obtained strains (Table 4). The presence of the *fbpA* and *mpl* genes was found in 32 (88.9%) bacilli studied, i.e., in 23 (88.5%) fish-isolated strains and nine (90.0%) from devices used in fish processing plants (Table 4). Two virulence profiles (A and B) were distinguished (Table 5). Profile A included 33 (91.7%) strains and it was significantly more frequent among isolates from the equipment used in a fish processing plant (10; 100.0%) than among strains isolated from fish (23, 88.5%) (Table 5). Profile B was distinguished only for three (11.54%) strains isolated from fish (Table 5).

Evaluation of drug resistance

The results obtained by the disk-diffusion method and the method of determining the MIC values were completely consistent (Tables 2 and 6). Among the 36 strains tested, 25 (69.44%) were sensitive to all antibiotics used. The remaining 11 (30.6%) were resistant to at least one of the antibiotics

Table 4 Frequency of selected genes occurrence encoding virulence factors among the studied *L. monocytogenes* strains

Gene	Number of strains depending on the origin [<i>n</i> (%)]			Total <i>n</i> = 36
	Fish <i>n</i> = 26	Equipment at the fish processing plant <i>n</i> = 10	<i>p</i> value	
<i>hlyA</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)
<i>plcA</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)
<i>fbpA</i>	23 (88.46%)	9 (90.0%)	0.725	32 (88.89%)
<i>iap</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)
<i>plcB</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)
<i>inlB</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)
<i>actA</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)
<i>inlA</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)
<i>mpl</i>	23 (88.46%)	9 (90.0%)	0.725	32 (88.89%)
<i>prfA</i>	26 (100.0%)	10 (100.0%)	1.000	36 (100.0%)

Table 5 Virulence profiles of *L. monocytogenes* strains tested

Virulence profile symbol	Virulence profile	Number of strains depending on the origin [n (%)]			Total n = 36
		Fish n = 26	Equipment at the fish processing plant n = 10	<i>p</i> value	
A	<i>hlyA, plcA, fbpA, iap, plcB, inlB, actA, inlA, mpl, prfA</i>	23 (88.46%)	10 (100.0%)	<i>0.001</i>	33 (91.67%)
B	<i>hlyA, plcA, iap, plcB, inlB, actA, inlA, prfA</i>	3 (11.54%)	0 (0.0%)	<i>0.001</i>	3 (8.33%)

p value indicating the significance of differences is set in italics

applied (Table 6). Most strains were resistant to penicillin ($n = 6$; 16.7%), i.e., four fish strains (15.4%) and two (20.0%) from processing plants. Resistance to erythromycin was confirmed in three strains isolated from fish (11.5%) and two (20.0%) from production equipment. Resistance to cotrimoxazole ($n = 2$, 5.6%) and ampicillin ($n = 2$, 5.6%) was found in one strain isolated from fish and one obtained from production equipment. Two strains isolated from fish were meropenem-resistant (7.7%), while none of the strains isolated from production equipment presented resistance to this antibiotic.

Seven drug-resistance profiles were distinguished (Table 7). The most numerous one, profile I included 25 strains (69.44%), which were susceptible to all antibiotics tested, and it was statistically more common among fish isolates (19 strains, 73.08%) as compared to isolates from production equipment (six strains, 60.0%) (Table 7). Two strains (5.6%) which presented simultaneous resistance to the three antibiotics tested, i.e., penicillin, ampicillin, and erythromycin, were classified in the IV drug-resistance profile. This type of resistance was found in one strain isolated from fish (3.9%) and one strain (10.0%) obtained from machinery used in fish processing (Tables 2 and 7). The V–VII profiles were the least frequent within the population studied (Table 7). Profile V was confirmed in one strain (10.0%) isolated from the production equipment. It was significantly more frequent in the population of isolates from production equipment than fish (Table 7). On the other hand, profiles VI and VII were more common in the population of fish isolates as compared to production equipment (Table 7).

The highest genetic similarity level (93%) within drug-resistance profile I was demonstrated for strains numbered 33 and 36 (Fig. 1). The similarity level of protein profiles of strains no. 33 and 36 was estimated to be around 0.5 (Fig. 2). The highest similarity at the level of protein profiles among representatives of profile I was demonstrated for the strains numbered 23 and 26; distance around 0.15 (Fig. 2). The genetic similarity rate of these strains was assessed to be around 82% (Fig. 1). Strains numbered 31 and 40, which were resistant to three antibiotics tested (IV profile) belonged to the IV and II phylogenetic groups, respectively (Fig. 1). The protein similarity level of these strains was about 0.57 (Fig. 2).

Assessment of coaggregation ability between *L. monocytogenes* isolates and *S. enteritidis* strain

It was reported that the coaggregation rate between *L. monocytogenes* and *S. enteritidis* reached from 16.5 to 36.3% (Table 2). The highest coaggregation level (36.3%) was found between *S. enteritidis* and strain no. 4 (Table 2). On the other hand, the lowest coaggregation rate (16.5%) was reported between *S. enteritidis* and strain no. 12 (Table 2). The level of coaggregation among strains isolated from the processing plant equipment reached from 16.5 to 35.1%, while among fish isolates from 19.3 to 36.3% (Table 2). The rate of coaggregation among the strains resistant to three antibiotics was 25.9% (strain no. 31) and 28.1% (strain no. 40), respectively (Tables 2 and 6). High and the same level of coaggregation was found among the first phylogenetic group (33.9%) and III (32.8%) strains. The highest level of coaggregation at 35.7% was demonstrated for strains no. 8 (II phylogenetic group) and no. 15 (IV phylogenetic group) (Fig. 1, Table 2).

Discussion

The contamination level of fish and fish products with *L. monocytogenes* is relatively high and amounts to about 7% (EFSA and ECDC 2016; EFSA 2017). Polish fish processing also has quite high levels of *L. monocytogenes* pollution, both in the case of raw materials and finished products, as confirmed by our own research and by the works of Wieczorek and Osek (2017) and Skowron et al. (2018). In our own study, it was shown that the level of fish contamination with *L. monocytogenes* was 13.2%. Similar contamination level of fresh and smoked fish was shown by Wieczorek and Osek (2017). In turn, Skowron et al. (2018) showed the presence of *L. monocytogenes* in 31.1% of fish samples.

L. monocytogenes bacilli isolated from fish, fish products, and fish processing plants most often belong to serotype 1/2a and 4b (Gudmundsdóttir et al. 2005; Jami et al. 2014). In our research, this tendency was confirmed. The affiliation of *L. monocytogenes* in the following serogroups was recorded:

Table 6 The MIC values for *L. monocytogenes* strains tested

Drug	MIC limit value (mg/L)*		MIC value (mg/L)																																				
	S ≤	R >	1	2	3	4	5	6	7	8	11	14	15	17	19	20	21	24	26	28	29	33	34	35	37	38	40	41	10	12	16	18	22	23	30	31	32	36	39
Penicillin	1	1	0.5	0.25	0.25	0.25	0.5	0.125	0.25	0.032	0.5	0.125	0.25	0.25	0.016	0.064	4	0.5	0.25	0.25	0.125	0.25	0.064	0.5	0.125	0.25	0.032	0.5	0.064	0.5	0.032	0.032	0.032	0.016	0.064	8	2	0.125	0.5
Ampicillin	1	1	0.25	0.25	0.5	0.125	0.125	0.25	0.25	0.125	0.064	0.032	0.5	0.064	0.125	0.064	1	0.5	0.032	0.032	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.032	0.032
Meropenem	0.25	0.25	0.008	0.016	0.016	0.008	0.016	0.064	0.016	0.064	0.008	0.125	0.064	0.125	0.064	0.064	0.125	0.064	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.008	0.064	
Erythromycin	1	1	0.25	0.5	0.125	0.064	0.064	0.064	0.125	0.032	0.032	0.064	0.125	0.064	0.032	0.032	0.125	0.016	0.016	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.016	0.016	
Cotrimoxazole	0.06	0.06	0.008	0.008	0.016	0.008	0.008	0.032	0.032	0.032	0.008	0.008	0.016	0.008	0.032	0.016	0.032	0.016	0.008	0.008	0.032	0.032	0.008	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.008	0.008		
Drug	MIC value (mg/L)																																						
Strain number																																							
Fish																																							
Production equipment at the fish processing plant																																							
Strain number																																							
Fish																																							
Production equipment at the fish processing plant																																							

* In accordance with the EUCAST v. 8.0 recommendations
 Numbers in italics—strain resistant to particular antibiotic

Table 7 Drug-resistance profiles of *L. monocytogenes* strains tested (S-sensitive, R-resistant, P-penicillin, AM-ampicillin, MEM-meropenem, E-erythromycin, SXT-cotrimoxazole)

Profile number	Drug-resistance profile	Number of strains depending on the origin [n (%)]			Total n = 36
		Fish n = 26	Equipment at the fish processing plant n = 10	<i>p</i> value	
I	R: -S; P, AM, MEM, E, SXT	19 (73.08%)	6 (60.0%)	<i>0.049</i>	25 (69.44%)
II	R: PS: AM, MEM, E, SXT	3 (11.54%)	1 (10.0%)	0.725	4 (11.11%)
III	R: ES: P, AM, MEM, SXT	1 (3.85%)	1 (10.0%)	0.087	2 (5.56%)
IV	R: P, AM, ES: MEM, SXT	1 (3.85%)	1 (10.0%)	0.087	2 (5.56%)
V	R: SXTS: P, AM, MEM, E	0 (0.0%)	1 (10.0%)	<i>0.001</i>	1 (2.78%)
VI	R: MEM, ES: P, AM, SXT	1 (3.85%)	0 (0.0%)	<i>0.048</i>	1 (2.78%)
VII	R: MEM, SXTS: P, AM, E	1 (3.85%)	0 (0.0%)	<i>0.048</i>	1 (2.78%)

p value indicating the significance of differences is set in italics

1/2a–3a, 1/2b–3b, and 4a–4d–4e. Most strains ($n = 30$, 80.3%) belonged to the group 1/2a–3a, followed by the group 4b–4d–4e ($n = 5$, 16.7%), and one strain (2.8%) to the group 1/2b–3b. In contrast to our research, Montero et al. (2015) reported that serotype 4b was dominant among the tested *L. monocytogenes* strains isolated from food products. However, the serogroup 1/2a–3a, dominant in our study, in the Montero et al. (2015) research was represented only by 32.0% of the population studied where 31.0% of the strains (5 of 18) were isolated from smoked fish. They also found the presence of seven isolates of serotype 1/2b (Montero et al. 2015). The data close to the results of our research was obtained by Martín et al. (2014) and Jamali et al. (2015). Martín et al. (2014) reported that the bacilli of *L. monocytogenes* isolated from meat products represented the following serological groups: 1/2a (36.8%), 1/2c (34.0%), 1/2b (17.9%), and 4b (11.3%). Jamali et al. (2015) reported the following serological affiliation: 1/2a–3a (61.1%); 1/2c–3c (27.8%), and 4b–4d–4e (11.1%). On the other hand, Martín et al. (2014) reported that bacilli of *L. monocytogenes* isolated from meat products represented the following serological groups: 1/2a (36.8%), 1/2c (34.0%), 1/2b (17.9%), and 4b (11.3%). In a study conducted by Wiczorek and Osek (2017), four serogroups of *L. monocytogenes* isolated from fish were identified molecularly. The highest number of strains belonged to serogroup 1/2a–3a (40 isolates, 70.2%), including 33 isolates from marine fish (71.7%) and seven from freshwater fish (63.6%). They showed that affiliation to the serogroup 1/2b–3b–7 was confirmed among 14 strains (24.6%), including 11 (23.9%) isolates from marine fish and three (27.3%) from freshwater fish (Wiczorek and Osek 2017). The results of the study Wiczorek and Osek (2017) are similar to the results of our own research among strains isolated from fish and processing plants.

In the present study, it was found that all strains tested had the following genes encoding virulence factors: *hlyA*, *plcB*,

actA, *inlA*, *inlB*, *iap*, *plcA*, and *prfA*. Also, Kosek-Paszowska et al. (2005) and Aurora et al. (2008) confirmed the presence of the *hlyA* gene among all *L. monocytogenes* strains tested, isolated from poultry meat and dairy RTE products. On the other hand, Park et al. (2012) confirmed the presence of seven *L. monocytogenes* isolates without the *hlyA* gene, but with other virulence genes. The *hlyA* gene in *L. monocytogenes* is coded constitutively and its absence may be the result of a mutation (Park et al. 2012). Jamali et al. (2015) confirmed the presence of the *hlyA*, *actA*, and *iap* genes in all *L. monocytogenes* strains evaluated. In contrast, Al-Nabulsi et al. (2015) found the *iap* gene in only 16.6% ($n = 4$) strains isolated from processed meat. Similar results were obtained by Wiczorek and Osek (2017) and Gelbíčová and Karpíšková (2012), who confirmed the presence of the *plcB* gene among the entire population studied. On the other hand, Jamali et al. (2015) detected the *plcA* gene in 41 (95.3%) strains isolated from fish. By using the PCR technique, Lotfollahi et al. (2014) found that the *plcA* gene was present at a 100.0% frequency among 130 *L. monocytogenes* strains isolated from milk and meat products. Jacquet et al. (2002) reported that the *actA* gene was present at a 100.0% frequency among *L. monocytogenes* strains isolated from food products. On the other hand, Gelbíčová and Karpíšková (2012) confirmed the presence of the *inlA* gene among four strains isolated from the natural environment. The *inlA* gene occurrence, at 88.6% frequency rate, was also confirmed in the study by Mureddu et al. (2014). The presence of the *inlB* gene was reported among 48.6% strains by Al-Nabulsi et al. (2015), however, Jacquet et al. (2002) found the presence of this gene among all 150 strains of *L. monocytogenes* isolated from food products. In the experiment by Jamali et al. (2015), the *prfA* gene was carried by 42 isolates (97.7%). The presence of this gene among all strains tested was also confirmed by Mureddu et al. (2014). The presence of *mpl* and *fbpA* genes was found among 32 (88.89%) strains evaluated in the present

study. In contrast, Montero et al. (2015) found that all analyzed isolates from food products, i.e., raw meat, smoked fish, fruit, and vegetables, had the *mpl* gene. Also, Das et al. (2013) confirmed the presence of the *mpl* gene among all *L. monocytogenes* isolates obtained from seafood.

In recent years, the increasing resistance of *L. monocytogenes* strains to antibiotics has become a serious problem, especially with those used during standard therapy of patients with diagnosed listeriosis (Chen et al. 2010). The *L. monocytogenes* strains investigated in the present study were mostly ($n = 25$, 69.4%) sensitive to each antibiotic used. On the other hand, according to Fallah et al. (2013), only 42 (15.1%) *L. monocytogenes* strains isolated from fish were sensitive to all antibiotics tested, whereas 11 (4.0%) isolates presented resistance to one or more antibiotics. Research conducted in the Polish processing plants demonstrated that all tested isolates (including those from fish) were resistant to ampicillin, gentamycin, amoxicillin, trimethoprim, erythromycin, chloramphenicol, sulfamethoxazole, rifampicin, and vancomycin (Korsak et al. 2012). In the present study, penicillin resistance was most often reported among strains ($n = 6$; 16.7%). Moreover, simultaneous resistance to three antibiotics was reported in two strains (5.6%). Jamali et al. (2015) reported the occurrence of *L. monocytogenes* strains, isolated from open fish markets, resistant to ampicillin ($n = 9/43$, 20.9%) and penicillin ($n = 7/43$, 16.3%). In contrast, Gelbičová and Karpíšková (2012) found no penicillin-resistant strains among the bacteria isolated from various food products. In the present study, resistance to erythromycin was detected in five (13.9%) strains. Research performed by Abdellrazeq et al. (2014) confirmed the resistance to erythromycin in two *L. monocytogenes* strains isolated from frozen fish. According to Jamali et al. (2015), resistance to this antibiotic was found in six isolates (14.0%) obtained from open fish markets. Moreover, they reported that the isolates were often resistant to two antibiotics ($n = 20/43$, 46.5%) (Jamali et al. 2015). Abdollahzadeh et al. (2016) found that all (100.0%) tested strains obtained from seafood and humans in Iran were resistant to ampicillin and cefotaxime, while 57.0% demonstrated resistance to penicillin. In the present study, resistance to meropenem and cotrimoxazole was reported in two strains (5.6%). However, in the study conducted by Ruiz-Bolivar et al. (2011), 44.0% strains, isolated from poultry, cheese, lettuce, spinach, and raw cow milk in Columbia, were resistant to meropenem. On the other hand, Majczynna and Białasiewicz (2006) found that none of the tested strains isolated from pork-minced meat, ready-made products, and frozen vegetables, was resistant to cotrimoxazole. Results of Fallah et al. (2013), Jamali et al. (2015), and Abdollahzadeh et al. (2016) indicate high resistance of *L. monocytogenes* to ampicillin and penicillin, i.e., the most commonly used antibiotics in patients with listeriosis.

Coaggregation plays an important role during surface colonization (including in processing plants) and the formation of biofilms (Kinder and Holt 1994). In the present study, it was found that the level of coaggregation between *L. monocytogenes* and *S. enteritidis* accounted for 16.5–36.3%. Research performed by Gómez et al. (2016) indicated the highest coaggregation rate (69.0%) between *L. monocytogenes* and *Lactobacillus curvatus*, while the lowest (53.4%) between *L. monocytogenes* and *Weissella viridescens*. Janković et al. (2012), on the other hand, found that the coaggregation rate between *Lactobacillus plantarum* and *L. monocytogenes* reached 6.5–39.7%.

The methods of determining the degree of genetic (PFGE) and protein (MALDI-TOF MS) similarity of studied strains obtained from food products and the processing environment were used in the study, which is an important epidemiological aspect. In our study, it was found that strains with a high degree of genetic similarity also showed a high degree of similarity at the level of protein profiles. For strains belonging to the first and third group of genetic similarity (accordingly on the level 69 and 66%), a high degree of coaggregation was found with *S. enteritidis* (accordingly 33.9 and 32.8%). It was found that the degree of protein similarity of strains resistant to three tested antibiotics was 0.57. Abdollahzadeh et al. (2016) showed a high level of genetic similarity of *L. monocytogenes* isolates from fish but did not find any correlation between the isolated pulsotypes of rods and their resistance to antibiotics. Also, Wiczorek and Osek (2017) showed no relation between the antibiotic resistance profile of *L. monocytogenes* and the genotype obtained by PFGE among isolates from Polish processing plants. Park et al. (2012), using the PFGE method and serotyping, found that *L. monocytogenes* tested strains are genetically and serologically heterogeneous. The study of Barbuddhe et al. (2008) shown that the isolated lines of genetic similarity (PFGE) were fully consistent with the data obtained using the MALDI-TOF MS technique. They demonstrated the repeatability, speed, and sensitivity of the analysis as significant advantages of the MALDI-TOF MS method. However, at present, the PFGE technique is the method of choice for studies determining the microorganisms responsible for epidemics (Barbuddhe et al. 2008).

In conclusion, due to the increasing number of recognized listeriosis incidents, there is an urge for continuous monitoring of food contamination with *L. monocytogenes*. Proper food storage, especially of raw fish material, as well as avoiding cross-contamination (also at the stage of retail sales) play an important role in ensuring consumer health safety. Moreover, it was found that the majority of strains isolated from fish processing industry belonged to the serological group 1/2a–3a, the most frequently implicated in human listeriosis. (Doumith et al. 2004) Consequently, secondary food contamination may be the main source of pathogenic bacilli in food processing plants.

Funding This research was financially supported by the Nicolaus Copernicus University with funds from the maintenance of the research potential of the Department of Microbiology DS-UPB no. 782.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not applicable.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- Abdellrazeq GS, Kamar AM, El-Houshy SM (2014) Molecular characterization of *Listeria* species isolated from frozen fish. *AJVS* 40(1):15
- Abdollahzadeh E, Ojagh SM, Hosseini H, Ghaemi EA, Irajian G, Naghizadeh Heidarlo M (2016) Antimicrobial resistance of *Listeria monocytogenes* isolated from seafood and humans in Iran. *Microb Pathog* 100:70–74
- Al-Nabulsi AA, Osaili TM, Awad AA, Olaimat AN, Shaker RR, Holley RA (2015) Occurrence and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw and processed meat products in Amman, Jordan. *CYTA-J FOOD* 13(3):346–352
- Aurora R, Prakash A, Prakash S, Rawool DB, Barbuddhe SB (2008) Comparison of PI-PLC based assays and PCR along with in vivo pathogenicity tests for rapid detection of pathogenic *Listeria monocytogenes*. *Food Control* 19(7):641–647
- Bansal NS, McDonnell FHY, Smith A, Arnold G, Ibrahim GF (1996) Multiplex PCR assay for the routine detection of *Listeria* in food. *Int J Food Microbiol* 33(2–3):292–300
- Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, Chkraborty T, Hain T (2008) Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl Environ Microbiol* 74(17):5402–5407
- Border PM, Howard JJ, Plaster GS, Siggins KW (1990) Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. *Lett Appl Microbiol* 11(3):158–162
- Camejo A, Carvalho F, Reis O, Leitão E, Sousa S, Cabanes D (2011) The arsenal of virulence factors deployed by *Listeria monocytogenes* to promote its cell infection cycle. *Virulence* 2(5):379–394
- Chen B-Y, Pyla R, Kim T-J, Silva JL, Jung Y-S (2010) Antibiotic resistance in *Listeria* species isolated from catfish fillets and processing environment. *Lett Appl Microbiol* 50:626–632
- Das S, Lalitha KV, Thampuran N, Surendran PK (2013) Isolation and characterization of *Listeria monocytogenes* from tropical seafood of Kerala, India. *Ann Microbiol* 63(3):1093–1098
- De Oliveira MMM, Brugnera FD, Alves E, Piccoli RH (2010) Biofilm formation by *Listeria monocytogenes* on stainless steel surface and biotransfer potential. *Braz J Microbiol* 41:97–106
- Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P (2004) Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J Clin Microbiol* 42(8):3819–3822
- European Centre for Disease Prevention and Control (2018) Multi-country outbreak of *Listeria monocytogenes* sequence type 8 infections linked to consumption of salmon products—25 October 2018. Stockholm: ECDC/Parma: EFSA
- European Committee on Antimicrobial Susceptibility Testing (2018) Breakpoints tables for interpretation of MICs and zones diameters. Version 8.0. <http://www.eucast.org>
- European Food Safety Authority (2017) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA J* 5(12):5077
- European Food Safety Authority and European Centre for Disease Prevention and Control (2016) The European Union summary report on trends and sources of zoonoses, Zoonotic agents and food-borne outbreaks in 2015
- Fallah AA, Saei-Dehkordi SS, Mahzounieh M (2013) Occurrence and antibiotic resistance profiles of *Listeria monocytogenes* isolated from seafood products and market and processing environments in Iran. *Food Control* 34(2):630–636
- Franciosa G, Maugliani A, Floridi F, Aureli P (2005) Molecular and experimental virulence of *Listeria monocytogenes* strains isolated from cases with invasive listeriosis and febrile gastroenteritis. *FEMS Immunol Med Microbiol* 43:431–439
- Gahan CG, Hill C (2005) Gastrointestinal phase of *Listeria monocytogenes* infection. *J Appl Microbiol* 98(6):1345–1353
- Gambarin P, Magnabosco C, Losio MN, Pavoni E, Gattuso A, Arcangeli G, Favretti M (2012) *Listeria monocytogenes* in Ready-to-eat seafood and potential hazards for the consumers. *International Journal of Microbiology Article ID 497635*, **10 pages**
- Gandhi M, Chikindas ML (2007) *Listeria*: a foodborne pathogen that knows how to survive. *Int J Food Microbiol* 113(1):1–15
- Gelbíčová T, Karpíšková R (2012) Outdoor environment as a source of *Listeria monocytogenes* in food chain. *Czech J Food Sci* 30(1):83–88
- Gómez NC, Ramiro JMP, Quecan BXV, de Melo Franco BCG (2016) Use of potential probiotic lactic acid Bacteria (LAB) biofilms for the control of *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7 biofilms formation. *Front Microbiol* 7:863
- Gudmundsdóttir S, Gudbjörnsdóttir B, Lauzon HL, Einarsson H, Kristinsson KG, Kristjánsson M (2005) Tracing *Listeria monocytogenes* isolates from cold-smoked salmon and its processing environment in Iceland using pulsed-field gel electrophoresis. *Int J Food Microbiol* 101(1):41–51
- Health Department of Republic of South Africa (2018) Listeriosis outbreak situation report - 27/04/2018. WHO Country Emergency Preparedness and Readiness Technical Meeting Listeriosis Outbreak in collaboration with MoH and Partners. Johannesburg 19–21 April 2018
- ISO 11290-1 (2017) Microbiology of food and animal feeding stuffs - horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method
- Jacquet C, Gouin E, Jeannel D, Cossart P, Rocoourt J (2002) Expression of ActA, Ami, InlB, and Listeriolysin O in *Listeria monocytogenes* of human and food origin. *Appl Environ Microbiol* 68(2):616–622
- Jamali H, Paydar M, Ismail S, Looi C, Wong W, Radmehr B, Abedini A (2015) Prevalence, antimicrobial susceptibility and virulotyping of *Listeria* species and *Listeria monocytogenes* isolated from open-air fish markets. *BMC Microbiol* 15:144

- Jami M, Ghanbari M, Zunabovic M, Doming KJ, Kneifel W (2014) *Listeria monocytogenes* in aquatic food products—a review. *Compr Rev Food Sci Food Saf* 13:798–813
- Janković T, Frece J, Abram M, Gobin I (2012) Aggregation ability of potential probiotic *Lactobacillus plantarum* strains. *Int J Sanit Eng Res* 6(1):19–24
- Kinder SA, Holt SC (1994) Coaggregation between bacterial species. *Methods Enzymol* 236:254–270
- Korsak D, Borek A, Daniluk S, Grabowska A, Pappelbaum K (2012) Antimicrobial susceptibilities of *Listeria monocytogenes* strains isolated from food and food processing environment in Poland. *Int J Food Microbiol* 158(3):203–208
- Kosek-Paszowska K, Bania J, Bystron J, Molenda J, Czerw M (2005) Occurrence of *Listeria* sp. in raw poultry meat and poultry meat products. *Bull Vet Inst Pulawy* 49:219–222
- Lotfollahi L, Pournajaf A, Irajian G, Nowrouzi J (2014) Polymerase chain reaction (PCR4)-based detection of *hly* and *plc-A* genes in *Listeria monocytogenes* isolated from dairy and meat products in Iran. *Afr J Microbiol Res* 8(10):1098–1101
- Majczyna D, Białasiewicz D (2006) Charakterystyka bakterii z rodzaju *Listeria* wyizolowanych z żywności. *Medycyna Doświadczalna i Mikrobiologia* 58(2):119–126
- Martín B, Perich A, Gómez D, Yangüela J, Rodríguez A, Garriga M, Aymerich T (2014) Diversity and distribution of *Listeria monocytogenes* in meat processing plants. *Food Microbiol* 44:119–127
- Montero D, Boderó M, Riveros G, Lapierre L, Gaggero A, Vidal RM, Vidal M (2015) Molecular epidemiology and genetic diversity of *Listeria monocytogenes* isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile. *Front Microbiol* 384
- Mureddu A, Mazza R, Fois F, Meloni D, Bacciu R, Piras F, Mazzette R (2014) *Listeria Monocytogenes* persistence in ready-to-eat sausages and in processing plants. *Ital J Food Saf* 3(1):1697
- Muskalska KB, Szymczak B (2015) Postępy badań nad bakteriami rodzaju *Listeria*. *Postępy Mikrobiologii* 54(2):123–132
- Park S, Jung J, Choi S, Oh Y, Lee J, Chae H, Ryu S, Jung H, Park G, Choi S, Kim B, Kim J, Zoo Chae Y, Jung B, Lee M, Kim H (2012) Molecular characterization of *Listeria monocytogenes* based on the PFGE and RAPD in Korea. *Adv Microbiol* 2(4):605–616
- PNL04 (2013) Standard Operating Procedure for PulseNet PFGE of *Listeria monocytogenes*
- Rawool DB, Malik SVS, Barbudde SB, Shakuntala I, Aurora RA (2007) A multiplex PCR for detection of virulence associated genes in *Listeria monocytogenes*. *Ital J Food Saf* 9:56–62
- Ruiz-Bolivar Z, Neuque-Rico MC, Poutou-Piñales RA, Carrascal-Camacho AK, Mattar S (2011) Antimicrobial susceptibility of *Listeria monocytogenes* food isolates from different cities in Colombia. *Foodborne Pathog Dis* 8(8):913–919
- Schuppler M, Loessner MJ (2010) The opportunistic pathogen *Listeria monocytogenes*: pathogenicity and interaction with the mucosal immune system. *Int J Inflam*. <https://doi.org/10.4061/2010/704321>
- Skowron K, Kwiecińska-Piróg J, Grudlewska K, Świeca A, Paluszak Z, Bauza-Kaszewska J, Wałęcka-Zacharska E, Gospodarek-Komkowska E (2018) The occurrence, transmission, virulence and antibiotic resistance of *Listeria monocytogenes* in fish processing plant. *Int J Food Microbiol* 282:71–83
- Suo B, He Y, Tu S-I, Shi X (2010) A multiplex real-time polymerase chain reaction for simultaneous detection of *Salmonella* spp., *Escherichia coli* O157, and *Listeria monocytogenes* in meat products. *Foodborne Pathog Dis* 7(6)
- Wałęcka-Zacharska E, Kosek-Paszowska K, Bania J, Karpíšková R, Stefaniak T (2013) Salt stress-induced invasiveness of major *Listeria monocytogenes* serotypes. *Lett Appl Microbiol* 56(3):216–221
- Wieczorek K, Osek J (2017) Prevalence, genetic diversity and antimicrobial resistance of *Listeria monocytogenes* isolated from fresh and smoked fish in Poland. *Food Microbiol* 64:164–171
- Wiegand I, Hilpert K, Hancock REW (2008) Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc* 3(2):163–175