Praca oryginalna ***Original paper***

Distribution of *Listeria monocytogenes* serogroups in food of animal origin produced in Poland during 2013-2022

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Received 14.11.2023 *Accepted 12.12.2023*

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Summary

***Listeria monocytogenes* is an important food-borne pathogen that causes severe disease in humans. Many different foods products may be vehicles of these pathogenic strains. The aims of this study were to identified serogroups *L. monocytogenes* isolates by using PCR and analyze the distribution of serogroups in different food products of animal origin. *L. monocytogenes* isolates from ready-to-eat (RTE) meat products (n = 1,571), non-meat RTE food (n = 237), and non-RTE products (n = 189) were collected during years 2013-2020. A total of 899 (45.0%) isolates, irrespective of the origin, were classified to serogroup IIa. The remaining 1,098 strains belonged either to serogroups IVb (478; 23.9%), IIc (347; 17.4%) or IIb (273; 13.7%), respectively. In the predominant group products, i.e. RTE food of meat origin, serogroup IIa (702; 44.7%) was the most common followed by IVb (334; 21.3%), IIc (300; 19.1%), and IIb (235; 14.9%), respectively. The statistical differences were observed between the prevalence of *L. monocytogenes* serogroup IIb in sausages and cold cuts ($P < 0.0001$), cold cuts and delicatessen food ($P < 0.001$), as well as strains of serogroup IVb from sausages and cold cuts ($P < 0.001$). The prevalence of serogroup IIa was statistically higher among strains of milk and milk products origin than RTE fish and fish products ($P < 0.05$). The differences ($P < 0.0001$) were also observed in the distribution of *L. monocytogenes* of serogroup IIa from mixed food and milk as well as fish products. Additionally, the occurrence of serogroup IVb in mixed food differed compared to other food categories, i.e. milk and fish products ($P < 0.0001$). The obtained results confirmed the presence of *L. monocytogenes* serogroups IIa and IVb in food of animal origin, including RTE products, that are responsible for listeriosis in humans and suggested that these food products may pose a potential health problem, particularly to sensitive groups of people.**

Keywords: *L. monocytogenes*, molecular serogroups, ready-to eat (RTE) food, non-RTE food

Listeria monocytogenes is one of the major food-borne pathogens in humans, mainly due to high rates of hospitalization and death. The bacteria are widely distributed in many environments, including soil and animals (15). The main route of transmission of this microorganism to humans is consumption of contaminated food, especially of animal origin. These products are exposed to the bacteria during different steps of processing, transportation and storage (24, 33). A special risk for public health constitutes ready-to-eat (RTE) foods in which *L. monocytogenes* can easily survive and even multiply. Recently, the consumption of RTE food has increased, among others, as a result of lifestyle changes, curiosity about various culinary dishes and the effects of the COVID-19 pandemic. The increase in consumer demand for RTE products has a positive impact on the retail and food industries. However, RTE foods may be more or less susceptible to microbiological hazards depending on the preparation method, and therefore, new microbiological food safety issues arising that need to be taken into account (6).

In the European Union the microbiological criteria presented in the Commission Regulation (EC) No. 2073/2005 specify microbiological requirements for various food categories in relation to the absence or the number of *L. monocytogenes* (30). Therefore, food industries carry out microbiological tests to determine the fulfillments with the above-mentioned criteria.

The incidence of *L. monocytogenes* varies depending on, e.g. food category and a sampling stage. According to the European Food Safety Authority (EFSA) and European Center for Disease Prevention and Control

(ECDC) recent report the highest occurrence (from 2% to 5%) of *L. monocytogenes* in RTE food in 2021 was observed in fish and fishery products, meat products from bovines or pigs, fruits, vegetables, and cheeses from sheep milk (14). Additionally, many multi-country outbreaks of listeriosis which caused several death cases have been resulted from the consumption of a wide range of foods, such as RTE meat, frozen vegetables, cheese and smoked fish (10-13, 28, 32).

All bacterial isolates identified as *L. monocytogenes* should be considered as pathogenic for humans; however, some strains are more often than others responsible for induction of severe symptoms of listeriosis. In particular, serotypes 4b and 1/2b (genetic lineage I) as well as 1/2a and 1/2c (genetic lineage II) are important from the public safety point of view since such strains are in the vast majority isolated from foods and patients with listeriosis (27).

The conventional *L. monocytogenes* serotyping using polyclonal antisera is time consuming and laborious; therefore, nowadays molecular techniques are increasingly applied to identify the major serotypes mentioned above. However, it should be underlined that the PCR tests based on the identification of differences in the nucleotide sequences of some *L. monocytogenes* genes do not enable distinguishing between particular serotypes, so the strains are classified to serogroups; i.e. serogroup IIa comprised of strains 1/2a and 3a serotypes, IIb serogroup covers 1/2b, 3b and 7 serotypes, serogroup IIc consists of 1/2c and 3c serotypes, and serogroup IVb includes serotypes 4b, 4d, and 4e (8, 27). Most listeriosis epidemics in the world are caused by *L. monocytogenes* serogroup IVb and, to a lesser extent, by serogroup IIa. Isolates of IIa serogroup are more common in food, which may be due to their higher resistance to adverse environmental conditions in food-production areas (27, 35).

The aims of this study were to: (i) assign molecular serogroups of *L. monocytogenes* originated from different food of animal origin produced in Poland, and (ii) assess the frequency of the presence of *L. monocytogenes* serogroups in different food products.

Material and methods

Bacterial strains. *L. monocytogenes* isolates were collected during the years 2013-2022 and were recovered from meat RTE food (n = 1,571), non-meat RTE food (n = 237), and non-RTE products (n = 189). In all these categories the foods were divided depending on whether they were heat-treated or not. The RTE meat products consist of sausages, cold cuts, and delicatessen food. Among not heat-treated sausages, various kinds of cured sausages were included, such as salami or meat. The cold cuts group consisted of dried, smoked and cooked whole muscle products like ham, tenderloin and bacon. The delicatessen food like beef tartare (as not-heat treated products) and different kinds of pork brawn, pate, lard, blood sausage (heat-treated food) were also source of *L. monocytogenes*.

The group of non-meat RTE products consisted of, among others, various types of soft, semi soft and hard cheeses from cow and goat milk which were divided according to whether they were produced from pasteurized or raw milk. The milk and milk products also contained butter, cream, ice cream, and fermented dairy products. Furthermore, *L. monocytogenes* was isolated from fish and fishery products such as heat and cold smoked, salted and marinated or other preserved fish, i.e. salmon, herring, mackerel, redfish, halibut, trout, vendace, and sturgeon. The mixed food category constituted of products such as salads, different kind of dumplings or croquettes.

The list of non-RTE food category included beef, pork, poultry and game (wild boar and deer) meats. The diverse elements such as thigh, leg, breast, carcass, loin, belly, and tenderloin were sources of *L. monocytogenes* depending on the kind of meat. The non-RTE fish comprised of raw herring, halibut, cod, and salmon.

All *L. monocytogenes* were isolated in veterinary official food control laboratories located throughout Poland using the International Organization for Standardization (ISO) 11290-1 or 2 methods (17-20). The strains were sent to the National Veterinary Research Institute in Pulawy and stored until further examination at –80°C in the Viabank cryoprotection system (BioMaxima, Lublin, Poland).

Multiplex PCR for serotyping of *L. monocytogenes*. The isolates were cultured on tryptone soya-yeast extract agar at 37°C for 18-24 h and DNA was extracted using the Genomic Mini kit and the manufacturer's protocol (A&A Biotechnology, Gdańsk, Poland), modified by adding 20 µL of lysozyme (10 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) and incubation of the samples at 37°C for 30 min. The classification of *L. monocytogenes* to molecular serogroups was determined with multiplex PCR as previously described (8, 25, 37). The gen targets, primer sequences, and PCR cycling conditions are shown in Tab. 1. The following positive and negative reference strains were used: *L. monocytogenes* (05CEB424LM and 13CEB102LM of serogroup

Tab. 1. The nucleotide primer sequences used for determination of *L. monocytogenes* serogroups

Target gene	Primer name	Product size (bp)	Primer sequence (5' → 3')	Serogroup specificity
lmo0737	lmo0737F	691	AGGGCTTCAAGGACTTACCC	IIa, IIc
	lmo0737R		ACGATTTCTGCTTGCCATTC	
lmo1118	lmo1118F	906	AGGGGTCTTAAATCCTGGAA	IIc
	lmo1118R		CGGCTTGTTCGGCATACTTA	
ORF2819	ORF2819F	471	AGCAAAATGCCAAAACTCGT	IIb, IVb
	ORF2819R		CATCACTAAAGCCTCCCATTG	
ORF2110	ORF2110F	597	AGTGGACAATTGATTGGTGAA	IVb
	ORF2110R		CATCCATCCCTTACTTTGGAC	
Prs	prsR	370	GCTGAAGAGATTGCGAAAGAAG	IIa, IIb, IIc, IVb
	prsF		CAAAGAAACCTTGGATTTGCGG	

Explanations: F – forward, R – reverse; PCR conditions: 94°C – 5 min., 30X (55°C – 1 min., 72°C – 2 min., 94°C – 1 min.), 55°C – 2 min., 72°C – 5 min.

Tab. 2. Temporal distribution of *L. monocytogenes* serogroups by the type of meat RTE food

Food group	Food category (no. of isolates)	Year of isolation	No. of isolates	Serogroup (no. of isolates)
Sausage	Not heat-treated (n = 326)	2014	9	IIa (6), IIc (3)
		2015	29	IIa (15), IIb (6), IIc (5), IVb (3)
		2016	59	IIa (29), IIb (4), IIc (14), IVb (12)
		2017	52	IIa (33), IIb (1), IIc (7), IVb (11)
		2018	47	IIa (21), IIb (1), IIc (12), IVb (13)
		2019	62	IIa (33), IIb (9), IIc (15), IVb (5)
		2020	33	IIa (14), IIb (4), IIc (12), IVb (3)
		2021	18	IIa (8), IIb (1), IIc (9)
		2022	17	IIa (4), IIb (2), IIc (c), IVb (8)
	Heat-treated or not specified (n = 273)	2014	3	IIb (2), IVb (1)
		2015	25	IIa (9), IIb (1), IVb (15)
		2016	58	IIa (35), IIb (6), IIc (10), IVb (7)
		2017	38	IIa (16), IIb (5), IIc (6), IVb (11)
		2018	33	IIa (16), IIb (7), IIc (4), IVb (6)
		2019	27	IIa (10), IIb (6), IIc (6), IVb (5)
		2020	47	IIa (10), IIb (15), IIc (13), IVb (9)
		2021	17	IIa (7), IIb (1), IIc (3), IVb (6)
		2022	25	IIa (14), IIb (4), IIc (2), IVb (5)
Cold cuts	Not heat-treated (n = 63)	2015	10	IIa (6), IIc (3), IVb (1)
		2016	4	IIa (4)
		2017	6	IIa (4), IIc (2)
		2018	16	IIa (13), IIb (1), IIc (2)
		2019	15	IIa (6), IIb (5), IIc (4)
		2020	7	IIc (2), IVb (5)
		2021	3	IIc (3)
		2022	2	IIa (1), IIc (1)
	Heat-treated or not specified (n = 614)	2013	7	IIa (4), IIc (2), IVb (1)
		2014	27	IIa (5), IIb (4), IIc (9), IVb (9)
		2015	77	IIa (31), IIb (7), IIc (17), IVb (22)
		2016	81	IIa (23), IIb (13), IIc (4), IVb (41)
		2017	24	IIa (6), IIb (2), IIc (3), IVb (13)
		2018	91	IIa (41), IIb (17), IIc (17), IVb (16)
		2019	122	IIa (60), IIb (33), IIc (20), IVb (9)
		2020	105	IIa (51), IIb (35), IIc (8), IVb (11)
		2021	32	IIa (18), IIb (6), IIc (4), IVb (4)
		2022	48	IIa (25), IIb (1), IIc (15), IVb (7)
Delicatessen food	Not heat-treated (n = 42)	2016	2	IIa (2)
		2018	3	IIa (2), IIb (1)
		2019	2	IIa (1), IIc (1)
		2020	2	IIc (2)
		2021	31	IIa (23), IIb (4), IIc (4)
		2022	2	IIc (2)
	Heat-treated or not specified (n = 253)	2013	7	IIa (1), IIb (1), IIc (2), IVb (3)
		2014	6	IIa (1), IIc (2), IVb (3)
		2015	21	IIa (14), IIb (3), IIc (1), IVb (3)
		2016	14	IIa (8), IIc (3), IVb (3)
		2017	23	IIa (4), IIb (2), IVb (17)
		2018	35	IIa (14), IIb (9), IIc (7), IVb (5)
		2019	62	IIa (21), IIb (5), IIc (8), IVb (28)
		2020	42	IIa (15), IIb (2), IIc (15), IVb (10)
		2021	17	IIa (9), IIb (5), IIc (2), IVb (1)
		2022	26	IIa (9), IIb (4), IIc (11), IVb (2)

IIa, 06CEB406LM and 06CEB435LM of serogroup IIb, 06CEB405LM, 13CEB1022LM of serogroup IIc, and 06CEB422LM and 16CEL724LM of serogroup IVb), *L. innocua* (ATCC 33090), and *L. ivanovii* (ATCC 19119). The strains was either obtained from the European Union Reference Laboratory for *Listeria* (ANSES, Maisons-Alfort, France) or were commercially available.

Statistical analysis. The statistical analyses were performed using Statistica version 10.0 (StatSoft, now TIBCO, Palo Alto, CA, USA) based on chi-squared and Fisher's exact tests. P-value < 0.05 was considered as significant.

Results and discussion

A total of 1,997 *L. monocytogenes* isolates originating from various food categories were examined using PCR towards their classification into molecular serogroups. Most of the *L. monocytogenes*, irrespective on the food group origin, belonged to the IIa serogroup to which a total of 899 (45.0%) isolates were classified. The remaining 1,098 strains belonged to IVb (478; 23.9%), IIc (347; 17.4%), or IIb (273; 13.7%) serogroups, respectively. The incidence of particular serogroups amongst different kind of foods are displayed on Figure 1. *L. monocytogenes* of serogroups IIa and IVb were more prevalent in non-RTE products, meat RTE, and non-meat RTE food. The third most common serogroup was IIc in two first mentioned above food groups whereas serogroup IIb was the third in non-meat RTE food (Fig. 1).

The vast majority of the samples tested were of RTE food (1,808; 90.5%) with the most predominant group RTE food of meat origin (1,571; 78.7%). Different numbers of isolates in each year was obtained, from 14 strains in 2013 to 290 isolates in 2019 (Tab. 2). The most numerous kind of food among this category was heat-treated or not specified cold cuts. Of the 614 *L. monocytogenes* strains from these origins, 264 (43.0%), 133 (21.7%), 118 (19.2%), and 99 (16.1%) were identified as serogroups IIa, IVb, IIb and IIc, respectively. The statistical differences were observed between the prevalence of *L. monocytogenes* IIb serogroup in sausages and cold cuts ($P < 0.0001$), cold cuts and delicatessen food ($P < 0.001$) as well as strains of serogroup IVb from sausages and cold cuts ($P < 0.001$).

The majority of samples classified as non-meat RTE origin belonged to milk and milk products (n = 136) and they were isolated in the years 2015-2022 (Tab. 3). Most strains classified to this food group were of milk product origin with the predominance of cheeses made of pasteurized milk. The characterization of the serogroups revealed the prevalence of *L. monocytogenes* of IIa (51.4%) and IVb (25.0%) serogroups followed by IIc (11.8%) and IIb (11.8%) serogroups isolated from milk and milk products. Among isolates originated from

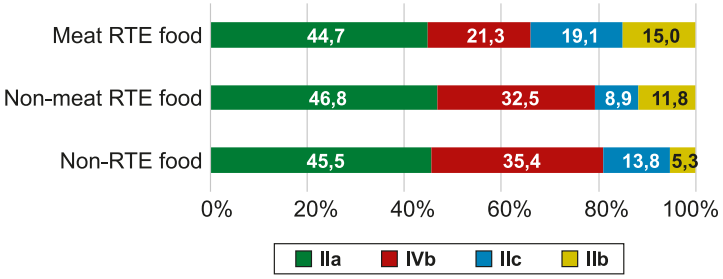

Fig. 1. Frequency of *L. monocytogenes* serogroups in different food categories tested

Tab. 3. Temporal distribution of *L. monocytogenes* serogroups by the type of non-meat RTE food

Food group	Food category (no. of isolates)	Year of isolation	No. of isolates	Serogroup (no. of isolates)
Milk and milk products	Cheeses from pasteurized milk (n = 69)	2015	6	IIa (6)
		2016	10	IIa (5), IIb (1), IIc (3), IVb (1)
		2018	5	IIa (1), IIb (4)
		2019	5	IIa (5)
		2020	7	IIa (4), IIb (1), IVb (2)
		2021	14	IIa (8), IIc (1), IVb (5)
		2022	22	IIa (9), IIb (2), IIc (6), IVb (5)
	Cheeses from unpasteurized milk (n = 53)	2016	12	IIb (5), IVb (7)
		2017	9	IIa (3), IIb (1), IIc (4), IVb (1)
		2018	16	IIa (12), IIb (2), IVb (2)
		2019	1	IVb (1)
		2020	7	IIa (2), IVb (5)
		2021	3	IIa (2), IVb (1)
		2022	5	IIa (3), IVb (2)
	Other (n = 14)	2015	1	IIb (1)
		2016	6	IIa (4), IIb (2)
		2018	2	IIa (2)
		2021	3	IIa (1), IVb (2)
		2022	2	IIa (2)
Fish and fish products (n = 50)		2014	1	IIa (1)
		2015	1	IIa (1)
		2016	8	IIa (6), IVb (4)
		2017	6	IIa (5), IIc (1)
		2018	2	IIa (1), IVb (1)
		2019	13	IIa (7), IVb (6)
		2020	10	IIa (8), IIc (1), IVb (1)
		2021	3	IIa (2), IVb (1)
		2022	6	IIa (4), IVb (2)
Mixed foods (n = 44)		2015	5	IIb (5)
		2018	5	IIa (3), IVb (2)
		2019	21	IIa (3), IVb (18)
		2020	2	IIc (2)
		2021	7	IIb (2), IVb (5)
		2022	4	IIc (1), IVb (3)
Sea foods (n = 7)		2015	6	IIb (4), IVb (2)
		2019	1	IIb (1)

cheeses from pasteurized milk, most strains belonged either to serogroups IIa (38; 55.1%) or IVb (13; 18.8%). The remaining 18 strains were classified either to IIc (10; 14.5%) or IIb (8; 11.6%) serogroups (Tab. 3).

In the RTE fish and fish products categories, the strains identified as IIa serogroup were predominant (35 of 50 strains; 70.0%). However, the prevalence of this serogroup was higher than among strains of milk and milk products origin ($P < 0.05$).

When the mixed food was taken into consideration, all serogroups tested were detected, with the most numerous IVb (28 out of 44 isolates; 63.6%) which presented differ in contribution compared to other food categories, i.e. milk and fish products ($P < 0.0001$). Similar differences ($P < 0.0001$) were observed in the distribution of *L. monocytogenes* of IIa serogroup between mixed food and milk as well as fish products.

Table 4 shows information about the presence of *L. monocytogenes* in non-RTE food. These samples were obtained during years 2013-2022. The highest number of isolates was investigated in 2017 when 40 strains, mainly from fish (n = 25) were tested. In total, the most numerous food category investigated was poultry meat with 45 isolates, mainly from mechanically deboned meat (n = 27). A total of 189 isolates were obtained from non-RTE food and they were classified mainly as IIa (86; 45.5%) or IVb (67; 35.4%), followed by IIc (26; 13.8%), and IIb (10; 5.3%) serogroups (Fig. 1). IIa serogroup was most frequently recorded among *L. monocytogenes* isolated from beef, pork poultry and mixed meat (Tab. 4).

Tab. 4. Temporal distribution of *L. monocytogenes* serogroups by the type of non-RTE food

Food group	Food category (no. of isolates)	Year of isolation	No. of isolates	Serogroup (no. of isolates)
Beef meat	Elements (n = 7)	2014	6	IIa (6)
		2016	1	IIc (1)
	Minced meat (n = 4)	2016	4	IIa (1), IIb (1), IIc (1), IVb (1)
	Other (n = 4)	2021	4	IIa (2), IIc (2)
Pork meat	Elements (n = 9)	2018	2	IVb (2)
		2019	1	IIa (1)
		2020	3	IIb (1), IIc (2)
		2022	3	IIa (1), IVb (2)
	Minced meat (n = 18)	2015	1	IIa (1)
		2016	7	IIa (6), IIc (1)
		2017	5	IIa (2), IIb (3)
		2019	1	IIb (1)
		2021	3	IIa (2), IIc (1)
		2022	1	IIc (1)
	Other (n = 2)	2018	1	IVb (1)
		2019	1	IIb (1)
Poultry meat	Elements (n = 15)	2015	1	IIa (1)
		2016	1	IVb (1)
		2017	3	IIa (2), IVb (1)
		2018	6	IIc (1), IVb (5)
		2019	2	IVb (1)
		2020	2	IIa (1)
	Mechanically deboned (n = 27)	2015	1	IIa (1)
		2016	2	IIa (2)
		2017	6	IIa (4), IIc (2)
		2018	5	IIa (4), IIc (1)
		2019	3	IIa (3)
		2020	9	IIa (9)
		2021	1	IIa (1)
	Other (n = 3)	2014	1	IIa (1)
		2017	2	IIa (1), IVb (1)
Game meat	Elements (n = 22)	2015	6	IIc (3), IVb (3)
		2016	3	IVb (3)
		2017	4	IIb (1), IVb (3)
		2018	1	IIc (1)
		2019	6	IIa (6)
		2020	2	IIa (1), IVb (1)
	Minced meat (n = 11)	2016	2	IIa (1), IVb (1)
		2017	4	IVb (4)
		2018	1	IIc (1)
		2019	4	IIa (3), IVb (1)
	Other (n = 7)	2016	2	IIa (2)
		2018	5	IIa (1), IIb (2), IVb (2)
Fish (n = 38)		2015	1	IIa (1)
		2017	25	IVb (25)
		2019	1	IVb (1)
		2020	2	IIa (1)
		2021	7	IIa (6), IVb (1)
		2022	2	IVb (2)
Mixed meat or not specified (n = 22)		2013	4	IIa (1), IIc (3)
		2014	3	IIa (2), IIb (1)
		2015	6	IIa (6)
		2016	6	IIc (2), IVb (4)
		2019	1	IIa (1)
		2021	2	IIc (2)

Taking into account all *L. monocytogenes* tested, it was shown that isolates of serogroup IIa, irrespective of the origin, were the most frequently identified, followed by serogroups IVb, IIc, and IIb. Other authors also confirmed that IIa serogroup predominated in different kinds of food but also in the food processing environments (1, 27, 35, 38). However, our results showed that in some food groups, i.e. non-meat RTE mixed food and non-RTE fish, strains belonging to IVb serogroup were predominant. The distribution of particular serogroups differs between other investigations and depends a lot on types of foods examined as well as varies in different geographic regions. For example, the study conducted by Braga et al. (4) showed that IIb and IVb serogroups were the most frequently identified among food which included frozen food, deli meats, ready-to-eat products, and cheeses. Serogrup IVb followed by IIb were predominant among *L. monocytogenes* isolated from domestic and imported foods such as beef, pork, poultry, meat products, fish, fish products, and natural cheese examined in Japan (31). On the other hand, Korsak et al. (21) defined IIa serogroup as the most frequently detected from a wide range of products such as fish, vegetables, cakes, dairy products, and meat. Similar distribution with the most prevalent serogroups IIa and IIc amongst meat and meat product in China were reported by Chen et al. (7). Arslan and Baytur (2) found that serotype 1/2a was predominant among beef and chicken meat samples which is in accordance with the present results, where serogroup IIa (which includes serotype 1/2a) was mostly identified. Interestingly, serotype 4b was not at all identified by these authors, contrary to detection of serogroup IVb (with this serotype) that was a frequently identified serogroup in poultry meat in our present investigation. It should be stressed that in the current study molecular classification to serogroups was used in contrast to classical serological methods applied by Arslan and Baytur (2).

The analysis of *L. monocytogenes* serogroup distribution within strains isolated from poultry meat also differ between our study and other investigations. Zeinali et al. (39) reported serogroup IIb as the most frequently isolated, which is in contrast to the current study in which serogroup IIa was the predominant and IIb was not identified at all in poultry meat. Orsi et al. (27) suggested that strains classified to IIa and IIc serogroups and belonging to lineage II are most often found in foods because of their better adaptation to various niches than *L. monocytogenes* which represented lineage I such as IVb serogroups. In contrast, some previous studies proved that the strains represented serogroups classified to different genetic lineages, i.e. IIa (lineage II) and IIb (lineage I) could also survive better in food processing environment and in food than isolates of other serological groups since they possess genes encoding resistance to biocides (9, 26). Strains of such serogroups were often identified during our present study, although they had not been tested towards the presence of biocide resistance genes.

A study conducted by Henriques et al. (16) regarding *L. monocytogenes* from meat RTE food revealed that IIb and IVb serogroups predominated in this kind of product. In the current study IIa and IVb serogroups were the most frequently identified and strains of serogroup IIb represented only 14.9% *L. monocytogenes* tested. Another investigation stated that *L. monocytogenes* classified to 1/2c serotype (belonging to IIc serogroup) were mainly isolated from beef and the beef processing environment (5) compared to IIa predominant serogroup amongst *L. monocytogenes* isolated from this food group in the current investigation.

The differences between our investigation and other research were also observed regarding the prevalence of *L. monocytogenes* serogroups in relation to non-RTE fish and RTE fish and fishery products. Current results showed that IVb and IIa serogroups predominated in two of the above mentioned food categories, respectively. However, Zakrzewski et al. (38) and our previous investigation indicated that serogroup IIa was much more common in these kind of samples than other serogroups (36). Moreover, our study showed that serogroup IVb was predominant in RTE mixed foods which is consistent with the findings of Szymczak et al. (34). Furthermore, strains of serogroup IVb were also identified in a wide range of sources in Poland and other countries, although such isolates are rather characteristic for clinical listeriosis because they are usually positive for the full length of the *inlA* gene encoding internalin, a protein critical for attachment of *L. monocytogenes* to human host cells (3, 22, 25, 27, 29). The presence of such strains in food, especially of RTE food, suggests that such kind of food may be a source of pathogenic for humans *L. monocytogenes* of the IVb serogroup. In our previous investigations we have also identified in food strains characterized by the presence of the intact *inlA* gene and other pathogenic markers and they were classified to hypervirulent clonal complexes (23). However, during the present study the IVb-positive *L. monocytogenes* were not further molecularly analyzed. All mentioned above results confirm a broad prevalence of such strains in the food production chain, including the final products, i.e. RTE or non-RTE foods.

In summary, the current study on a large group of *L. monocytogenes* isolated from different food categories during a 10-year period revealed that food of animal origin may be a source of strains potentially pathogenic for humans. Several hundred isolates were classified into IVb serogroup which was previously shown to be hypervirulent. Furthermore, a high number of isolates belonged to IIa and IIb serological groups, that are often responsible for human listeriosis cases. Therefore, studies on the prevalence and serological characterization of *L. monocytogenes* isolates of food origin should be further performed.

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