International Journal of Food Microbiology 289 (2019) 1–6

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Antimicrobial resistance and genotypic characteristics of *Listeria monocytogenes* isolated from food in Poland

Maciej Sosnowski, Beata Lachtara, Kinga Wieczorek*, Jacek Osek

Department of Hygiene of Food of Animal Origin, National Veterinary Research Institute, Partyzantow 57, 24-100 Pulawy, Poland

ARTICLE INFO

Keywords:
Listeria monocytogenes
Antimicrobial resistance
Virulence genes
Molecular typing
PFGE
MLST

ABSTRACT

The aim of the study was to determine antimicrobial resistance and genotypic characteristics of *L. monocytogenes* isolated from food of animal origin from different parts of Poland during years 2013–2016. A total of 146 isolates were tested using a microbroth dilution method, whereas virulence genes and molecular serogroups were identified by PCR. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) methods were used to analyze the genotypic relationship of the strains. Altogether, 102 pulsotypes grouped into 7 clusters and 24 sequence types, including 3 new types, were identified. Most of the strains clustered into individual patterns were originated from different food products and were isolated in different geographical regions at various time. *L. monocytogenes* was mostly resistant to oxacilin (90.4% strains), clindamycin (54.1%) and ceftriaxone (49.3%). A multiresistance patterns, mainly to ceftriaxone, oxacillin together with other antimicrobials, were observed among 27.4% strains. Antimicrobial resistance and presence of virulence genes suggest that food of animal origin contaminated with *L. monocytogenes* may present a risk for public health.

1. Introduction

Listeria monocytogenes, an important foodborne pathogen, is responsible for human listeriosis which is especially dangerous for infants, pregnant women, immunosuppressed individuals and elderly (Alonso-Hernando et al., 2012; Henriques et al., 2017). A high listeriosis-related mortality in humans contributed to recognize of *L. monocytogenes* as one of the major bacterial pathogen (Jamali and Thong, 2014; Liu et al., 2007).

These bacteria, due to tolerance to extreme pH, temperature and salt concentrations, can survive during many food manufacturing processes, which may result to their presence in ready-to-eat and heat-to-eat food which were associated with several listeriosis outbreaks (Gomez et al., 2014; Harakeh et al., 2009; Jamali and Thong, 2014; Li et al., 2016; Liu et al., 2007; Martin et al., 2014).

Resistance of *L. monocytogenes* to many antimicrobial agents is increasingly observed (Alonso-Hernando et al., 2012; Gomez et al., 2014; Zhang et al., 2007). The resistance genes can be transferred between *Listeria* and other Gram-positive and Gram-negative bacteria; therefore, it is important to monitor the prevalence and transmission of resistant *L. monocytogenes* strains (Li et al., 2016). A combination of penicillin or ampicillin with aminoglycosides and trimethoprim-sulfamethoxazole are antimicrobials used for treatment of human *Listeria* infections. Resistance to these antimicrobial agents may be a result of their common usage in food producing animals for treatment of bacterial diseases (Su et al., 2016).

Thirteen *L. monocytogenes* serovars have been identified but usually only four of them were recovered from patients and food: 1/2a, 1/2b, 1/2c and 4b (Neves et al., 2008). Among them, 4b is responsible for the majority of human listeriosis outbreaks, while 1/2a is the most prevalent serovar in food (Zhang et al., 2007). Several virulence genes responsible for bacterial entry and replication within the host cells as well as microbial adherence and invasion have been identified in *L. monocytogenes*. It has been shown that internalins A and B, encoded by the *inlA* and *inlB* genes play a key role in the initial stages of infection (Jacquet et al., 2002; Wu et al., 2015). The *llsX* gene responsible for listeriolysin expression is considered to be an important factor in the survival of *L. monocytogenes* in polymorphonuclear neutrophils and in the pathogenesis of human listeriosis (Cotter et al., 2008; Wu et al., 2015; Wu et al., 2016). Furthermore, many other gene markers, i.e. *plcA*, *plcB*, *hly*, *lmo*2672, *prfA* have been found as a key virulence genes playing a role in the development of the disease (Heras et al., 2011; Jamali and Thong, 2014; Liu, 2006; Liu et al., 2007; Tamburro et al., 2015).

Molecular subtyping and assession of genetic relatedness of *L. monocytogenes* are mainly performed by pulsed-field gel electrophoresis

* Corresponding author.
E-mail address: kinga.wieczorek@piwet.pulawy.pl (K. Wieczorek).

https://doi.org/10.1016/j.ijfoodmicro.2018.08.029
Received 12 December 2017; Received in revised form 9 July 2018; Accepted 30 August 2018
Available online 31 August 2018
0168-1605/

(PFGE) which is characterized by a high reproducibility and discriminatory (Neves et al., 2008; Jamali and Thong, 2014). Furthermore, to investigate the genetic diversity of the isolates, the multilocus sequence typing (MLST) method based on sequencing of housekeeping genes is also applied. Each allele is designated by a number and the combination of the alleles is defined as sequence type (ST) of the tested strain. The STs, that are different in one allele, create a clonal complex (CC) (Henri et al., 2016; Martin et al., 2014).

The aim of this study was to determine antimicrobial resistance and genotypic characterization of *L. monocytogenes* isolated from food in Poland.

2. Materials and methods

2.1. Collection of isolates

A total of 146 *L. monocytogenes* isolates from various types of food of animal origin were selected from a group of strains isolated in all 16 voivodeships (administrative regions) of Poland during 2013–2016. A comparable number of isolates collected in each year, belonging to 4 main serogroups 1/2a, 1/2b, 1/2c and 4b were taken to the present study. They were recovered from ready-to-eat food (n = 105), raw meat (n = 17), raw sausages (n = 16) and seafood (n = 8). The group of ready-to-eat products contained heat-treated sausages, delicatessen, salads, and packed dinner dishes whereas the other kinds of food were non thermal treated. Fish and shrimps in brine and smoked fish were defined as seafood. All *L. monocytogenes* isolates were obtained from different food samples collected by official food control laboratories according to the Standard ISO 11290-1 method (ISO, 1996) and sent to National Veterinary Research Institute in Pulawy. The isolates were streaked directly on Tryptone Soya Yeast Extract Agar (Bio-Rad, USA), incubated at 37 °C for 24 ± 2 h and then stored at −80 °C in Viabank (BioMaxima, Poland).

2.2. Serogrouping

Multiplex PCR to determine *L. monocytogenes* molecular serogroups was applied as described previously (Doumith et al., 2004, Wieczorek et al., 2012a). The DNA amplification was performed in a thermal cycler (Biometra, Germany) under the following conditions: initial DNA denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. The final cycle was carried out at 55 °C for 2 min and 72 °C for 5 min.

2.3. Detection of virulence genes

The virulence marker genes *inlA*, *inlC*, *inlJ*, *lmo2672*, *llsX*, *prfA*, *plcA*, *hlyA*, *mplA*, *actA*, *plcB*, *inlB* were identified using PCR as described previously (Chen et al., 2009; Gray and Kroll, 1995; Kim et al., 2004; Volokhov et al., 2002; Wieczorek et al., 2012b; Wieczorek and Osek, 2017). After initial DNA denaturation at 95 °C for 5 min, the following amplification conditions (30 cycles) were used: 94 °C for 20 s, 55 °C for 20 s, 72 °C for 55 s (*inlA*, *inlC*, *inlJ*); 94 °C for 20 s, 60 °C for 20 s, 72 °C for 45 s (*lmo2672*); 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min (*llsX*); and 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min (*plcA*, *hlyA*, *mplA*, *actA*, *plcB*, *inlB*, *flaA*, *prfA*).

2.4. Antimicrobial resistance

Antimicrobial resistance was tested using the microbroth dilution method as described before (Wieczorek and Osek, 2017). Sensititre GPN3F plates (Thermo Scientific, USA) containing 17 antimicrobials (dilution range in mg/l) were used: ampicillin (AMP; 0.12–16), ceftriaxone (AXO; 8–64), ciprofloxacin (CIP; 0.5–2), clindamycin (CLI; 0.12–2), erythromycin (ERY; 0.25–4), gatifloxacin (GAT; 1–8), gentamicin (GEN; 2–16), levofloxacin (LEVO; 0.25–8), linezolid (LZD; 0.5–8), oxacillin (OXA; 0.25–8), penicillin (PEN; 0.06–8), quinupristin/dalfopristin (SYN; 0.12–4), rifampicin (RIF; 0.5–4), streptomycin (STR; 512–1024), tetracycline (TET; 2–16), trimethoprim/sulfamethoxazole (SXT; 0.5–4), vancomycin (VAN; 1–128). The minimal inhibitory concentration (MIC) records were read using the Sensititre Vizion System (Trek, UK). Antimicrobial resistance of the isolates was determined according to the guidelines of the Clinical and Laboratory Standards Institute, adopting the criteria set for *Staphylococcus* spp. and *Enterococcus* spp. expect for ampicillin, penicillin and trimethoprim, where specific *L. monocytogenes* breakpoints are defined (CLSI, 2012; CLSI, 2016; Escolar et al., 2017; Lyon et al., 2008).

2.5. MLST

L. monocytogenes isolates were typed using the MLST scheme as described in the Institut Pasteur MLST database (http://bigsdb.pasteur.fr/listeria; Moura et al., 2016). The BioNumerics software version 7.6 (Applied Maths, Belgium) was used to assemble the sequences and to obtain the allele identifiers and sequence types (STs) together with clonal complex (CC) information by connecting with pubMLST.net via BioNumerics MLST online plugin. The obtained data were elaborated and the minimum spanning tree was generated using BioNumerics.

2.6. PFGE

The strains were tested using the PFGE protocol developed by the European Union Reference Laboratory for *L. monocytogenes* with some modifications (Marault, 2008). Briefly, bacterial DNA was digested by two restriction enzymes: *Asc*I and *Apa*I (Thermo Scientific) and separation of the generated DNA fragments was performed on CHEF DR II (Bio-Rad) at 6 V/cm with initial and final time 4–40 s over 18 h in 1% SeaKem® Gold agarose (Lonza, Switzerland) gels in 0.5× TBE buffer (Sigma Aldrich, USA). The PFGE images were analyzed with BioNumerics. *Salmonella* Branderup H9812 ATCC BAA-664 was used for band normalization. A combined dendrogram for *Asc*I and *Apa*I restriction enzymes were generated by the unweighted-pair group method with arithmetic means (UPGMA) with BioNumerics and converted using Interactive Tree of Life (iTOL: http://itol.embl.de/. Accessed 10 May 2017). The pulsotypes were considered identical when the patterns were indistinguishable.

3. Results

3.1. Serogroups and virulence genes

Among 146 *L. monocytogenes* isolates tested 43 (29.5%) belonged to 1/2a, 32 (21.9%) to 1/2b, 35 (24.0%) to 1/2c and 36 (24.6%) to 4b serogroups, respectively. The following virulence genes were identified in all strains: *inlA*, *inlC*, *inlJ*, *lmo2672*, *prfA*, *plcA*, *hly*, *mpl*, *actA*. Furthermore, the majority of the isolates were positive for the *plcB* (145, 99.3%) and *inlB* (130, 89.0%) virulence markers. On the other hand, only 31 (21.2%) of *L. monocytogenes* tested had the *llsX* gene. The lack of the *llsX* gene was observed in all strains of serogroups 1/2a and 1/2c. The isolates that were negative for the *inlB* gene belonged only to 1/2b serogroup, whereas one strain without the *plcB* marker was of 4b serogroup.

3.2. Antimicrobial resistance

Most of the isolates were resistant to oxacilin (132, 90.4%), followed by clindamycin (79, 54.1%) and ceftriaxone (72, 49.3%). Only few strains showed resistance to linezolid (5, 3.4%) and ciprofloxacin, gatifloxacin, gentamycin and tetracycline (1, 0.7% of each). In addition, intermediate resistance to ceftriaxone and clindamycin was demonstrated in several isolates (70, 47.9% and 56, 38.4%, respectively). On the other hand, all strains were sensitive to ampicillin, erythromycin,

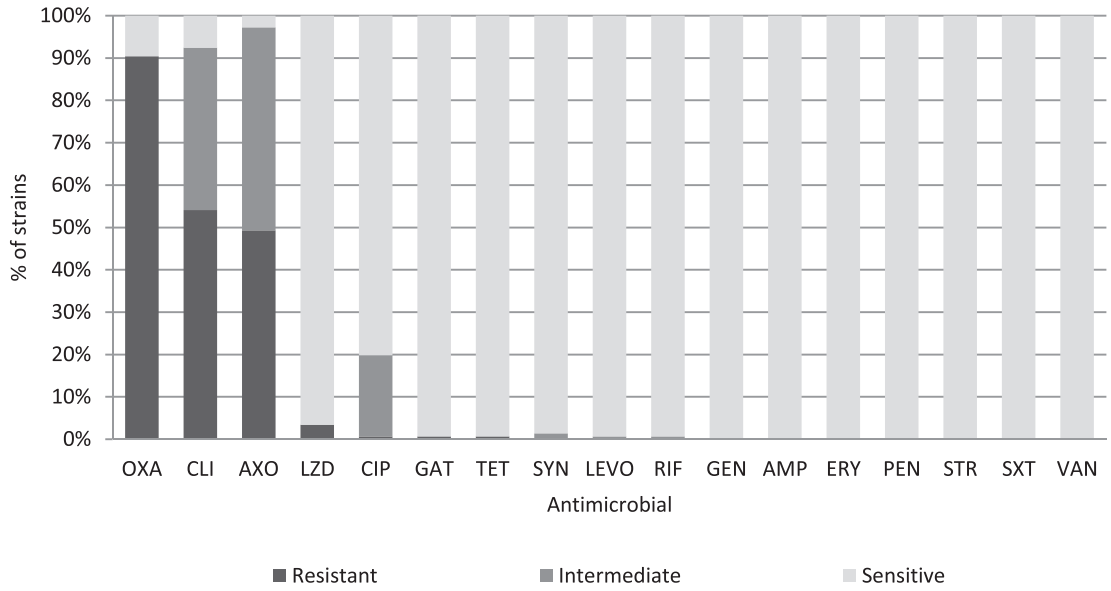

Fig. 1. Sensitivity of *L. monocytogenes* to antimicrobials: oxacillin (OXA), clindamycin (CLI), ceftriaxone (AXO), linezolid (LZD), ciprofloxacin (CIP), gatifloxacin (GAT), tetracycline (TET), quinupristin/dalfopristin (SYN), levofloxacin (LEVO), rifampicin (RIF), gentamicin (GEN), ampicillin (AMP), erythromycin (ERY), penicillin (PEN), streptomycin (STR), trimethoprim/sulfamethoxazole (SXT), and vancomycin (VAN).

Table 1
Antimicrobial resistance profiles among *L. monocytogenes* strains in relation to serogroup and sequence type.

Profile no.	Antimicrobial resistance profile	Number (%) of resistant strains n = 146					Sequence type	Clonal complex
		1/2a	1/2b	1/2c	4b	Total		
1	Not resistant for all	3 (2.1)	0	1 (0.7)	0	4 (2.7)	ST9, ST12, ST37, ST155	CC7,CC9, CC37, CC155
2	AXO	3 (2.1)	0	2 (1.4)	0	5 (3.4)	ST9, ST37, ST121, ST193, ST1268	CC9, CC37, CC121, CC193
3	CLI	2 (1.4)	0	1 (0.7)	0	3 (2.0)	ST8, ST9, ST155	CC8, CC9, CC155
4	OXA	15 (10.3)	9 (6.2)	4 (2.7)	2 (1.4)	30 (20.5)	ST2, ST3, ST5, ST14, ST21, ST121, ST155, ST580	CC2, CC3, CC5, CC9, CC14, CC21, CC121, CC155
5	AXO-OXA	3 (2.1)	14 (9.6)	0	10 (6.8)	27 (18.5)	ST1, ST2, ST3, ST5, ST6, ST31, ST87, ST121, ST145, ST193	CC1, CC2, CC3, CC5, CC6, CC31, CC87, CC121, CC193
6	CLI-OXA	9 (6.2)	3 (2.1)	22 (15.1)	2 (1.4)	36 (24.7)	ST2, ST5, ST8, ST9, ST122, ST145, ST155, ST580	CC2, CC5, CC8, CC9, CC155
7	CLI-LZD	1 (0.7)	0	0	0	1 (0.7)	ST155	CC155
8	AXO-CIP-TET	1 (0.7)	0	0	0	1 (0.7)	ST199	CC199
9	AXO-CLI-OXA	5 (3.4)	6 (4.1)	5 (3.4)	18 (12.3)	34 (23.3)	ST1, ST2, ST3, ST5, ST8, ST9, ST121, ST145, ST427, ST580, ST1266, ST1267	CC1, CC2, CC3, CC5, CC6, CC8, CC9, CC29, CC121
10	AXO-CLI-OXA-LZD	0	0	0	4 (2.7)	4 (2.7)	ST1, ST6, ST145	CC1, CC2, CC6
11	AXO-CLI-OXA-GAT	1 (0.7)	0	0	0	1 (0.7)	ST155	CC155

Ampicillin (AMP), ceftriaxone (AXO), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gatifloxacin (GAT), gentamicin (GEN), levofloxacin (LEVO), linezolid (LZD), oxacillin (OXA), penicillin (PEN), quinupristin/dalfopristin (SYN), rifampicin (RIF), streptomycin (STR), tetracycline (TET), trimethoprim/sulfamethoxazole (SXT), and vancomycin (VAN).

gentamycin, penicillin, streptomycin, trimethoprim/sulfamethoxazole and vancomycin (Fig. 1). Eleven antimicrobial resistance profiles were defined, including 4 strains (2.7%) that were susceptible and intermediate resistant (Table 1, profile 1). A total of 38 strains (26.0%) were resistant to one antimicrobial only, whereas the remaining 104 (71.2%) isolates were resistant to more than one antimicrobial. Among them, 40 (27.4%) strains displayed the multiresistance pattern (Table 1). No correlation between the susceptibility profiles and *L. monocytogenes* serogroups was observed.

3.3. MLST

A total of 24 different sequence types (STs) were identified among the tested strains that were grouped into 18 clonal complexes (CCs). The distribution of STs and relatedness of isolates from various *L. monocytogenes* serogroups and STs are illustrated in the minimum spanning tree (Fig. 2). The most common STs were ST9 (17, 11.6% isolates), followed by ST3 (16, 11.0%) and ST580 (15, 10.3%). Furthermore, 3 new STs, with one strain in each, were detected (ST1266, ST1267 and ST1268) and were assigned to the defined clonal complexes CC8, CC5 and CC9, respectively. Four CCs with more than one ST were identified: CC2 (ST2, ST145), CC5 (ST5, ST1267), CC8 (ST8, ST1266) and CC9 (ST9, ST122, ST580, ST1268). Analysis of *L. monocytogenes* serogroups and STs demonstrated that strains of 1/2a serogroup were classified into 12 sequence types whereas isolates 1/2b, 1/2c and 4b were represented by 4 STs each. A relationship between STs and antimicrobial resistance profiles revealed that the most common patterns CLI-OXA (36 isolates), AXO-CLI-OXA (34 strains), OXA (30 isolates) and AXO-OXA (27 strains) contained predominantly *L. monocytogenes* of ST9, ST1/ST145, ST121 and ST3, respectively (Table 1).

3.4. PFGE

All 146 *L. monocytogenes* isolates were grouped by PFGE into 102

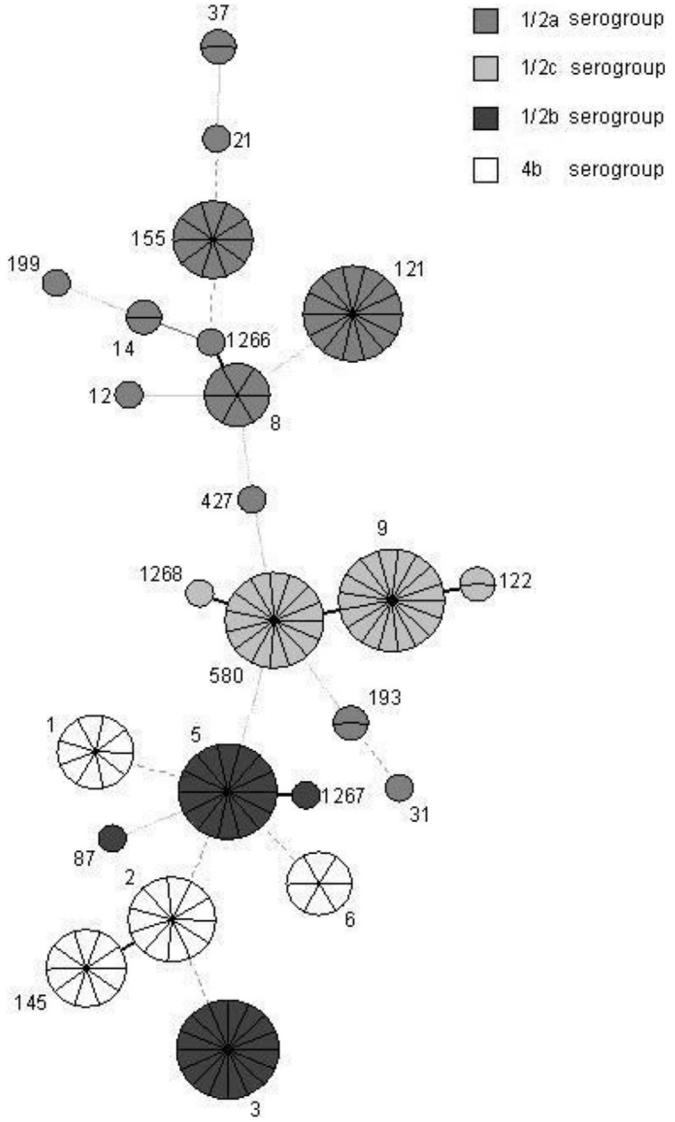

Fig. 2. Minimum spanning tree (MST) generated on the multilocus sequence typing (MLST) method of *L. monocytogenes* strains grouped by sequence types (ST) and serogroups (color-coded). STs are shown as circles. The size of each circle indicates the number of isolates within this particular type. The lines infer relatedness by stating the number of differing alleles, dotted line combines STs that has no alleles in common; bold line indicates STs in the same clonal complex (CC).

different pulsotypes and 7 major clusters. The PFGE dendrogram (Fig. 3) presents the distribution of macrorestriction profiles according to serogroups, STs and antimicrobial resistance of the isolates. Among all pulsotypes, 84 were unique with only one strain. The remaining 18 PFGE profiles with 62 strains contained from 2 to 11 isolates. A correlation between pulsotypes and serotypes was identified, e.g. strains with the identical PFGE profiles were classified to the same *L. monocytogenes* molecular serogroup. Cluster I (47 isolates with 27 profiles) included mostly strains of serogroup 1/2c (33 isolates; 70.2%), which were classified into 11 different STs and 7 CCs (Fig. 3). The second most numerous cluster V (33 isolates with 23 PFGE profiles) was represented mainly by strains of 1/2b serogroup which were classified into 6 STs and 5 CCs. All these isolates were resistant to one (OXA), two (AXO-OXA; CLI-OXA) or three (AXO-CLI-OXA) antimicrobials used in the study. In cluster II (9 PFGE pulsotypes; 13 isolates) several antimicrobial resistance profiles were identified and all these strains belonged to 1/2a serogroup classified into 4 STs and 3 CCs. Among them, two isolates displayed multiresistance pattern (profiles 8 and 11) (Fig. 3, Table 1).

4. Discussion

L. monocytogenes is generally susceptible to many antimicrobials except tetracyclines, fluoroquinolones and cephalosporins (Jamali and Thong, 2014; Morvan et al., 2010). In the present study resistance to ceftriaxone, which belongs to the 3rd generation of cephalosporins, was confirmed (49.3% resistant and 47.9% intermediate resistant strains). On the other hand, the vast majority of the isolates were susceptible to tetracyclines and fluoroquinolones. Jamali and Thong (2014) demonstrated resistance of *L. monocytogenes* to penicillin, erythromycin and vancomycin (53.1%, 6.3% and 9.3% isolates, respectively) but in the present study none of the strains was resistant to these antimicrobials. In another study (Davis and Jackson, 2009) a high resistance rate to oxacillin (99.0%) and ceftriaxone (72.0%) was shown and such resistant strains were also identified in the current investigation (90.4% and 49.3% strains, respectively). On the other hand, in both studies all isolates were sensitive to penicillin, streptomycin and trimethoprim/sulfamethoxazole. Resistance to oxacillin, the most common in the present study, was also identified in the vast majority of the strains by other authors (Gomez et al., 2014; Jamali and Thong, 2014).

Resistance to penicillin as well as to other antimicrobials which are used in human and animal therapy (tetracycline, erythromycin, ampicillin, rifampicin) was previously identified among *L. monocytogenes* of food origin (Escolar et al., 2017; Harakeh et al., 2009; Li et al., 2016). However, susceptibility to vancomycin, considered as a drug of last choice, was demonstrated in most of the studies (Escolar et al., 2017; Harakeh et al., 2009; Li et al., 2016; Morvan et al., 2010) but there are few reports on the presence of *L. monocytogenes* resistant to this antibiotic (Jamali and Thong, 2014; Harakeh et al., 2009).

Multiresistance, defined as resistance to antimicrobials of 3 or more classes, was observed among 40 (27.4%) of tested strains. Such resistance was also identified in another studies among *L. monocytogenes* of food origin although the percentages of multiresistant isolates was lower than in the present investigation, i.e. from 0.6% to 14.1% (Dmowska et al., 2013; Jamali et al., 2015; Li et al., 2016; Wieczorek and Osek, 2017; Zhang et al., 2007).

The virulence genes identified during the current study (*inlA*, *inlC*, *inlJ*, *lmo2672*, *prfA*, *plcA*, *hly*, *mpl*, *actA*, *plcB* and *inlB*) were also detected previously in all

L. monocytogenes strains recovered from humans and food (Almeida et al., 2017; Henriques et al., 2017; Jamali and Thong, 2014; Su et al., 2016; Wieczorek and Osek, 2017). The listeriolysin encoding gene (*llsX*), which was shown in 21.2% strains tested in the present study, was also identified by other authors at different rates (6.7%–44.8%), mostly among *L. monocytogenes* of 1/2b serogroup (Shen et al., 2013; Su et al., 2016; Wu et al., 2015). Furthermore, the strains negative for the *llsx* and *inlB* markers were not classified to 4b serogroup which is often involved in human listeriosis cases (Jeffers et al., 2001).

The PFGE analysis revealed that *L. monocytogenes* isolated from food in Poland was genetically diverse and heterogenous. This typing method has been reported as the gold standard for molecular differentiation and subtyping of *L. monocytogenes* strains (Jamali and Thong, 2014; Neves et al., 2008; Revazishvili et al., 2004). However, Revazishvili et al. (2004) showed that the discriminatory ability of MLST is greater than that of PFGE. Therefore, to provide more reliable results on molecular relationship of the isolates both methods have been used in the present investigation. The MLST analysis indicated that *L. monocytogenes* sequence types ST9, ST3, ST580, ST5 and ST121 were predominant among the strains tested, covering a total of 42.5% isolates. The study of Martin et al. (2014) has also shown that *L. monocytogenes* of ST9 were mainly identified among strains from food production environment. Similar results on the prevalence of *L. monocytogenes* ST9 and ST121 isolated from food in France were obtained by

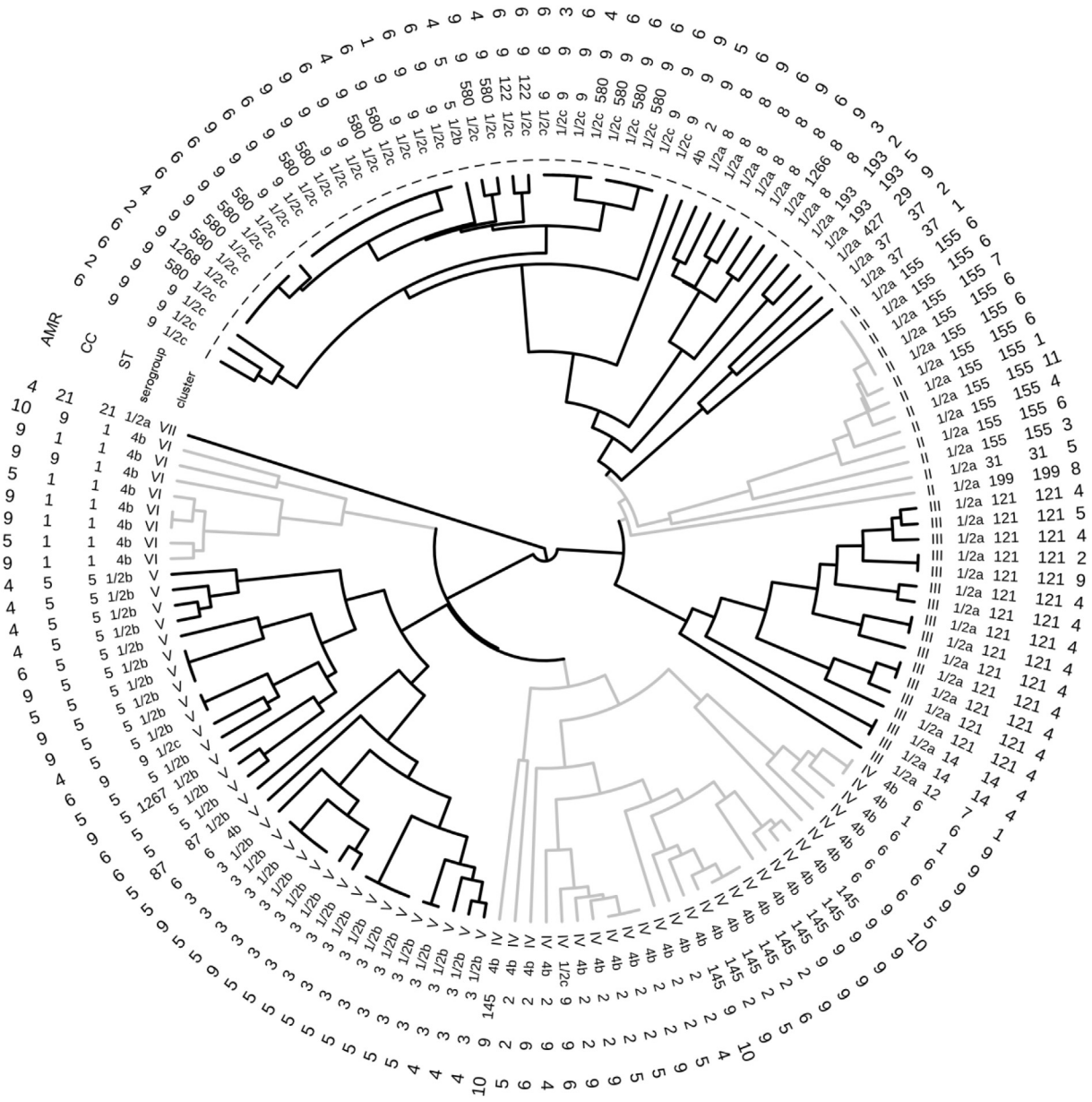

Fig. 3. A combined dendrogram for *Asc*I and *Apa*I PFGE restriction enzymes generated by the unweighted-pair group method with arithmetic means (UPGMA). The pulsotypes considered identical for indistinguishable patterns. The dendrogram includes serogroup, cluster number, sequence type (ST), clonal complex (CC) and antimicrobial resistance profile numbers (AMR) as shown in Table 1.

Henri et al. (2016). Strains from food and clinical cases of human listeriosis with such sequence types were also identified in other countries which may suggest their high virulence potential (Hein et al., 2011; Holch et al., 2013). Some STs (ST1, ST8, ST87) identified in few strains in the present study were often detected among isolates in China (20.0%, 30.0%, 8.8%, respectively) (Wu et al., 2016). However, the most common STs in Poland were very seldom found in the Chinese *L. monocytogenes*.

All but 3 new (ST1266, ST1267, ST1268) STs identified in the present study were already included in the *L. monocytogenes* Institute Pasteur MLST database (http://bigsdb.pasteur.fr/perl/bigsdb/bigsdb.pl?db=pubmlst_listeria_isolates_public). The most often isolated ST3, ST9, ST121, ST5, and ST580 were reported among 256, 138, 97, 54 and 2 strains, respectively. All of them were isolated from food or humans with listeriosis (except ST580). It is worth noting that 17 out of 24 (70.8%) STs detected in the present investigation were responsible for human infections in various world regions on all continents, indicating that these *L. monocytogenes* STs have a pathogenic potential (http://bigsdb.pasteur.fr/listeria/). Therefore, the MLST typing should be used for epidemiological and public health studies of *L. monocytogenes*.

In conclusion, the present study showed that population structure of *L. monocytogenes* isolated from food in Poland was diverse. Antimicrobial resistance, especially identification of multiresistant isolates and prevalence of virulence genes suggest that food of animal origin contaminated with *L. monocytogenes* may present a risk for public health.

Acknowledgements

The study was funded by KNOW (Leading National Research Centre) Scientific Consortium “Healthy Animal - Safe Food”, Decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

References

Almeida, R.M., Barbosa, A.V., Castro Lisbôa, R., Mercês Santos, A.F., Hofer, E., Vallim, D.C., Hofer, C.B., 2017. Virulence genes and genetic relationship of *L. monocytogenes* isolated from human and food sources in Brazil. Braz. J. Infect. Dis. 21, 282–289.

Alonso-Hernando, A., Prieto, A., Garcia-Fernandez, C., Alonso-Calleja, C., Capita, R., 2012. Increase over time in the prevalence of multiple antibiotic resistance among isolates of *Listeria monocytogenes* from poultry in Spain. Food Control 23, 37–41.

Chen, J., Luo, X., Jiang, L., Jin, P., Wei, W., Liu, D., Fang, W., 2009. Molecular characteristics and virulence potential of *Listeria monocytogenes* isolates from Chinese food systems. Food Microbiol. 26, 103–111.

CLSI (Clinical and Laboratory Standards Institute), 2012. Performance Standards for Antimicrobial Susceptibility Testing Twenty Second Informational Supplement. vol. 33, no. 3 Clinical and Laboratory Standards Institute, Wayne, PA, USA (Document M100-S22-20).

CLSI (Clinical and Laboratory Standards Institute), 2016. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. Clinical and Laboratory Standards Institute, Wayne, PA, USA (Document M45).

Cotter, P.D., Draper, L.A., Lawton, E.M., Daly, K.M., Groeger, D.S., Casey, P.G., Ross, R.P., Hill, C., 2008. Listeriolysin S, a novel peptide haemolysin associated with a subset of lineage I *Listeria monocytogenes*. PLoS Pathog. 4, e1000144. https://doi.org/10.1371/journal.ppat.1000144.

Davis, J.A., Jackson, C.R., 2009. Comparative antimicrobial susceptibility of *Listeria monocytogenes*, *L. innocua*, and *L. welshimeri*. Microb. Drug Resist. 15, 27–32.

Dmowska, K., Wieczorek, K., Lynch, O., Osek, J., 2013. Typing of *Listeria monocytogenes* isolated from slaughtered cattle and beef meat. Bull. Vet. Inst. Pulawy 57, 179–183.

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, 3819–3822.

Escolar, C., Gómez, D., Del Carmen Rota García, M., Conchello, P., Herrera, A., 2017. Antimicrobial resistance profiles of *Listeria monocytogenes* and *Listeria innocua* isolated from ready-to-eat products of animal origin in Spain. Foodborne Pathog. Dis. 14, 357–363.

Gomez, D., Azón, E., Marco, N., Carramiñana, J.J., Rota, C., Ariño, A., Yangüela, J., 2014. Antimicrobial resistance of *Listeria monocytogenes* and *Listeria innocua* from meat products and meat-processing environment. Food Microbiol. 42, 61–65.

Gray, D.I., Kroll, R.G., 1995. Polymerase chain reaction amplification of the *fla*A gene for the rapid identification of *Listeria* spp. Lett. Appl. Microbiol. 20, 65–68.

Harakeh, S., Saleh, I., Zouhairi, O., Baydoun, E., Barbour, E., Alwan, N., 2009. Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products. Sci. Total Environ. 407, 4022–4027.

Hein, I., Klinger, S., Dooms, M., Flekna, G., Stessl, B., Leclercq, A., Hill, C., Allerberger, F., Wagner, M., 2011. Stress survival islet 1 (SSI-1) survey in *Listeria monocytogenes* reveals an insert common to *Listeria innocua* in sequence type 121 *L. monocytogenes* strains. Appl. Environ. Microbiol. 77, 2169–2173.

Henri, C., Félix, B., Guillier, L., Leekitcharoenphon, P., Michelon, D., Mariet, J., Aarestrup, F.M., Mistou, M., Hendriksen, R.S., Roussel, S., 2016. Population genetic structure of *Listeria monocytogenes* strains as determined by pulsed-field gel electrophoresis and multilocus sequence typing. Appl. Environ. Microbiol. 82, 5720–5728.

Henriques, A.R., Cristino, J.M., Fraqueza, M.J., 2017. Genetic characterization of *Listeria monocytogenes* isolates from industrial and retail ready-to-eat meat-based foods and their relationship with clinical strains from human listeriosis in Portugal. J. Food Prot. 80, 551–560.

Heras, A., Cain, R.J., Bielecka, M.K., Vazquez-Boland, J.A., 2011. Regulation of *Listeria* virulence: prfA master and commander. Curr. Opin. Microbiol. 14, 118–127.

Holch, A., Webb, K., Lukjancenko, O., Ussery, D., Rosenthal, B.M., Gram, L., 2013. Genome sequencing identifies two nearly unchanged strains of persistent *Listeria monocytogenes* isolated at two different fish processing plants sampled 6 years apart. Appl. Environ. Microbiol. 79, 2944–2951.

Interactive Tree of Life (iTOL) http://itol.embl.de/, Accessed date: 15 June 2018.

ISO, 1996. Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* - Part 1: Detection Method. (EN-ISO 11290-1:1996). International Organization for Standardization, Geneva, Switzerland.

Jacquet, C., Gouin, E., Jeannel, D., Cossart, P., Rocourt, J., 2002. Expression of ActA, Ami, InlB, and listeriolysin O in *Listeria monocytogenes* of human and food origin. Appl. Environ. Microbiol. 68, 616–622.

Jamali, H., Thong, K.L., 2014. Genotypic characterisation and antimicrobial resistance of *Listeria monocytogenes* from ready-to-eat foods. Food Control 44, 1–6.

Jamali, H., Paydar, M., Ismail, S., Looi, C.Y., Wong, W.F., Radmehr, B., Abedini, A., 2015. Prevalence, antimicrobial susceptibility and virulotyping of *Listeria* species and *Listeria monocytogenes* isolated from open-air fish markets. BMC Microbiol. 144, 1–7.

Jeffers, G.T., Bruce, J.L., McDonough, P.L., Scarlett, J., Boor, K.J., Wiedmann, M., 2001. Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal listeriosis cases. Microbiology 147, 1095–1104.

Kim, S.H., Bakko, M.K., Knowles, D., Borucki, M.K., 2004. Oral inoculation of A/J mice for detection of invasiveness differences between *Listeria monocytogenes* epidemic and environmental strains. Infect. Immun. 72, 4318–4321.

Li, L., Olsen, R.H., Ye, L., Wang, W., Shi, L., Yan, H., Meng, H., 2016. Characterization of antimicrobial resistance of *Listeria monocytogenes* strains isolated from a pork processing plant and its respective meat markets in southern China. Foodborne Pathog. Dis. 13, 262–268.

Liu, D., 2006. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. J. Microbiol. Methods 55, 645–659.

Liu, D., Lawrence, M.L., Austin, F.W., Ainsworth, A.J., 2007. A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. J. Microbiol. Methods 71, 133–140.

Lyon, S.A., Berrang, M.E., Fedorka-Cray, P.J., Fletcher, D.L., Meinersmann, R.J., 2008. Antimicrobial resistance of *Listeria monocytogenes* isolated from a poultry further processing plant. Foodborne Pathog. Dis. 5, 253–259.

Marault, M., 2008. In House Method: *Listeria monocytogenes* Pulsed Field Gel Electrophoresis Subtyping Method Developed by European Reference Laboratory for *L. monocytogenes*. Afssa-Lerquap Ceb.04.

Martin, B., Perich, A., Gomez, 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.

Morvan, A., Moubareck, C., Leclercq, A., Herve-Bazin, M., Bremont, S., Lecuit, M., Courvalin, P., Le Monnier, A., 2010. Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. Antimicrob. Agents Chemother. 54, 2728–2731.

Moura, A., Criscuolo, A., Pouseele, H., Maury, M.M., Leclercq, A., Tarr, C., Björkman, J.T., Dallman, T., Reimer, A., Enouf, V., Larsonneur, E., Carleton, H., Bracq-Dieye, H., Katz, L.S., Jones, L., Touchon, M., Tourdjman, M., Walker, M., Stroika, S., Cantinelli, T., Chenal-Francisque, V., Kucerova, Z., Rocha, E.P.C., Nadon, C., Grant, K., Nielsen, E.M., Pot, B., Gerner-Smidt, P., Lecuit, M., Brisse, S., 2016. Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. Nat. Microbiol. 2, 16185.

Neves, E., Lourenco, A., Silva, A.C., Coutinho, R., Brito, L., 2008. Pulsed-field gel electrophoresis (PFGE) analysis of *Listeria monocytogenes* isolates from different sources and geographical origins and representative of the twelve serovars. Syst. Appl. Microbiol. 31, 387–392.

Revazishvili, T., Kotetishvili, M., Stine, O.C., Kreger, A.S., Morris, J.G., Sulakvelidze, A., 2004. Comparative analysis of multilocus sequence typing and pulsed-field gel electrophoresis for characterizing *Listeria monocytogenes* strains isolated from environmental and clinical sources. J. Clin. Microbiol. 42, 276–285.

Shen, J., Rumpb, L., Zhang, Y., Chen, Y., Wang, X., Meng, J., 2013. Molecular subtyping and virulence gene analysis of *Listeria monocytogenes* isolates from food. Food Microbiol. 35, 58–64.

Su, X., Zhang, J., Shi, W., Yang, X., Li, Y., Pan, H., Kuang, D., Xu, X., Shi, X., Meng, J., 2016. Molecular characterization and antimicrobial susceptibility of *Listeria monocytogenes* isolated from foods and humans. Food Control 70, 96–102.

Tamburro, M., Sammarco, M.L., Ammendolia, M.G., Fanelli, I., Minelli, F., Ripabelli, G., 2015. Evaluation of transcription levels of *inl*A, *inl*B, *hly*, *bsh* and *prf*A genes in *Listeria monocytogenes* strains using quantitative reverse-transcription PCR and ability of invasion into human CaCo-2 cells. FEMS Microbiol. Lett. 362. https://doi.org/10.1093/femsle/fnv018.

Volkhov, D., Rasooly, A., Chumakov, K., Chizhikov, V., 2002. Identification of Listeria species by microarray-based assay. J. Clin. Microbiol. 40, 4720–4728.

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.

Wieczorek, K., Dmowska, K., Osek, J., 2012a. Characterization and antimicrobial resistance of *Listeria monocytogenes* isolated from retail beef meat in Poland. Foodborne Pathog. Dis. 9, 681–685.

Wieczorek, K., Dmowska, K., Osek, J., 2012b. Prevalence, characterization, and antimicrobial resistance of *Listeria monocytogenes* isolates from bovine hides and carcasses. Appl. Environ. Microbiol. 78, 2043–2045.

Wu, S., Wu, Q., Zhang, J., Chen, M., Yan, Z., Hu, H., 2015. *Listeria monocytogenes* prevalence and characteristics in retail raw foods in China. PLoS One 10, e0136682.

Wu, S., Wu, Q., Zhang, J., Chen, M., Guo, W., 2016. Analysis of multilocus sequence typing and virulence characterization of *Listeria monocytogenes* isolates from Chinese retail ready-to-eat food. Front. Microbiol. 7, 1–11.

Zhang, Y., Yeh, E., Hall, G., Cripe, J., Bhagwat, A.A., Meng, J., 2007. Chracterisation of *Listeria monocytogenes* isolated from retail foods. Int. J. Food Microbiol. 113, 47–53.