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Short communication

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

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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*, *lmo2672*, *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

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(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,