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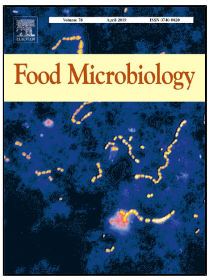

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Molecular characterization of *Listeria monocytogenes* isolates from a small-scale meat processor in Montenegro, 2011-2014

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Abstract

The presence of *Listeria monocytogenes* was evaluated in a small-scale meat processing facility in Montenegro during 2011-2014. *L. monocytogenes* isolates from traditional meat products and environmental swabs were subjected to a) molecular characterization b) serotyping by both multiplex PCR and next generation sequencing (NGS) c) potential antimicrobial resistance (AMR) was assessed by extraction of specific genes from NGS data and d) screening for the presence of some disinfectant resistance markers. Overall, traditional meat products were contaminated, most likely from incoming raw materials, with 4 major specific STs of *L. monocytogenes* (ST515, ST8, ST21, ST121) representing 4 clonal complexes (CC1, CC8, CC21, CC121) identified during the four-year period. These strains belonged to serogroup IIa which predominated, followed by IVb (ST515, CC1). The strains from environmental swabs belonged, exclusively, to ST21 and were isolated from cutting board and floor swabs in 2011. Furthermore, we found Tn*6188*, a novel transposon conferring tolerance to BC, to be specific to sequence type ST121. In addition, antimicrobial resistance genes *mprF* and *fosX* were present in clonal complexes CC21 and CC121, while complexes CC8 and CC1 exclusively harbored the *mprF* antimicrobial resistance gene.

1. Introduction

Listeria monocytogenes is a facultatively anaerobic, gram-positive, non-spore-forming, psychrophilic, salt-tolerant, facultative intracellular pathogen of humans and animals, causing clinical manifestations that include gastroenteritis, encephalitis, meningitis, abortion and septicemia (Ruppitsch et al., 2015; Lakicevic et al., 2014a). Typically, pregnant women, the elderly, and immunocompromised individuals are at greatest risk (Hyden et al., 2016; Lakicevic et al., 2014b). Among all *Listeria* species, *L. monocytogenes* remains the only one of importance to human health with lineages I and II isolates (serotypes 4b, 1/2a, 1/2b) responsible for about 99% of all human cases of foodborne listeriosis (Orsi et al., 2011; Kasper et al*.,* 2009). However, the genetic diversity, evolution and geographic distribution of *L. monocytogenes* clones remains largely unknown (Chenal-Franasque et al., 2011; Yin et al., 2015). The ability of *L. monocytogenes* to produce biofilms at low temperatures helps to facilitate persistent dissemination of this pathogen during food production (Allerberger et al., 2015). *L. monocytogenes* is transmitted from environmental sources outside the processing facility (incoming raw materials, animals, soil, dust and water) into the food processing environments (FPEs). Like other bacteria, *L. monocytogenes* can persist in biofilms on stainless steel surfaces and can be isolated from equipment, floors and cold storage areas over long periods of time. Having colonised an FPE, *L. monocytogenes* may spread throughout the facility via aerosols, personnel, food workflows, and contaminated contact materials leading to its persistent presence if sanitation procedures are insufficient (Alali and Schaffner, 2013). FPEs often display a multitude of niche environments that are challenging to effectively clean and sanitize. The problem is enhanced by inappropriate design of equipment, niche adaptation and biofilm formation leading to long-term persistence of the bacterium

(Carpentier and Cerf, 2011; Lakicevic and Nastasijevic, 2017) and recurrent cross-contamination of food products (Ferreira et al., 2014).

During the investigation of a listeriosis outbreak, rapid and accurate subtyping methods are essential for identification of the infection source and subsequent elimination of the contaminated food (Pichler et al., 2011). Martín et al. (2018) highlighted that molecular typing of *L. monocytogenes* isolates has an important role in meat processing plants in order to trace the source of contamination and transmission routes. The ongoing evolution of sequencing technologies from Sanger sequencing to next-generation sequencing (NGS) enables analysis on a whole-genome level (Ruppitsch et al., 2015). Several studies using different bacteria have shown that whole-genome sequence (WGS)–based typing, using single nucleotide variant (SNVs) approaches (Turabelidze et al., 2013; Eyre et al., 2012) or gene-by-gene allelic profiling of core genome genes, frequently named core genome MLST (cgMLST) or MLST^{+} (Mellmann et al., 2011; Maiden et al., 2013), are particularly attractive diagnostic tools for strain typing (FAO, 2016; Ruppitsch et al., 2015; Moura et al., 2016). These technologies for tracing the source of listeriosis, as well as the development and implementation of effective listeriosis prediction, monitoring, and risk assessment methods, are of great importance in the prevention and control both animal and human listeriosis (Yin et al., 2015).

This study aimed to: a) molecularly characterize selected *L. monocytogenes* strains obtained from Montenegrin meat processing establishment, b) determine the distribution of serogroups among isolates by multiplex PCR and NGS data, c) assess the presence of antimicrobial resistance (AMR) genes from NGS data and d) identify the presence of some disinfectant resistance markers (*bcrABC* cassette, *tetR*, *qacH*, *tnpABC*) (Müller et al., 2013).

2. Materials and Methods

2.1. Meat processing plant

The commercial meat processing facility in Montenegro produced about 100 tons per year of traditional pork products, including dry-cured ham (*Njeguški pršut*), pork tenderloin, pancetta (thin dry bacon) and sausages. The products were manufactured using authentic traditional methods that included cutting the meat/soft fat and connective tissue trimming, salting, removing the water under pressure, beech wood smoking, drying and ripening in Lovcen mountain air for 9 months. These deli meat products (whole or sliced package) primarily sold on the domestic market. In total, 671 samples were taken during the slaughter and preparation of dry and smoked meat products from 2011 to 2014 in the last quarter of each year (Table 1). Samples from the processing environment (swabs from surfaces and drains) were also simultaneously collected and tested. These environmental samples (taken from easily and hardly accessible non-food and food-contact surfaces) were collected in the early morning hours, before the beginning of production process and after regular sanitation had been conducted the previous day (pre-operational collection of

samples). The collected samples were transported to the laboratory within 2 h, in a cold bin at < 4°C.

2.2. Microbiological method

The food samples (an amount of 25 g), each consisting of 5 representative sample units collected from a production batch, were analyzed according to ISO 11290-1 (1996). Each environmental swab sample was taken from the total surface of 25 cm^2 and/or 100 cm^2 within the meat establishment. Wet-dry swabs (Dryswab™, MWE, UK) were used for the sampling according to ISO 18593:2004 (2004).

2.3. Biofilm formation ability

All *L. monocytogenes* isolates were examined for their ability to form biofilms using the microplate assay (Borucki et al., 2003). Each strain were inoculated into the wells (4 wells for each strain) of sterile flat-bottom microtiter plates (Nunc, Rosklide, Denmark) and incubated for 72 h at 30°C. Cut-off optical density (ODc) was defined as three standard deviations above the mean OD of the negative control. Isolates were classified as follows: non-biofilm producers ($OD \leq ODc$); weak biofilm producers ($ODc < OD \leq 2 \times ODc$); moderate biofilm producers ($2 \times ODc < OD \leq 4 \times ODc$) or strong biofilm producers ($4 \times ODc < OD$) (Stepanovic et al., 2004). Optical density (OD) was measured spectrophotometrically (Lasystems Multiscan® MCC/340) using 595 filter. Based on the results obtained from the microplate assay, one isolate of *L. monocytogenes* (randomly selected), prepared in Tryptone soya broth (TSB; Oxoid Ltd., Basingstoke, UK) (incubated 72 h at 30°C) was chosen for the visualization of biofilm by scanning electron microscopy. The sample was gold coated with a sputter coater (Sputter Coater, BAL TEC SCD 005, Liechtenstein) (working time 100 s, used current 30 mA) prior to SEM analysis (JEOL JSM 6390 LV, Japan).

2.4. Bacterial strains and DNA extraction

Isolate origin, biofilm formation ability (including mean OD ± SD) and period of isolation are listed in Table 2. All strains were cultured overnight at 37°C on RAPIDL.Mono agar (Bio-Rad, Vienna, Austria) for species confirmation and were sub-cultured on Columbia blood agar plates (BioMérieux, Marcy I'Etoile, France) prior to high quality DNA extraction using the MagAttract HMW DNA Kit, according to the instructions of the manufacturer (Qiagen, Hilden, Germany).

2.5. Whole-genome sequencing, assembly and data analysis

Sequencing libraries were prepared using NexteraXT chemistry (Illumina Inc., San Diego, CA, USA) for a 2 × 300 bp sequencing run on an Illumina MiSeq sequencer. Samples were sequenced over a minimum coverage of 70-fold using Illumina's recommended standard protocols. The resulting FASTQ files were first quality trimmed and then de novo assembled using the Velvet assembler (Zerbino and Birney, 2008) integrated in Ridom SeqSphere software (Ruppitsch et al., 2015) (version 3.1; Ridom GmbH, Münster, Germany). Sequence reads were trimmed at their 5′- and 3′-ends until an average PHRED value of 30 was reached in a window of 20 bases. The

assembly was performed with the Velvet assembler, with the k-mer values and coverage cutoffs automatically optimized for each genome, based on the average length of contigs with >1000 bp. Contigs with an overall length less than 200 bp or an average coverage below five were discarded.

Assembled genomes were compared by a recently developed core genome MLST scheme using SeqSphere$^+$ as described previously (Ruppitsch et al., 2015). Minimum spanning tree was visualized in SeqSphere$^+$ and colored in InkScape v 0.91.

For serogroup determination, relevant information was extracted from WGS data as described previously (Hyden et al., 2016) and a five-plex PCR (Doumith et al., 2004).

Antimicrobial resistance genes were identified using The Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al., 2013).

2.6. Nucleotide sequence accession number

All raw reads generated were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk /ena/) under the study accession numbers 3003770 and 3000198.

2.7. PCR screening for Tn6188 and bcrABC

In total, 20 *L. monocytogenes* strains were screened for the presence of transposon Tn*6188* conferning tolerance to benzalkonium chloride (BC). PCR primers targeting the *qacH* gene from Tn*6188* and the flanking *radC* gene, into which Tn*6188* is integrated, were designed based on available Tn*6188* and *L. monocytogenes* genome sequences (Müller et al., 2013). PCR conditions were as follows: 0.2 pmol/µl of each primer, 2mM $MgCl_2$, 1mM dNTP-Mix, 0.625U Platinum Taq DNA polymerase (Life Technologies). PCR cycling conditions were: initial denaturation for 5 min at 95°C; 30 cycles of denaturation at 94°C for 40s, annealing at 56°C for 40s and elongation at 72°C (for *qacH* 25s; for *radC* 165s); final elongation at 72°C for 5 min. Negative controls (no DNA added) and positive controls (genomic DNA from *L. monocytogenes* 6179) were included in all PCR reactions. The presence and size of amplification products was checked with agarose gel electrophoresis using SYBR Safe (Life Technologies) or ethidium bromide (Merck) –staining.

These same 20 strains were also screened for the presence of the *bcrABC* resistance cassette using primers BcF5 and BcR targeting the *bcrABC* genes (Müller et al., 2013). PCR conditions were as described above using an annealing temperature of 62°C and elongation for 45s. PCR products obtained were sequenced by LGC Genomics.

3. Results and discussion

Whole genome sequence based typing of all 20 *L. monocytogenes* isolates originating from the same Montenegrin meat establishment clearly grouped isolates into three different complexes and one singleton based on seven housekeeping genes (*abcZ*, *bglA*, *cat*, *dapE*, *dat*, *ldh*, and *lhkA*)

(Salcedo et al., 2003) (Figure 1). Significantly, isolates within ST complexes are closely related indicating that these isolates persist since several years in the establishment. WGS also provided information on the antibiotic resistance gene profiles. The predicted proteins were analyzed by BLASTp against the Comprehensive Antibiotic Resistance Database (CARD) to predict potential antibiotic resistance. Complex 1, the largest complex, comprised isolates belonging to serogroup IIa (lineage II), cluster type 5746, sequence type (ST21) and clonal complex (CC21). Isolates of complex 1 differed by a maximum allelic difference of 3 genes and harbored both the antibiotic target-modifying enzyme (*mprF*) that changes cell wall charge, and an antibiotic target-modifying enzyme (*fosX*) that is a determinant of fosfomycine resistance. *L. monocytogenes* ST21 was linked to Montenegrin dry pork sausage, pork Prosciutto and environmental swabs and was isolated during 2011 and 2013 (Table 3). Two environmental *L. monocytogenes* isolates (out of 140), were genetically identical and identified from the cutting board and floor swabs during the 2011. According to Chenal-Franasque et al., (2011), strains of CC21 were isolated in Africa (Algeria, 1988), Europe (The Netherlands and Germany), North America (Canada, 1954), predominantly from human sources. No information is currently available regarding ST21 in meat products and food processing environments.

Complex 2 contained isolates belonging to serogroup IIa (lineage II), CT 5747 and 5750, sequence type (ST121), and clonal complex (CC121). Isolates of complex 2 differed by a maximum allelic difference of 10 genes and contained both the *mprF* and *fosX*, antimicrobial resistance genes. *L. monocytogenes* ST121, highly abundant in food and food production environments (Rychly et al., 2017), was linked to Montenegrin pork dry sausage, dry pancetta, pork tenderloin and dry pork neck with this recurrent sequence type identified in 2012, 2013 and 2014 (Table 3). Despite a high number of available *L. monocytogenes* genome sequences, only a few studies have focused on analyses of *L. monocytogenes* ST121 genomes (Holch et al., 2013; Ortiz et al., 2016; Schmitz-Esser et al., 2015). Henri et al. (2016) suggested that ST121 strains may be better adapted genetically to persist in food and food processing environments but that they are less virulent for humans due to mutations in the internalin A gene. This observation may be directly relevant for refining risk analysis models for better management of food safety. Additionally, the apparent expansion of CC121 may represent the impact of certain geographic regions (Bergholz et al., 2018).

Complex 3 comprised isolates of serogroup IIa (lineage II), different cluster types (CT295, CT1358 and CT5748), sequence type ST8 and clonal complex CC8. Isolates of complex 3 differed by a maximum allelic difference of 4 genes and possessed only the *mprF* antimicrobial resistance gene. *L. monocytogenes* ST8 was isolated from minced meat, Montenegrin dry pork sausage, pork Prosciutto, and pork tenderloin in 2012 and 2013 (Table 3). Only one isolate (number 9) belonged to CT5749, sequence type (ST515), and clonal complex (CC1) and was PCR-positive for serogroup IVb (Table 3). This singleton, belonging to lineage I, encodes *mprF* protein which is involved in resistance against cationic antimicrobial peptides (CAMP) and was isolated from dry pancetta (whole meat) in 2012 (Table 3). Strains belonging to CC1 and serotype 4b (clinical associated clone) are widely distributed globally, have a high risk of causing listeriosis (Yin et al., 2015). For

example, serotype 4b *L. monocytogenes* strain F2365 was involved in the 1985 U.S. outbreak associated with Mexican-style soft cheese (Nelson et al., 2004), LL195 was involved in the 1983–1987 Switzerland outbreak associated with Vacherin Mont d'Or soft cheese (Weinmaier et al., 2013), and Scott A was involved in the 1983 U.S. outbreak associated with a clinical isolate (Bries et al., 2011). Clip80459 caused 85 deaths among 279 infected people associated with jellied pork tongue in France from 1999 to 2000 (de Valk et al., 2001). Interestingly, clonal complex CC1 is hypervirulent, strongly associated with central nervous system and materno-neonatal infections, whereas clone CC121 is associated with bacteremia and is more often isolated from highly immunocompromised patients (Maury et al., 2016). The same authors stated that most lineage I strains are overrepresented in human clinical infections and ruminant neurolisteriosis, while a majority of lineage II strains are associated with contaminated food and the environment. A recent landmark study, has revealed that *L. monocytogenes* CC1 strains harbour listeriolysin S (lls) and particular alleles of internalin (inl) F and inlJ which are not present in CCs commonly isolated from food and the environment (Rupp et al., 2017). Also, CC8 is globally distributed (Haase et al., 2014). In Switzerland, CC8 was the most prevalent clone during 2011–2013 (Althaus et al., 2014) and in Canada a CC8/ST120 clone caused both sporadic cases and outbreaks during 1988–2010 (Knabel et al., 2012).

Our results are partly similar to a recently conducted study in Serbia (Nastasijevic et al., 2017) where serotypes 1/2a, 1/2c and 4b, belonging to clonal complexes CC26, CC9 and CC1, were present in FPEs within a facility that manufacturers delicatessen meats made from pork. Notably, MLST-based phylogenetic analysis showed that serotype 1/2b and 4b strains are genetically related and have an "interval-type" evolution pattern, while serotype 1/2a and 1/2c strains have a "progressive-type" evolution pattern (Yin et al., 2015).

According to Kasper et al. (2009), 96 % of all reported human listeriosis cases are caused by lineage I and II (serotypes 4b, 1/2a, 1/2b) isolates. The overwhelming preponderance of serotypes 4b, 1/2a, and 1/2b among clinical and food isolates, clearly points to differences in ability to survive in foods and/or cause disease (Bergholz et al., 2018).

All 20 *L. monocytogenes* strains were assayed for biofilm formation by using a microtiter plate assay. Intracomplex variabililty in biofilm formation was seen; but variation in biofilm-forming capacity at the serogroup level was not observed. The isolate (originated from dry sausage, sample code: 152/1), identified as a strong biofilm producer, was not able to form a biofilm on stainless steel (Figure 2). This finding was previously confirmed by Midelet and Carpentier (2002) who reported greater attachment of *L. monocytogenes* to polyvinyl chloride and polyurethane compared to stainless steel. According to Chen et al (2006), lineage I isolates were 100 times more likely to cause listeriosis than lineage II strains and are also more capable of forming biofilms (Borucki et al., 2003).

A total of 20 *L. monocytogenes* isolates were screened for presence/absence of a novel transposon Tn*6188* and *bcrABC* resistance cassette by PCR (Table 4). Overall, Tn*6188* was present in 6 strains belonging to sequence type (ST) 121, clonal complex (CC) 121 and were classified by PCR into serogroup IIa. The presence of resistance genes might contribute to the high prevalence of ST121 in food processing plant (Pasquali et al., 2018; Palma et al., 2017; EFSA, 2018).

Similar observation was reported by Rychli et al. (2017) who identified several candidate genes possibly involved in survival of ST121 *L. monocytogenes* strains in food and food production environments; like the transposon Tn*6188*, which confers increased tolerance towards various quaternary ammonium compounds. The authors concluded that Tn*6188* is particularly abundant among ST121 strains, which is in line with other recent studies (Ebner et al., 2015; Moura et al., 2016; Müller et al., 2013; Leong et al., 2015). However, we did not detect the *bcrABC* cassette, another mostly plasmid-borne genetic feature responsible for tolerance against quaternary ammonium compounds. These findings were confirmed by Rychli et al. (2017), with Meier et al. (2017) reporting benzalkonium chloride resistance in 12.3% of Swiss and 10.6% of Finnish *L. monocytogenes* strains. In both countries, BC-resistance was most prevalent among serotype 1/2c strains. The *bcr*ABC resistance cassette has been identified in a strain related to the 1998/1999 hot dog associated listeriosis outbreak in the USA (Elhanafi et al., 2010). Its presence in nonpathogenic *Listeria* spp. indicates the possibility of these strains as reservoirs of BC and other resistance determinants for *L. monocytogenes* as a result of conjugative transfer (Katharios-Lanwermeyer et al., 2012). In one clone (CC8) that includes strains implicated in the 2008 deli meat outbreak in Canada, tolerance to quaternary ammonium disinfectants was found to be mediated by *ermE,* harbored on a chromosomal island. Expression of the *emrE* gene is upregulated in the presence of BC, raising the concern of possible adaptation and persistence of *Listeria* strains harboring this gene in the food processing environment (Kovacevic et al. 2015).

Tn*6188* is related to *Tn554* from *Staphylococcus aureus* and other Tn*554*-like transposons such as Tn*558*, Tn*559* and Tn*5406* found in various *Firmicutes*. Tn*6188* comprises 5117 bp, is integrated chromosomally within the *radC* gene and consists of three transposase genes (tnpABC) as well genes encoding a putative transcriptional regulator (*tetR*), quaternary ammonium compound resistance protein (QacH), and a small multidrug resistance protein family transporter (SMR) (Müller et al., 2013). Genes and gene cassettes conferring tolerance to quaternary ammonium disinfectants and to phage appear to have been acquired from other bacteria. Strains harboring these genes may have enhanced fitness and persistence in manufacturing facilities (Buchanan et al., 2017).

4. Conclusion

Due to the fact that several clones exist in Montenegrin meat establishment source identification should be significantly enhanced with the aid of an effective, reliable and powerful core genome

MLST method which is beneficial to the food industry and may help to improve consumer safety. The Montenegrin meat processing company yielded 4 different specific STs (ST515, ST8, ST21 and ST121) representing 4 major clonal complexes (CC1, CC8, CC21 and CC121). All of these strains belonged to molecular serogroup IIa (lineage II), except one (ST515, CC1), which belonged to serogroup IVb (lineage I) and was isolated from dry pancetta in 2012. Tn*6188* - a novel transposon conferring tolerance to BC, was found in 6 of the 20 strains, all of which belonged to specific sequence type ST121. These six strains were isolated from pancetta, sausage, tenderloin and pork neck during 2012, 2013 and 2014. Different meat products, including minced meat, were contaminated with *L. monocytogenes* ST8 in 2012 and 2013. Interestingly, the isolates from environmental swabs (cutting board and floor swabs) and from Montenegrin pork, dry sausage and pork Prosciutto belonged exclusively to ST21. In contrast, *bcrABC* was not detected in any of the strains tested. Two antimicrobial resistance genes, *mprF* and *fosX,* were present in clonal complexes CC21 and CC121, while complexes CC8 and CC1 exclusively harbored antimicrobial resistance gene *mprF*. Based on these findings, contaminated incoming raw materials led to contamination of the final Montenegrin meat products, suggesting that animals might be carrying these strains. Collectively, these results could help food processors and food agency investigators more quickly identify those *Listeria* strains that are likely to possess enhanced tolerances to certain stresses and persist long-term in food processing environments.

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References

Adzitey, F., Huda, N., 2010. *Listeria monocytogenes* in foods: incidences and possible control measures. Afr. J. Microbiol. Res. 4, 2848 – 2855.

Alali, W. Q., Schaffner, D. W., 2013. Relationship between *Listeria monocytogenes* and *Listeria* spp. in seafood processing plants. J. Food Protect. 76, 1279 – 1282.

Allerberger, F., Bagó, Z., Huhulescu, S., Pierzka, A., 2015. Listeriosis: the dark side of refrigeration and ensiling, In SingA (ed), Zoonoses - infections affecting humans and animals focus on public health aspects (pp. 249 – 286). Springer Verlag,Heidelberg, Germany.

Althaus, D., Lehner, A., Brisse, S., Maury, M., Tasara, T., Stephan, R., 2014. Characterization of *Listeria monocytogenes* strains isolated during 2011 – 2013 from human infections in Switzerland. Foodborne Pathog. Dis. 11, 753 – 758.

Bergholz, T. M., Shah, M. K., Burall, L. S., Rakic Martinez, M., Datta, A. R., 2018. Genomic and phenotypic diversity of *Listeria monocytogenes* clonal complexes associated with human listeriosis. Appl. Microbiol. Biotechnol. 102, 3475 – 3485.

Borucki, M. K., Peppin, J. D., White, D., Loge, F., Call, D. R., 2003. Variation in biofilm formation among strains of *Listeria monocytogenes*. Appl. Environ. Microbiol. 69, 7336 – 7342.

Briers, Y., Klumpp, J., Schuppler, M., Loessner, M. J., 2011. Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak. J. Bacteriol. 193, 4284 – 4285.

Buchanan, R. L., Gorris, L. G. M., Hayman, M. M., Jackson, T. C., Whiting, R. C., 2017. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control 75, 1 – 13.

Carpentier, B., Cerf, O., 2011. Review – Persistence of *Listeria monocytogenes* in food industry equipment and premises. Int. J. Food Microbiol. 145, 1 – 8.

Chen, Y., Ross, W. H., Gray, M. J., Wiedmann, M., Whiting, R. C., Scott, V. N., 2006. Attributing risk to *Listeria monocytogenes* subgroups: dose response in relation to genetic lineages. J. Food Protect. 69, 335 – 344.

Chenal-Francisque, V., Lopez, J., Cantinelli, T., Caro, V., Tran, C., Leclercq, A., Lecuit, M., Brisse, S., 2011. Worldwide distribution of major clones of *Listeria monocytogenes*. Emerg. Infect. Dis. 17, 1110 – 1112.

de Valk, H., Vaillant, V., Jacquet, C., Rocourt, J., Le Querrec, F., Stainer, F., Quelquejeu, N., Pierre, O., Pierre, V., Desenclose, J. C., Goulet, V., 2001. Two consecutive nationwide outbreaks of Listeriosis in France, October 1999 – February 2000. Am. J. Epidemiol. 154, 944 – 950.

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.

Ebner, R., Stephan, R., Althaus, D., Brisse, S., Maury, M., Tasara, T., 2015. Phenotypic and genotypic characteristics of *Listeria monocytogenes* strains isolated during 2011 – 2014 from different food matrices in Switzerland. Food Control 57, 321 – 326.

EFSA., 2018. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in EU. EFSA J. 16, 5134.

354 Elhanafi, D., Dutta, V., Kathariou, S., 2010. Genetic characterization of plasmid-associated
355 benzalkonium chloride resistance determinants in a *Listeria monocytogenes* strain from the 1998 –
356 1999 outbreak. Appl. Environ. Microbiol. 76, 8231 – 8238.

357 Eyre, D. W., Golubchik, T., Gordon, N. C., Bowden, R., Piazza, P.; Batty, E. M., Ip, C. L. C.,
358 Wilson, D. J., Didelot, X., O'Connor, L., Lay, R., Buck, D., Kearns, A. M., Shaw, A., Paul, J.,
359 Wilcox, M. H., Donnelly, P. J., Peto, T. E. A., Walker, A. S. Crook, D. W., 2012. A pilot study of
360 rapid benchtop sequencing of *Staphylococcus aureus* and *Clostridium difficile* for outbreak
361 detection and surveillance. B. M. J. Open 2:pii=e001124.10.1136/bmjopen-2012-001124.

362 Ferreira, V., Wiedmann, M., Teixeira, P., Stasiewicz, M. J., 2014. *Listeria monocytogenes*
363 Persistence in Food-Associated Environments: Epidemiology, Strain Characteristics, and
364 Implications for Public Health. J. Food Protect. 77, 150 – 170.
365
366 Food and Agriculture Organization of the United Nations., 2016. Applications of Whole Genome
367 Sequencing in food safety management. Technical Background paper.

368 Haase, J. K., Didelot, X, Lecuit, M., Korkeala, H., Achtman, M., Leclercq, A., 2014. The
369 ubiquitous nature of *Listeria monocytogenes* clones: A large-scale multilocus sequence typing
370 study. Environ. Microbiol. 16, 405 – 416.

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

375 Holch, A., Webb, K., Lukjancenko, O., Ussery, D., Rosenthal, B. M., Gram, L., 2013. Genome
376 sequencing identifies two nearly unchanged strains of persistent *Listeria monocytogenes* isolated
377 at two different fish processing plants sampled 6 years apart. Appl. Environ. Microbiol. 79(9), 2944
378 – 2951.

379 Hyden, P., Pietzka, A., Lennkh, A., Murer, A., Springer, B., Blaschitz, M., Indra, A., Huhulescu,
380 S., Allerberger, F., Ruppitsch, W., Sensen, C. W., 2016. Whole genome sequence-based
381 serogrouping of *Listeria monocytogenes* isolates. J. Biotechnol. 235, 181 – 186.

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

385 International Organization for Standardization – ISO., 2004. Microbiology of Food and Animal
386 Feeding Stuffs – Horizontal Methods for Sampling Techniques from Surfaces using Contact Plates
387 and Swabs (Geneva, Switzerland).

388 Katharios-Lanwermeyer, S., Rakic-Martinez, M., Elhanafi, D., Ratani, S., Tiedje, J. M., Kathariou,
389 S., 2012. Coselection of cadmium and benzalkonium chloride resistance in conjugative transfers
390 from nonpathogenic *Listeria* spp. to other Listeriae. Appl. Environ. Microbiol. 78, 7549 – 7556.

391 Kasper, S., Huhulescu, S., Auer, B., Heller, I., Karner, F., Würzner, R., Wagner, M., Allerberger,
392 F., 2009. Epidemiology of listeriosis in Austria. Wien. Klin. Wochenschr. 121, 113–119.

393 Kovacevic, J., Ziegler, J., Walecka-Zacharska, E., Reimer, A., Kitts, D. D., Gilmour, M. W.,
394 2015.Tolerance of *Listeria monocytogenes* to quaternary ammonium sanitizers is mediated by a
395 novel efflux pump encoded by *emrE*. Appl. Environ. Microbiol. 82, 939 – 953.

396 Knabel, S. J., Reimer, A., Verghese, B., Lok, M., Ziegler, J., Farber, J., 2012. Sequence typing
397 confirms that a predominant *Listeria monocytogenes* clone caused human listeriosis cases and
398 outbreaks in Canada from 1988 to 2010. J. Clin. Microbiol. 50, 1748 – 1751.

399 Lakicevic, B., Nastasijevic, I., 2017. *Listeria monocytogenes* in retail establishments:
400 contamination routes and control strategies. Food Rev. Int. 33, 247 – 269.

401 Lakicevic, B., Buncic, O., Katic, V., Lepsanovic, Z., Petrovic, Lj., Jankovic, V., Ikonic, P., 2014a.
402 Detection of *Listeria* spp. during production and ripening of *Petrovská klobása*. Acta Vet. 64, 367
403 – 377.

404 Lakicevic, B., Velebit, B., Jankovic, V., Spiric, D., Baltic, T., Mitrovic, R., Babic, J., 2014b. Taq
405 Man Real Time PCR detection of *Listeria monocytogenes*: a study of enrichment incubation time
406 affecting sensitivity in experimental dry fermented sausages. Tehn. mesa 55, 60 – 65.

407 Leong, D., Alvarez-Ordonez, A., Zaouali, S., Jordan, K., 2015. Examination of *Listeria*
408 *monocytogenes* in Seafood Processing Facilities and Smoked Salmon in the Republic of Ireland. J.
409 Food Protect. 78, 2184 – 2190.

410 Maiden, M. C., van Rensburg, M. J., Bray, J. E., Earle, S. G., Ford, S. A., Jolley, K. A., McCarthy,
411 N. D., 2013. MLST revisited: the gene-by-gene approach to bacterial genomics. Nat. Rev.
412 Microbiol. 11, 728 – 736. doi:10.1038/nrmicro3093.

413 Martín, B., Bover, C. S., Aymerich, T., 2018. MLVA subtyping of *Listeria monocytogenes* isolates
414 from meat products and meat processing plant. Food Res. Int. 106, 225 – 232.

415 Maury, M. M., Tsai, Y. H., Charlier, C., Touchon, M., Chenal-Francisque, V., Leclercq, A.,
416 Criscuolo, A., Gaultier, C., Roussel, S., Brisabois, A., Disson, O., Rocha, E. P. C., Brisse, S., 2016.
417 Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat. Genet. 48,
418 308 – 313.

419 McArthur, A. G., Waglechner, N., Nizam, F., Yan, A., Azad, M. A., Baylay, A. J., Bhullar, K.,
420 Canova, M. J., De Pascale, G., Ejim, L., Kalan, L., King, A. M., Koteva, K., Morar, M., Mulvey,
421 M. R., O'Brien, J. S., Pawlowski, A. C., Piddock, L. J., Spanogiannopoulos, P., Sutherland, A. D.,

Tang, I., Taylor, P. L., Thaker, M., Wang, W., Yan, M., Yu, T., Wright, G. D., 2013. The comprehensive antibiotic resistance database. Antimicrob. Agents Chemother. 57, 3348–3357.

Meier, A. B., Guldimann, C., Markkula, A., Pöntinen, A., Korkeala, H., Tasara, T., 2017. Comparative Phenotypic and Genotyping Analysis of Swiss and Finnish *Listeria monocytogenes* Isolates with Respect to Benzalkonium Chloride Resistance. Front. Microbiol. https://doi.org/10.3389/fmicb.2017.00397

Mellmann, A., Harmsen, D., Cummings, C. A., Zentz, E. B., Leopold, S. R., Rico, A., Prior, K., Szczepanowski, R., Ji, Y., Zhang, W., McLaughlin, S. F., Henkhaus, J. K., Leopold, B., Bielaszewska, M., Prager, R., Brzoska, P. M., Moore, R. L., Guenther, S., Rothberg, J. M., Karch H., 2011. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. PloS One 6:e22751.10.1371/journal.pone.0022751.

Midelet, G., Carpentier, B., 2002. Transfer of microorganisms, including *Listeria monocytogenes*, from various material to beef. Appl. Environ. Microbiol. 68, 4015 – 4024.

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., 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.

Müller, A., Rychli, K., Muhterem-Uyar, M., Zaiser, A., Stessl, B., Guinane, C. M., Cotter, P. D., Wagner, M., Stephan Schmitz-Esser, S., 2013. Tn6188 – A Novel Transposon in *Listeria monocytogenes* Responsible for Tolerance to Benzalkonium Chloride. PloS One 8(10): e76835. doi:10.1371/journal.pone.0076835.

Nastasijevic, I., Milanov, D., Velebit, B., Djordjevic, V., Swift, C., Painset, A., Lakicevic, B., 2017. Tracking of *Listeria monocytogenes* in meat establishment using Whole Genome Sequencing as a food safety management tool: A proof of concept. Int. J. Food Microbiol. 257, 157 – 164.

Nelson, K. E., Fouts, D. E., Mongodin, E. F., Ravel, J., DeBoy, R. T., Kolonay, J. F., Rasko, D. A., Angiuoli, S. V., Gill, S. R., Paulsen, I. T., Peterson, J., White, O., Nelson, W. C., Nierman, W., Beanan, M. J., Brinkac, L. M., Daugherty, S. C., Dodson, R. J., Durkin, A. S., Madupu, R., Haft, D. H., Selengut, J., Van Aken, S., Khouri, H., Fedorova, N., Forberger, H., Tran, B., Kathariou, S., Wonderling, L. D., Uhlich, G. A., Bayles, D. O., Luchansky, J. B., Fraser, C. M., 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. Nucleic Acids Res. 32, 2386 – 2395.

Orsi, R. H, den Bakker, H. C., Wiedmann, M., 2011. *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. Int. J. Med. Microbiol. 301, 79 – 96.

Ortiz, S., Lopez-Alonso, V., Rodriguez, P., Martinez-Suarez, J. V., 2016. The connection between persistent, disinfectant-resistant *Listeria monocytogenes* strains from two geographically separate Iberian pork processing plants: Evidence from comparative genome analysis. Appl. Environ. Microbiol. 82, 308 – 317.

Palma, F., Pasquali, F., Lucchi, A., De Cesare, A., Manfreda, G., 2017. Whole genome sequencing for typing characterisation of *Listeria monocytogenes* isolated in a rabbit meat processing plant. Ital. J. Food Saf. 6:6879. doi: 10.4081/ijfs.2017.6879.

Pasquali, F., Palma, F., Guillier, L., Lucchi, A., De Cesare, A., Manfreda, G., 2018. *Listeria monocytogenes* sequence types 121 and 14 repeatedly isolated within one year of sampling in a rabbit meat processing plant: persistence and ecophysiology. Front. Microbiol. 9:596. doi: 10.3389/fmicb.2018.00596

Pichler, J., Appl, G., Pietzka, A., Allerberger, F., 2011. Lessons to be learned from an outbreak of foodborne listeriosis, Austria 2009 – 2010. Food Prot. Trends 31, 268–273.

Rupp, S., Bärtschi, M., Frey, J., Oevermann, A., 2017. Hyperinvasiveness and increased intercellular spread of *Listeria monocytogenes* sequence type 1 are independent of listeriolysin S, internalin F and internalin J1. J. Med. Microbiol. 66, 1053 – 1062.

Ruppitsch, W., Pietzka, A., Prior, K., Bletz, S., Fernandez, H. L., Allerberger, F., Harmsen, D., Mellmann, A., 2015. Defining and Evaluating a Core Genome Multilocus Sequence Typing Scheme for Whole-Genome Sequence-Based Typing of *Listeria monocytogenes*. J. Clin. Microbiol. 53, 2869 – 2876.

Rychli, K., Wagner, E. M., Ciolacu, L., Zaiser, A., Tasara, T., Wagner, M., Schmitz-Esser, S., 2017. Comparative genomics of human and non-human *Listeria monocytogenes* sequence type 121 strains. PloS One, 12,e0176857.

Salcedo, C., Arreaza, L., Alcala, B., de la Fuente, L., Vazquez, J. A., 2003. Development of a multilocus sequence typing method for analysis of *Listeria monocytogenes* clones. J. Clin. Microbiol. 41, 757 – 762.

Schmitz-Esser, S., Muller, A., Stessl, B., Wagner, M., 2015. Genomes of sequence type 121 *Listeria monocytogenes* strains harbor highly conserved plasmids and prophages. Front. Microbiol. 6:380. https://doi.org/10.3389/fmicb.2015.00380

Stepanovic, S., Cirkovic, I., Ranin, L., Svabic-Vlahovic, M., 2004. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett. Appl. Microbiol. 38, 428 – 432.

491 Turabelidze, G., Lawrence, S. J., Gao, H., Sodergren, E., Weinstock, G. M., Abubucker, S., Wylie,
492 T., Mitreva, M., Shaikh, N., Gautom, R., Tarr, P. I., 2013. Precise dissection of an *Escherichia coli*
493 O157:H7 outbreak by single nucleotide polymorphism analysis. J. Clin. Microbiol. 51, 3950 –
494 3954.

495 Yin, Y., Tan, W., Wang, G., Kong, S., Zhou, X., Zhao, D., Jia, Y., Pan, Z., Jiao, X., 2015.
496 Geographical and longitudinal analysis of *Listeria monocytogenes* genetic diversity revaeals its
497 correlation with virulence and unique evolution. Microbiol. Res. 175, 84 – 92.

498 Weinmaier, T., Riesing. M., Rattei, T., Bille, J., Arguedas-Villa, C., Stephan, R., Tasara, T., 2013.
499 Complete genome sequence of *Listeria monocytogenes* LL195, a serotype 4b strain from the 1983
500 – 1987 Listeriosis epidemic in Switzerland. Genome Announc. 1, e00152 – 212.

501 Zerbino, D. R., Birney, E., 2008. Velvet: algorithms for de novo short read assembly using de
502 Brujin graphs. Genome Res. 18, 821-829. 10.1101/gr.074492.107.

503

504 Table 1. The list of collected food and environmental samples during the four - year period

Year	2011	2012	2013	2014	∑
Dry sausage	18	20	34	18	90
Dry tenderloin	25	32	10	20	87
Pršut- dry cured ham	82	77	15	15	189
Dry pancetta	15	16	30	25	86
Dry neck	22	17	10	30	79
Swabs	40	58	22	20	140
∑	202	220	121	128	671

505

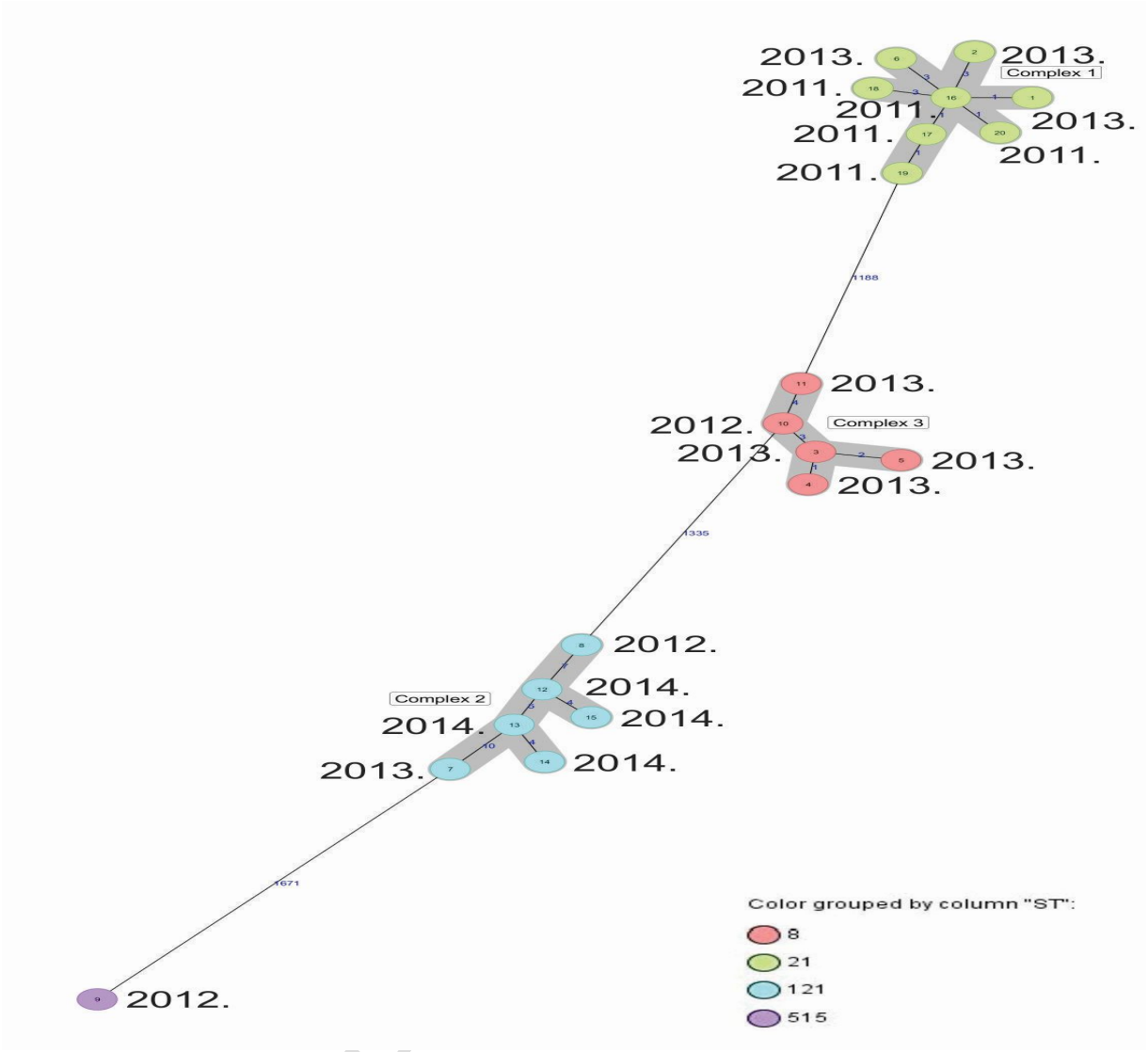

Figure 1: Minimum spanning tree analysis based on cgMLST allelic profiles of 20 *L. monocytogenes* isolates from the Montegrin meat establishment. Each circle represents an allelic profile based on sequence analysis of 1,701 genes. The numbers on the connecting lines illustrate the numbers of target genes with differing alleles. Isolates belonging to a cluster are called "complex" and these closely related genotypes (≤ 10 allele difference) are shown with a grey shadow. The different ST groups of strains are distinguished by the colors of the circles according to year of isolation.

517 Table 2. *L. monocytogenes* strains used in this study, indicating number of isolates testing, their
518 origin, biofilm formation ability with mean OD±SD and period of isolation

ID	Sample code	Origin	Biofilm formation ability	Mean OD±SD[£]	Collection year
1	152/1	Montenegrin dry sausage (pork)	strong[a]	0,724±0,029	2013
2	152/3	Montenegrin dry sausage (pork)	strong[a]	0,738±0,055	2013
3	256/2	Minced meat, from machine, for fill sausage	strong[a]	0,707±0,066	2013
4	256/3	Minced meat from Mixer	moderate[b]	0,386±0,032	2013
5	256/4	Montenegrin dry sausage (pork)	moderate[b]	0,467±0,042	2013
6	256/5	Montenegrin dry sausage (pork)	moderate[b]	0,577±0,024	2013
7	328/1	Dry pancetta - sliced	moderate[b]	0,542±0,051	2013
8	728/49	Montenegrin dry sausage (pork)	moderate[b]	0,417±0,056	2012
9	728/50	Dry pancetta – whole meat	moderate[b]	0,331±0,054	2012
10	728/51	Pork Prosciutto - sliced	moderate[b]	0,408±0,053	2012
11	1208	Dry pork Tenderloin	strong[a]	0,704±0,073	2013
12	2018/1-5	Dry pork Tenderloin	moderate[b]	0,472±0,057	2014
13	2019/1-5	Dry pancetta	moderate[b]	0,555±0,065	2014
14	2020/1-5	Dry pork neck	moderate[b]	0,380±0,026	2014
15	2052/43	Dry pork neck	moderate[b]	0,357±0,101	2014
16	3564/3	Montenegrin dry sausage (pork)	moderate[b]	0,404±0,056	2011
17	3565/7	Cutting board swab	moderate[b]	0,377±0,043	2011
18	3565/15	Floor swab – salting room	moderate[b]	0,361±0,049	2011
19	3356/1	Pork Prosciutto - sliced	moderate[b]	0,410±0,036	2011
20	3356/4	Pork Prosciutto - chopped	moderate[b]	0,333±0,019	2011

519 [£]The optical density values were displayed for Tryptone soya broth (TSB) incubated 72h at 30°C; ODc (TSB) = 0.161
520 [a]strong biofilm producer: OD > 0.644
521 [b]moderate biofilm producer:0.322 < OD ≤ 0.644
522
523

524 Table 3 *L. monocytogenes* strains used in this study, indicating their origin, antimicrobial
525 resistance, Accession number and sequence type according to year of isolation

ID	Origin	AMR	ARO Category	ARO Accession	Sequence Type	Collection year
1	Montenegrin dry sausage (pork)	mprF	Antibiotic target modifying enzyme, gene altering	3003770	21	2013
2	Montenegrin dry sausage (pork)	mprF	Antibiotic target modifying enzyme, gene altering	3003770	21	2013
3	Minced meat, from machine, for fill sausage	mprF	Antibiotic target modifying enzyme, gene altering	3003770	8	2013
4	Minced meat from Mixer	mprF	Antibiotic target modifying enzyme, gene altering	3003770	8	2013
5	Montenegrin dry sausage (pork)	mprF	Antibiotic target modifying enzyme, gene altering	3003770	8	2013
6	Montenegrin dry sausage (pork)	mprF	Antibiotic target modifying enzyme, gene altering	3003770	21	2013
7	Dry pancetta – sliced	mprF	Antibiotic target modifying enzyme, gene altering	3003770	121	2013
8	Montenegrin dry sausage (pork)	fosX	Deteriminant of fosfomycin resistance	3000198	121	2012
9	Dry pancetta – whole meat	mprF	Antibiotic target modifying enzyme, gene altering	3003770	515	2012
10	Pork Prosciutto - sliced	mprF	Antibiotic target modifying enzyme, gene altering	3003770	8	2012
11	Dry pork Tenderloin	mprF	Antibiotic target modifying enzyme, gene altering	3003770	8	2013
12	Dry pork Tenderloin	fosX	Deteriminant of fosfomycin resistance	3000198	121	2014
13	Dry pancetta	fosX	Deteriminant of fosfomycin resistance	3000198	121	2014
14	Dry pork neck	mprF	Antibiotic target modifying enzyme, gene altering	3003770	121	2014
15	Dry pork neck	mprF	Antibiotic target modifying enzyme, gene altering	3003770	121	2014
16	Montenegrin dry sausage (pork)	mprF	Antibiotic target modifying enzyme, gene altering	3003770	21	2011
17	Cutting board swab	mprF	Antibiotic target modifying enzyme, gene altering	3003770	21	2011
18	Floor swab – salting room	mprF	Antibiotic target modifying enzyme, gene altering	3003770	21	2011
19	Pork Prosciutto - sliced	fosX	Deteriminant of fosfomycin resistance	3000198	21	2011
20	Pork Prosciutto – chopped	fosX	Deteriminant of fosfomycin resistance	3000198	21	2011

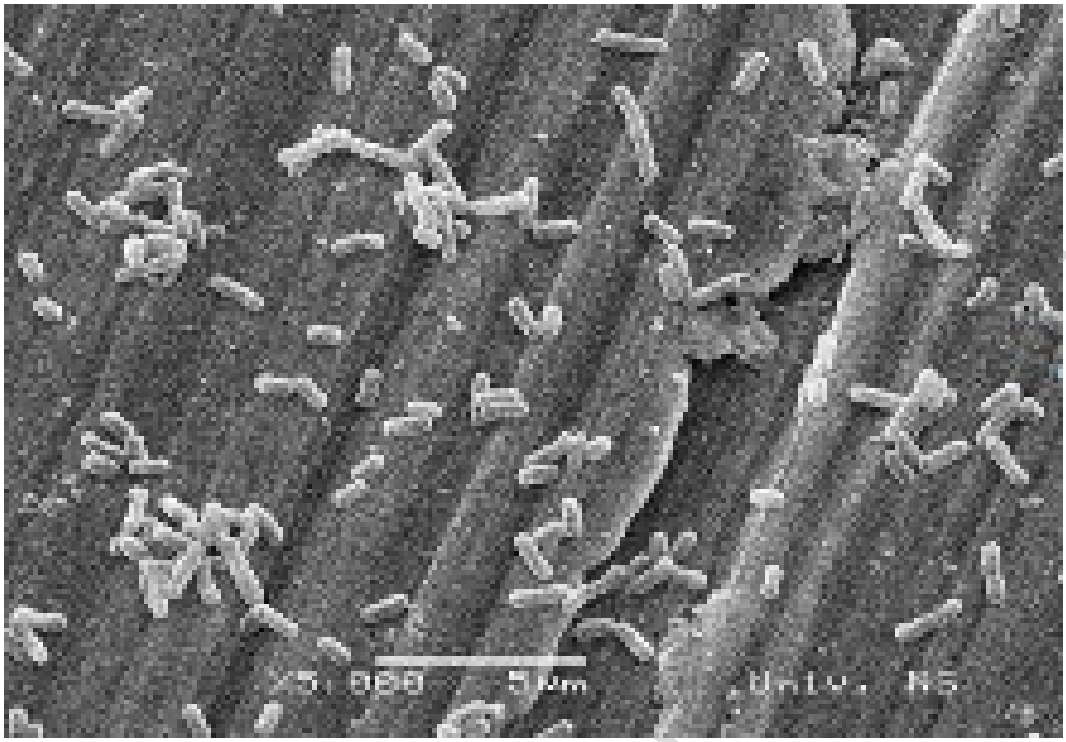

Figure 2: Scanning Electron Microscopy (SEM) – Single cells and diplo-forms producing V or Y shapes strain on stainless steel surface (bar 5 μm) after incubation of *L. monocytogenes* isolate in TSB for 72 h days at 30 °C (JEOL JSM 6390 LV, Japan).

Table 4. The connection between Core Genome Multilocus Sequence Typing, AMR and some disinfectant resistance markers

ID (NRL Graz)	ID	ST	Cluster Type	Serogroup	CC	Profile	abcZ	bglA	cat	dapE	dat	ldh	lhkA	bcrA	bcrB	bcrC	tetR	qacH	tnpC	tnpB	tnpA
MRL-17-01214	1	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-
MRL-17-01215	2	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-
MRL-17-01216	3	8	295	IIa	CC8	5, 6, 2, 9, 5, 3, 1	5	6	2	9	5	3	1	-	-	-	-	-	-	-	-
MRL-17-01217	4	8	1358	IIa	CC8	5, 6, 2, 9, 5, 3, 1	5	6	2	9	5	3	1	-	-	-	-	-	-	-	-
MRL-17-01218	5	8	1358	IIa	CC8	5, 6, 2, 9, 5, 3, 1	5	6	2	9	5	3	1	-	-	-	-	-	-	-	-
MRL-17-01219	6	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-
MRL-17-01220	7	121	5747	IIa	CC121	7, 6, 8, 8, 6, 37, 1	7	6	8	8	6	37	1	-	-	-	+	+	+	+	+
MRL-17-01221	8	121	5750	IIa	CC121	7, 6, 8, 8, 6, 37, 1	7	6	8	8	6	37	1	-	-	-	+	+	+	+	+
MRL-17-01222	9	515	5749	IVb	CC1	3, 1, 1, 39, 3, 1, 3	3	1	1	39	3	1	3	-	-	-	-	-	-	-	-
MRL-17-01223	10	8	5748	IIa	CC8	5, 6, 2, 9, 5, 3, 1	5	6	2	9	5	3	1	-	-	-	-	-	-	-	-
MRL-17-01224	11	8	1358	IIa	CC8	5, 6, 2, 9, 5, 3, 1	5	6	2	9	5	3	1	-	-	-	-	-	-	-	-
MRL-17-01225	12	121	5750	IIa	CC121	7, 6, 8, 8, 6, 37, 1	7	6	8	8	6	37	1	-	-	-	+	+	+	+	+
MRL-17-01226	13	121	5747	IIa	CC121	7, 6, 8, 8, 6, 37, 1	7	6	8	8	6	37	1	-	-	-	+	+	+	+	+
MRL-17-01227	14	121	5750	IIa	CC121	7, 6, 8, 8, 6, 37, 1	7	6	8	8	6	37	1	-	-	-	+	+	+	+	+
MRL-17-01228	15	121	5750	IIa	CC121	7, 6, 8, 8, 6, 37, 1	7	6	8	8	6	37	1	-	-	-	+	+	+	+	+
MRL-17-01229	16	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-
MRL-17-01230	17	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-
MRL-17-01231	18	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-
MRL-17-01232	19	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-
MRL-17-01233	20	21	5746	IIa	CC21	7, 7, 3, 10, 5, 6, 1	7	7	3	10	5	6	1	-	-	-	-	-	-	-	-

ST: Sequence Type; CC: Clonal Complex; *abcZ* (ABC transporter), *bglA* (beta-glucosidase), *cat* (catalase), *dapE* (Succinyl diaminopimelate desuccinylase), *dat* (D-amino acid aminotransferase), *ldh* (lactate deshydrogenase), *lhkA* (histidine kinase), *bcrABC* (benzalkonium chloride resistance cassette), *tetR* (transcriptional regulator), *qacH* (Quaternary ammonium compoundresistance protein), *tnpABC* (transposase), + present; - absence

Molecular characterization of *Listeria monocytogenes* isolates from a small-scale meat processor in Montenegro, 2011-2014

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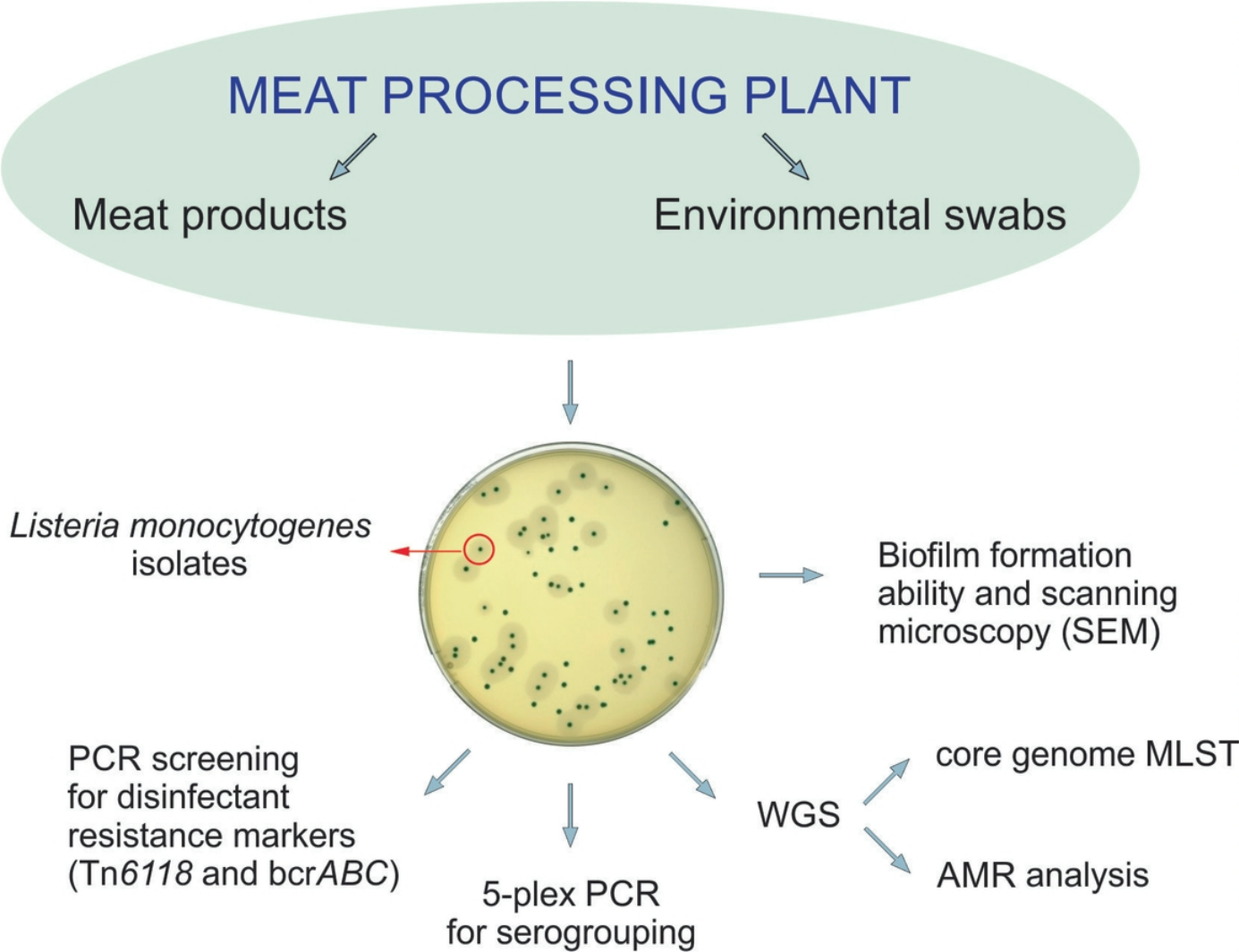
Highlights

- Genetic diversity and biofilms of *Listeria monocytogenes* isolated from traditional meat products and environmental swabs
- The Montenegrin meat processing company yielded 4 different specific STs (ST515, ST8, ST21 and ST121) representing 4 major clonal complexes (CC1, CC8, CC21 and CC121)
- Strains were predominantly from serogroup IIa, followed by IVb
- Only ST 121 strains contained benzalkonium chloride (BC) resistance transposon Tn*6188*
- Two antimicrobial resistance genes, *mprF* and *fosX*, were present in some sequences


MEAT PROCESSING PLANT
Meat products
Environmental swabs
Listeria monocytogenes isolates
Biofilm formation ability and scanning microscopy (SEM)
PCR screening for disinfectant resistance markers (Tn6118 and bcrABC)
5-plex PCR for serogrouping
WGS
core genome MLST
AMR analysis