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Abstract: Listeria monocytogenes is widely distributed in meat products and the meat-processing industry thus posing a risk to consumers. The aim of this study was to evaluate the suitability of the multilocus variable-number tandem-repeat analysis (MLVA) for use as a L. monocytogenes subtyping technique for surveillance and routine control in meat products and meat processing plants. A collection of 113 isolates (including control strains and isolates from meat products and meat processing plants) were subject to MLVA analysis using two different platforms for fragment sizing: 1.) ABI 3730xl DNA analyzer (Life Technologies) as the reference method and 2.) the QIAxcel Advanced System (Qiagen). Although discrepancies in fragment sizing were observed it was possible to standardize the results in order to assign the same allele for a given fragment independently of the platform used for fragment sizing. MLVA and multilocus sequence typing (MLST) results were compared and yielded Simpson's diversity indices of 0.907 and 0.872, respectively. The congruence between both typing methods was measured with the adjusted Wallace coefficient (AW). Using MLVA as the primary method, AW= 0.946 suggested that MLVA can predict the sequence type with high accuracy. Given its discriminatory power and high throughput, MLVA could be considered a rapid, reliable, and high-throughput alternative to existing subtyping methods for surveillance and control of L. monocytogenes in the meat-processing industry.

Highlights

QIAxcel sizing platform is a trustworthy alternative to automated sequencers

Twenty-four MLVA unique profiles were determined among the meat industry isolates

MLVA had more discriminatory power than MLST, especially for serotype 1/2c isolates

MLVA can predict the sequence type with high accuracy

MLVA is a reliable method to type and trace *L.monocytogenes* in the meat industry

MLVA subtyping of *Listeria monocytogenes* isolates from meat products and meat processing plants

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ABSTRACT

Listeria monocytogenes is widely distributed in meat products and the meat-processing industry thus posing a risk to consumers. The aim of this study was to evaluate the suitability of the multilocus variable-number tandem-repeat analysis (MLVA) for use as a *L. monocytogenes* subtyping technique for surveillance and routine control in meat products and meat processing plants. A collection of 113 isolates (including control strains and isolates from meat products and meat processing plants) were subject to MLVA analysis using two different platforms for fragment sizing: 1.) ABI 3730xl DNA analyzer (Life Technologies) as the reference method and 2.) the QIAxcel Advanced System (Qiagen). Although discrepancies in fragment sizing were observed it was possible to standardize the results in order to assign the same allele for a given fragment independently of the platform used for fragment sizing. MLVA and multilocus sequence typing (MLST) results were compared and yielded Simpson's diversity indices of 0.907 and 0.872, respectively. The congruence between both typing methods was measured with the adjusted Wallace coefficient (AW). Using MLVA as the primary method, AW= 0.946 suggested that MLVA can predict the sequence type with high accuracy. Given its discriminatory power and high throughput, MLVA could be considered a rapid, reliable, and high-throughput alternative to existing subtyping methods for surveillance and control of *L. monocytogenes* in the meat-processing industry.

1. Introduction

Listeria monocytogenes is a ubiquitous bacterium that is known as the causative agent of human listeriosis, an important foodborne disease with a high fatality rate particularly in high-risk population such as the elderly, immunocompromised patients, pregnant woman and newborn infants. In The European Union, there has been an increasing trend of listeriosis during the period 2008-2015. A total of 1,524 confirmed human listeriosis cases were reported in 2015 with a fatality rate of 17.7% (EFSA-ECDC, 2016).

L. monocytogenes is widely distributed in food-processing environments (Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014; Martin et al., 2014; Paoli, Bhunia, & Bayles, 2005; Tompkin, 2002) thus posing a risk of contamination of food products. Contaminated foods are consider the main vehicle for listeriosis (Scallan et al., 2011) particularly ready-to-eat (RTE) foods, which are intended to be consumed without further processing. Outbreaks and sporadic cases of listeriosis are generally associated to the consumption of those RTE foods such as soft cheese, smoked fish, vegetables and meat and meat products (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017; EFSA-ECDC, 2016).

Characterization of *L. monocytogenes* strains is needed in order to determine its virulence potential, for surveillance purposes and epidemiological tracking (Kathariou, 2002; Swaminathan & Gerner-Smidt, 2007). Among the 13 described serotypes of *L. monocytogenes*, serotypes 1/2a, 1/2b and 4b are implicated in most cases of human listeriosis and outbreaks (Clark et al., 2010; Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004; EFSA-ECDC, 2015, 2016; Pagotto, Ng, Clark, & Farber, 2006). On the other hand, serotype 1/2c are commonly described in food-processing environments and food products (Gelbicova & Karpiskova, 2009; Martin et al., 2014; Thévenot et al., 2006) but it has rarely been implicated in human listeriosis cases (Orsi, den Bakker, & Wiedmann, 2011).

Molecular typing methods are crucial for the identification and monitoring clonal groups of *L. monocytogenes* along the food chain. There are many molecular methods currently available for typing *L. monocytogenes* isolates differing in discriminatory power and epidemiological concordance (EFSA BIOHAZ Panel, 2013; van Belkum et al., 2007). Pulsed-field gel electrophoresis (PFGE) has been long considered the "gold standard" method for *L. monocytogenes* subtyping because of its high discriminatory power, reproducibility and repeatability (Gerner-Smidt et al., 2006; Lukinmaa, Aarnisalo, Suihko, & Siitonen, 2004). However, PFGE is considered a laborious and time-consuming technique with limited data portability (EFSA BIOHAZ Panel, 2013; Heir, Lindstedt, Vardund, Wasteson, & Kapperud, 2000). Nowadays, sequence-based typing methods provide unambiguous and portable data that can be useful not only for typing purposes, but also for the study of the population structure and evolution of this pathogen (Nightingale, 2010). Multi-locus sequence typing (MLST) is a well stablished sequenced-based typing method for studying population genetics of *L. monocytogenes* (Salcedo, Arreaza, Alcala, de la Fuente, & Vazquez, 2003) providing an easy and unambiguous inter-laboratory exchange of data through public databases (Nightingale, 2010). Multi-virulence-locus sequence typing (MVLST) has also been used as a sequence-based approach for *L. monocytogenes* genotyping showing an excellent epidemiological concordance (Y. Chen, Zhang, & Knabel, 2007; Zhang, Jayarao, & Knabel, 2004). Nevertheless these techniques generally show a limited discriminatory power (den Bakker, Didelot, Fortes, Nightingale, & Wiedmann, 2008; EFSA BIOHAZ Panel, 2013; Ragon et al., 2008) for their use in the surveillance of *L*. *monocytogenes*. The advent of next generation sequencing technologies has dramatically reduced the cost of DNA sequencing making whole-genome sequencing (WGS) a convenient tool for molecular epidemiology and foodborne outbreak investigations (Datta, Laksanalamai, & Solomotis, 2013); thus, it is rapidly becoming the method of choice for *L. monocytogenes* genotyping in national reference laboratories. On the other hand, WGS is still prohibitive for most routine laboratories and generates massive amount of

data requiring intensive bioinformatic analysis, especially for testing a large number of isolates. Recently, multiple-locus variable number of tandem repeat analysis (MLVA) has been emerged as a powerful method to subtype food-borne pathogens such as *Salmonella enterica* serotypes Typhimurium and Enteritidis (Heck, 2009; Lindstedt, Heir, Gjernes, & Kapperud, 2003), *Escherichia coli* O157:H7 (Cooley et al., 2007) and *L. monocytogenes* (Lindstedt et al., 2008; Miya et al., 2008; Murphy et al., 2007; Sperry, Kathariou, Edwards, & Wolf, 2008). The approach is based on the detection of the number of tandem repeats at multiple variable-number tandem repeat loci distributed along the genome. Typically, multiplex PCR is used to amplify the tandem repeats and flanking regions and the amplification products are sized using capillary electrophoresis. MLVA is considered an easy and low-cost method which provides rapid and portable results with a high discriminatory power (Lindstedt et al., 2008; Sperry et al., 2008).

In this study, we have applied MLVA to subtype a collection of *L. monocytogenes* strains isolated from meat-processing plants using both an automatic sequencer and/or capillary electrophoresis for amplicon sizing. MLVA results were compared to those previously obtained with MLST to evaluate the discriminatory power of the technique for its implementation in systematic environmental and product monitoring in meat processing plants.

2. Material and methods

2.1. L. monocytogenes *isolates*

A total of 106 isolates of *L*. *monocytogenes* were obtained from meat products and meat processing plants. Ninety-six isolates were collected from 18 meat processing plants, including 53 isolates from RTE meat products (fermented sausages, dry ham, blood sausages and other cured pork products), 10 isolates from raw meat products (beef and pork) and 33 isolates from food contact surfaces. In addition, 10 isolates

from the IRTA collection (recovered from meat products) were also analyzed. Seven control strains of *L. monocytogenes,* EGDe, ScottA, ATCC 35152 (equivalent to CIP104794), ATCC 19112 (equivalent to SLCC2372), ATCC 19114 (equivalent to SLCC2374), ATCC 19117 and CECT4032 (equivalent to F646/86) were used to calibrate the MLVA method.

The serotype and sequence type (ST) of the 106 isolates were previously determined by us (Martin et al., 2014). For control strains, ST and serotype was obtained from the Listeria MLST Database hosted at the web site of the Institut Pasteur (http://bigsdb.pasteur.fr/listeria/) and from Chenal-Francisque et al. (2011) and Cantinelli et al. (2013).

2.2. DNA extraction from L. monocytogenes *isolates.*

Genomic DNA was extracted using DNeasy® blood and Tissue kit (Qiagen GmbH, Hilden, Germany) and the QIAcube® automated sample preparation system (Qiagen). Cultures were grown overnight at 37ºC in Triptic Soy Broth (BD, Sparks, MD) and 1 ml was centrifuged at 9,000 x g for 5 min; the pellets obtained were resuspended in 180 µl of lysis buffer (35 mg/ml lysozyme in 20 mM Tris-HCl pH 8.0, 2 mM EDTA, 1.2% Triton) and the enzymatic lysis step was programmed at 37ºC for 1 h. DNA was eluted in 150 µl of AE buffer (10 mM Tris-HCl, 0.5 mM EDTA pH 9.0), quantified using Quant-It™ high sensitivity DNA assay kit (Invitrogen, Merelbeke, Belgium) and adjusted to 20 ng/µl. The DNA was split into aliquots and stored at -20ºC for further use in PCR.

2.3. MLVA

The MLVA procedure was conducted using the eight primer pairs proposed by Sperry et al. (Sperry et al., 2008; Sperry, Kathariou, Edwards, & Wolf, 2009) and two different multiplex-PCR protocols were performed. The first protocol consisted in two 4-plex PCR reactions as in the reference protocol but using different dyes to label forward primers (Table 1), the Type-it Microsatellite PCR Kit (Qiagen), 0.2 µM of each

primer (all purchased at Life Technologies) and 2 µl of DNA. After amplification, fluorescent PCR products were resolved by automated capillary electrophoresis on an ABI 3730xl DNA analyzer (Life Technologies) with GeneScan-500 LIZ size standard (Life Technologies) using commercial GeneScan service (Macrogen Inc). Fragment sizes obtained were then analyzed using the Peakscanner software version 1.0 (ThermoFisher Scientific). The second protocol consisted in 3 different multiplex-PCR reactions of 25 µl final volume using also the Type-it Microsatellite PCR Kit and the same primers (0.2 µM each) but not labelled. PCR reaction one (PCR1) contained primers for amplification of locus Lm-2, Lm-23 and Lm-32, the second PCR (PCR2) contained primers for locus Lm-3 and Lm-5 and the third PCR (PCR3) contained primers for locus Lm-8, Lm-10 and Lm-11. Thermal cycling conditions consisted of a denaturation step at 95ºC for 5 min and 35 cycles of 30 s at 94ºC, 30 s at 50ºC and 30 s at 72ºC and a final extension step at 72ºC for 5 min. All amplification reactions were performed in a thermal cycler GeneAmp PCR System 2700. The high-resolution capillary electrophoresis device QIAxcel Advanced System (Qiagen) was used to determine the size of PCR products. After PCR amplification, PCR plates were directly loaded onto the system. The QIAxcel DNA High Resolution kit was used applying the method OM800 (separation time of 920 s at 5 kV) with the Alignment Marker 15-600 bp (Qiagen). Each QIAxcel run included a QX DNA size Marker 25-600 bp (Qiagen). Fragment sizing was performed using software QIAxcel Screengel v 1.4.0 (Qiagen).

2.4. Data analysis

Estimated fragment size, dye (when present), peak height, and area data for each isolate were exported into Bionumerics Software v. 7.6 (Applied Maths, Sint-Martens-Latem, Belgium). With the first protocol (fragments size determined with ABI 3730xl DNA analyzer), seven control strains (EGDe, ScottA, ATCC 35152, ATCC 19112, ATCC 19114, ATCC 19117 and CECT4032) were used to calibrate the system and

adjust the offsets (Table 1) to obtain the same copy number for each VNTR locus as the reference study (Sperry et al., 2008). Null alleles were coded as negative (-2).

Results obtained with the second protocol (fragments size determined with QIAxcel advanced System) were calibrated according the results obtained with the automated sequencer. A look-up table was constructed for allele assignment containing the different alleles obtained for each locus and the corresponding observed fragment size (Table 2). This table was used as a mapping list in Bionumerics MLVA plugin for determining VNTR copy numbers.

The relationship among *L. monocytogenes* isolates was studied with the minimum spanning tree (MST) using categorical coefficient and the unweighted-pair group method with arithmetic mean (UPGMA). Both analysis were performed with Bionumerics software. Simpson's diversity index (SID) as described by Hunter and Gaston (1988) was used to measure genetic diversity among isolates. An index greater than 0.90 is considered desirable if the typing results are to be interpreted with confidence (Hunter & Gaston, 1988). The degree of congruence among MLVA and MLST was calculated using the adjusted Wallace coefficient (AW), which indicates the probability that two strains classified as the same type by one typing method will also be identified as the same type by the other method (Carrico et al., 2006). Both index were calculated using the Comparing Partitions tool available online (http://www.comparingpartitions.info/).

3. Results

3.1. Optimization of L. monocytogenes *MLVA*

A total of 106 isolates previously recovered from meat processing plants and meat products (RTE and raw products) were submitted to MLVA typing. In addition, 7 control strains were also analyzed to first stablish the correct offset to obtain the same copy number for each locus. *L. monocytogenes* ATCC 19117 was not analyzed in the study

carried out by Sperry et al. (2008), but as the complete genome sequence of this strain (Sumrall, Klumpp, Shen, & Loessner, 2016) together with that of strains EGDe (Glaser et al., 2001) and ATCC 19112 (Kuenne et al., 2013) have been determined, the real fragment size of the amplified fragments (determined *in silico*) were also taken into account for the establishment of the offsets. The MLVA typing system using ABI 3730xl for fragment sizing could be adjusted to obtain the same MLVA profiles previously published except for strain EGDe, which showed a different copy number for locus Lm-23 (20 instead of 19). In our hands, fragment analysis of locus Lm-23 in EGDe using ABI 3730xl automated sequencer was 229.70 bp, instead of 221.99 bp as previously described by Sperry et al. (2008) using a different sizing platform. Nevertheless, *in silico* MLVA analysis of EGDe showed a fragment of 231 bp for locus Lm-23, closer to our results and for that reason we decided to maintain the difference in that locus.

Fragment sizing was also carried out using QIAxcel Advanced System. Overall, discrepancies between QIAxcel results and the reference values obtained with ABI 3730xl were observed (Table 2), being QIAxcel values consistently lower than the reference values. Differences augmented along with the increase of the PCR fragment length. Highest differences were observed among PCR fragments longer than 380 bp (mainly locus Lm-2), showing a difference from expected values of 4 to 14 bp. To overcome discrepancies among sizing platforms, a look-up table containing fragment size ranges for all alleles was constructed taking into account the results obtained with the reference method (using ABI 3730xl automated sequencer). This look-up table was used in Bionumerics software to assign the number of repeats at each locus. Using this approach, the concordance between both sizing methods was 100%.

3.2. L. monocytogenes *distribution in meat products and meat processing plants by MLVA.*

A total of 27 different MLVA profiles (Fig. 1) were obtained considering all the isolates from meat products, food processing plants and control strains (N=113).

Without control strains (N=106), the number of MLVA unique profiles was 24. Most common MLVA profiles were 19-3-5-4-6-2-20-13 assigned to MLVA Type (MT) 1 (22.3% of isolates) and 15-3-5-4-1-4-37-14 (MT2, 15.2% of isolates). Isolates from the IRTA collection (recovered from RTE meat products in different years) displayed 7 different MTs, but common to the MTs displayed by others isolates from meat products and /or food-contact surfaces (MT1, MT2, MT7, MT8 and MT16), except for MT15 (which included two IRTA collection isolates) and MT19 (one single isolate).

The suitability of MLVA to stablish the contamination patterns of *L. monocytogenes* in meat processing plants was also evaluated. Fig. 2 shows the dendrogram generated with the isolates (N=96) recovered from the 18 meat processing plants evaluated on the basis of MLVA data, the source information, in addition to serotype and ST. The isolates showed 21 different MTs distributed throughout the meat processing plants. MT1 was the most frequent genotype, represented by 23 isolates and detected in 12 of the plants studied. MT2 (corresponding to ST121) was represented by 16 isolates recovered from several plants (8 out of 18). In contrast, 11 different MTs were only detected in one single plant. Although, MLVA identified more types than MLST, similar results were obtained regarding to the source tracking of isolates, and 25 of the isolates recovered from meat products showed the same genotype as isolates collected from food contact surfaces of their respective production plants.

3.3. Evaluation of the MLVA subtyping method in comparison with MLST

The discriminatory power of the MLVA subtyping method was evaluated comparing the results with the previously obtained by MLST (Martin et al., 2014). The number of MLVA unique profiles obtained was 27 corresponding to the 22 different allelic profiles previously identified by MLST. Most STs could be clearly differentiated or even further subtyped into different MTs; ST9 could be split in four different MTs and ST8, ST3 and ST1 in two unique MTs each. In contrast, ST122 (corresponding to control strain ATCC 19112) could not be distinguished from ST9 isolates.

Clustering analysis of MLVA data was highly consistent with MLST analysis and interestingly, isolates belonging to the same ST clustered together. MLVA identified some clonal complexes (CC) in accordance with MLST data, CC9 (that included ST9, ST625 and ST35 isolates), CC8 (including ST8 and ST16 isolates), CC3 (composed by two MTs of ST3 isolates) CC1 (composed by three MTs) and CC87 (composed by three MTs). In contrast, two different ST isolates (ST12 and ST204) clustered together, while by MLST they belong to different CCs. Table 3 shows the different MLVA profiles found in relation with the serotypes and STs previously described. Simpson's diversity index (SID) was calculated based on different genotypes obtained to assess discrimination ability of both MLST and MLVA. The diversity of the complete panel of isolates (N=113) was SID=0.907 by MLVA and slightly lower by MLST (SID=0.872). Differences in diversity were observed among serotypes (Table 3). Serotype 1/2a (N=41) demonstrated the highest diversity, regardless the subtyping method used (SID=0.796 by MLVA and SID=0.778 by MLST). Serotype 1/2b (N=19) also showed a high diversity index (SID=0.754) when using MLVA, but clearly lower by MLST (SID=0.556). Serotype 4b (N=14) showed also a higher diversity index by MLVA than by MLST, SID=0.659 and 0.513 respectively. However, the most relevant difference was in serotype 1/2c (N=37), where diversity was almost double by MLVA (SID=0.493) compared to MLST (SID=0.246). The adjusted Wallace coefficients (AW) obtained were $AW_{MLVA \rightarrow MLST}$=0.946 (CI=0.857-1.000) and $AW_{MLST \rightarrow MLVA}$=0.660 (CI=0.518-0.802). These values indicated that ST was well predicted by MLVA but MT was less well predicted by MLST.

4. Discussion

Molecular typing of *L. monocytogenes* isolates has an important role in meat-processing plants in order to identify contamination and spread routes of this pathogen. Currently typing methods used including PFGE, MLST and recently WGS, present several drawbacks for their use in food control laboratories, such as the intensively

work required and the high cost. MLVA is an alternative molecular typing method with practical advantages including its rapidity, ease of use and low cost (EFSA BIOHAZ Panel, 2013; van Belkum et al., 2007). In this study, we have optimized a MLVA protocol to discriminate among *L. monocytogenes* isolates recovered from meat products and meat processing plants using two different sizing technologies and comparing the results with those of MLST.

MLVA is based on the accurate sizing of PCR amplified DNA fragments by capillary electrophoresis. Nevertheless, important differences between measured and real fragment lengths are commonly observed (Larsson, Torpdahl, Nielsen, & Group, 2013) mainly attributed to the different capillary electrophoresis platforms and reagents used for fragment sizing (Hyytia-Trees, Lafon, Vauterin, & Ribot, 2010). In this sense, the use of a standardized MLVA method to normalized fragment size data is a must. Efforts for standardization have been carried out for MLVA analysis of some food-borne pathogens (Hyytia-Trees et al., 2010; Larsson et al., 2013) and although several MLVA schemes for typing *L. monocytogenes* have been published (S. Chen, Li, Saleh-Lakha, Allen, & Odumeru, 2011; Chenal-Francisque et al., 2013; Lindstedt, 2005; Murphy et al., 2007; Saleh-Lakha et al., 2013) none of them has been established as the standard method. Among the MLVA methods available, we chose the MLVA scheme developed by Sperry et al. (2008) intended to be implemented in Pulsenet Laboratories and adapted it to be used with ABI 3730xl. Offsets had to be adjusted in order to maintain the copy numbers of VNTR loci previously published to obtain comparable results. Nevertheless, it was not possible to calculate an offset for locus Lm-23 to maintain the copy number of this locus for all control strains (EGDe, showed a higher copy number, 20 instead of 19). Some authors recommends the use of an established set of calibration strains to convert the discrepancies between real (sequenced) and measured fragment length to obtain comparable results between laboratories independently of the capillary electrophoresis platform used (Larsson et al., 2013). This

implies that each new allele must be verified by sequencing but it would avoid errors in copy number assignation due to discrepancies on measured fragment lengths.

Apart from the results obtained using ABI 3730xl, MLVA was also carried out using QIAxcel Advanced System (developed by Qiagen), an automated capillary electrophoresis platform that constitutes an alternative to the methods based on separation of fluorescent-labelled PCR fragments using automated sequencers. This platform has been successfully used for MLVA genotyping of several bacterial species other than *L. monocytogenes* (De Santis et al., 2013; Gauthier et al., 2015; Nikolayevskyy et al., 2016; Takahashi et al., 2014) leading to shorter turnaround times, technical simplicity and cost effectiveness what can be of great interest for small food control laboratories. In this study, discrepancies on size fragment were observed between both platforms, mainly in fragments higher than 380 bp. The results obtained with ABI 3730xl were taken as reference values and a look-up table containing fragment size ranges was constructed for allele assignment as previously suggested to overcome discrepancies on fragment size data among platforms (Hyytia-Trees et al., 2010). In this way, concordance between both sizing platforms was 100%, concluding than QIAxcel is an effective and affordable alternative to automated sequencers that exhibit adequate performance after accurate validation.

MLVA was compared with the widely used MLST scheme of Institute Pasteur that stablishes a common nomenclature for *L. monocytogenes* genotypes. High level of congruence was obtained between both molecular typing methods suggesting that MLVA can be routinely used as an alternative method to MLST. Similar results of agreement between MLVA and MLST was also obtained by other authors (Chenal-Francisque et al., 2013) although a different MLVA scheme was applied for genotyping *L. monocytogenes* isolates recovered from different sources (mainly human). Most STs could be differentiated by MLVA and all MLST clonal complexes could be also identified. This level of congruence could be explained by a similar level of variation in

both MLVA and MLST markers (Chenal-Francisque et al., 2013) and by the low level of genetic recombination in *L. monocytogenes* (Cantinelli et al., 2013; den Bakker et al., 2010; Ragon et al., 2008). Recombination is more prevalent in lineage II than in lineage I, which is more affected by positive selection (Cantinelli et al., 2013; den Bakker et al., 2008). Regarding most prevalent STs found, MLVA confirmed the clonal origin of ST121 isolates whereas ST9 was more diversified by MLVA than by MLST. Interestingly, MLVA could also distinguished one single ST9 isolate that showed a different virulence type (VT95) from the others as reported in a previous study (Martin et al., 2014) . From our results, it is possible to deduce ST from the MT obtained although this encompass the MLST analysis of every newly described MT.

Discriminatory power of MLVA varies depending on the MLVA scheme used and the collection of isolates evaluated. In our study, MLVA showed a higher discriminatory power than MLST and differences were observed regarding the serotype and ST of the isolates. Several studies showed a discriminatory power of MLVA similar or even higher than PFGE (Li et al., 2013; Saleh-Lakha et al., 2013) whereas others showed lower discrimination of MLVA (Chenal-Francisque et al., 2013; Sperry et al., 2008). Chenal- Francisque et al. (2013) showed that discrimination power of MLVA is dependent on the clonal complex, being highly efficient in the discrimination of CC9 and CC4 isolates. In accordance with this results, we also showed a high diversity among ST9 isolates, the most prevalent among the isolates studied, what could underline the utility of MLVA for subtyping *L. monocytogenes* isolates in meat-processing environments to identify the sources of contamination and improve the safety of meat products.

5. Conclusions

Our results show that MLVA could be used as a rapid and reliable subtyping method for *L. monocytogenes* isolates in meat-processing plants. It permits to track the spread of clones and the persistence of particular subtypes and can be an efficient and

high- throughput tool to improve food safety control and *L*. *monocytogenes* surveillance.

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References

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.

Cantinelli, T., Chenal-Francisque, V., Diancourt, L., Frezal, L., Leclercq, A., Wirth, T., Brisse, S. (2013). "Epidemic Clones" of *Listeria monocytogenes* are widespread and ancient clonal groups. *Journal of Clinical Microbiology, 51*(11), 3770-3779.

Carrico, J. A., Silva-Costa, C., Melo-Cristino, J., Pinto, F. R., de Lencastre, H., Almeida, J. S., & Ramirez, M. (2006). Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*. *Journal of Clinical Microbiology, 44*(7), 2524-2532.

Chen, S., Li, J., Saleh-Lakha, S., Allen, V., & Odumeru, J. (2011). Multiple-locus variable number of tandem repeat analysis (MLVA) of *Listeria monocytogenes* directly in food samples. *International Journal of Food Microbiology, 148*(1), 8-14.

Chen, Y., Zhang, W., & Knabel, S. J. (2007). Multi-virulence-locus sequence typing identifies single nucleotide polymorphisms which differentiate epidemic clones

and outbreak strains of *Listeria monocytogenes*. *Journal of Clinical Microbiology, 45*(3), 835-846.

Chenal-Francisque, V., Diancourt, L., Cantinelli, T., Passet, V., Tran-Hykes, C., Bracq-Dieye, H., Brisse, S. (2013). Optimized Multilocus Variable-Number Tandem-Repeat Analysis Assay and Its Complementarity with Pulsed-Field Gel Electrophoresis and Multilocus Sequence Typing for Listeria monocytogenes Clone Identification and Surveillance. *Journal of Clinical Microbiology, 51*(6), 1868-1880.

Clark, C. G., Farber, J., Pagotto, F., Ciampa, N., Dore, K., Nadon, C., Ng, L. K. (2010). Surveillance for *Listeria monocytogenes* and listeriosis, 1995-2004. *Epidemiology and Infection, 138*(4), 559-572.

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M. T., Myers, C., Rose, C., Mandrell, R. E. (2007). Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. *Plos One, 2*(11), e1159.

Datta, A. R., Laksanalamai, P., & Solomotis, M. (2013). Recent developments in molecular sub-typing of *Listeria monocytogenes*. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment, 30*(8), 1437-1445.

De Santis, R., Ancora, M., De Massis, F., Ciammaruconi, A., Zilli, K., Di Giannatale, E., Lista, F. (2013). Molecular strain typing of *Brucella abortus* isolates from italy by two VNTR allele sizing technologies. *Molecular Biotechnology, 55*(2), 101-110.

den Bakker, H. C., Cummings, C. A., Ferreira, V., Vatta, P., Orsi, R. H., Degoricija, L., Wiedmann, M. (2010). Comparative genomics of the bacterial genus *Listeria*: Genome evolution is characterized by limited gene acquisition and limited gene loss. *Bmc Genomics, 11*, 688-708.

den Bakker, H. C., Didelot, X., Fortes, E. D., Nightingale, K. K., & Wiedmann, M. (2008). Lineage specific recombination rates and microevolution in *Listeria monocytogenes*. *Bmc Evolutionary Biology, 8*, 277-290.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., & Martin, P. (2004). Differentiation of the major *Listeria monocytogenes* serovars by Multiplex PCR. *J. Clin. Microbiol., 42*(8), 3819-3822.

EFSA-ECDC. (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA Journal, 13*(1), 3991-4153.

EFSA-ECDC. (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal, 13*(12), 4329.

EFSA BIOHAZ Panel. (2013). Scientific Opinion on the evaluation of molecular typing methods for major food-borne microbiological hazards and their use for attribution modelling, outbreak investigation and scanning surveillance: Part 1 (evaluation of methods and applications). *EFSA Journal, 11*(12), 3508-3584.

Ferreira, V., Wiedmann, M., Teixeira, P., & Stasiewicz, M. J. (2014). *Listeria monocytogenes p*ersistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection, 77*(1), 150-170.

Gauthier, M., Bidault, F., Mosnier, A., Bablishvili, N., Tukvadze, N., Somphavong, S., Berland, J.-L. (2015). High-throughput mycobacterial interspersed repetitive-unit-variable-number tandem-repeat genotyping for *Mycobacterium tuberculosis* epidemiological studies. *Journal of Clinical Microbiology, 53*(2), 498-503.

Gelbicova, T., & Karpiskova, R. (2009). Occurrence and characteristics of *Listeria monocytogenes* in ready-to-eat food from retail market in the Czech Republic. *Czech Journal of Food Sciences, 27*, 3-7.

Gerner-Smidt, P., Hise, K., Kincaid, J., Hunter, S., Rolando, S., Hyytia-Trees, E., PulseNet, T. (2006). PulseNet USA: A five-year update. *Foodborne Pathogens and Disease, 3*(1), 9-19.

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Cossart, P. (2001). Comparative genomics of *Listeria* species. *Science, 294*(5543), 849-852.

Heck, M. (2009). Multilocus variable number of tandem repeats analysis (MLVA) - a reliable tool for rapid investigation of *Salmonella typhimurium* outbreaks. *Eurosurveillance, 14*(15), 2-2.

Heir, E., Lindstedt, B. A., Vardund, T., Wasteson, Y., & Kapperud, G. (2000). Genomic fingerprinting of shigatoxin-producing *Escherichia coli* (STEC) strains: comparison of pulsed-field gel electrophoresis (PFGE) and fluorescent amplified-fragment-length polymorphism (FAFLP). *Epidemiology and Infection, 125*(3), 537-548.

Hunter, P. R., & Gaston, M. A. (1988). Numerical Index of the Discriminatory Ability of Typing Systems: an Application of Simpson's Index of Diversity. *Journal of Clinical Microbiology, 26*(11), 2465-2466.

Hyytia-Trees, E., Lafon, P., Vauterin, P., & Ribot, E. M. (2010). Multilaboratory validation study of standardized multiple-locus variable-number tandem repeat analysis protocol for shiga toxin-producing *Escherichia coli* O157: a novel approach to normalize fragment size data between capillary electrophoresis platforms. *Foodborne Pathogens and Disease, 7*(2), 129-136.

Kathariou, S. (2002). *Listeria monocytogenes* virulence and patogenicity, a food safety perspective. *Journal of Food Protection, 65*, 1811-1829.

Kuenne, C., Billion, A., Abu Mraheil, M., Strittmatter, A., Daniel, R., Goesmann, A., Chakraborty, T. (2013). Reassessment of the *Listeria monocytogenes* pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome. *BMC Genomics, 14*.

Larsson, J. T., Torpdahl, M., Nielsen, E. M., & Group, M. W. (2013). Proof-of-concept study for successful inter-laboratory comparison of MLVA results. *Eurosurveillance, 18*(35), 20-27.

Li, X. J., Huang, B. X., Eglezos, S., Graham, T., Blair, B., & Bates, J. (2013). Identification of an optimized panel of variable number tandem-repeat (VNTR) loci for *Listeria monocytogenes* typing. *Diagnostic Microbiology and Infectious Disease, 75*(2), 203-206.

Lindstedt, B. A. (2005). Multiple-locus variable number tandem repeats analysis for genetic fingerprinting of pathogenic bacteria. *Electrophoresis, 26*(13), 2567-2582.

Lindstedt, B. A., Heir, E., Gjernes, E., & Kapperud, G. (2003). DNA fingerprinting of *Salmonella enterica* subsp *enterica* serovar Typhimurium with emphasis on phage type DT104 based on variable number of tandem repeat loci. *Journal of Clinical Microbiology, 41*(4), 1469-1479.

Lindstedt, B. A., Tham, W., Danielsson-Tham, M.-L., Vardund, T., Helmersson, S., & Kapperud, G. (2008). Multiple-locus variable-number tandem-repeats analysis of *Listeria monocytogenes* using multicolour capillary electrophoresis and comparison with pulsed-field gel electrophoresis typing. *Journal of Microbiological Methods, 72*(2), 141-148.

Lukinmaa, S., Aarnisalo, K., Suihko, M. L., & Siitonen, A. (2004). Diversity of *Listeria monocytogenes* isolates of human and food origin studied by serotyping, automated ribotyping and pulsed-field gel electrophoresis. *Clinical Microbiology and Infection, 10*(6), 562-568.

Martin, B., Perich, A., Gomez, D., Yangüela, J., Rodriguez, A., Garriga, M., & Aymerich, T. (2014). Diversity and distribution of *Listeria monocytogenes* in meat processing plants. *Food Microbiology, 44*, 119-127.

Miya, S., Kimura, B., Sato, M., Takahashi, H., Ishikawa, T., Suda, T., Wiedmann, M. (2008). Development of a multilocus variable-number of tandem repeat typing method for Listeria monocytogenes serotype 4b strains. *International Journal of Food Microbiology, 124*(3), 239-249.

Murphy, M., Corcoran, D., Buckley, J. F., O'Mahony, M., Whyte, P., & Fanning, S. (2007). Development and application of Multiple-Locus Variable Number of tandem repeat Analysis (MLVA) to subtype a collection of *Listeria monocytogenes*. *International Journal of Food Microbiology, 115*(2), 187-194.

Nightingale, K. (2010). *Listeria monocytogenes*: knowledge gained through DNA sequence-based subtyping, implications, and future considerations. *Journal of Aoac International, 93*(4), 1275-1286.

Nikolayevskyy, V., Trovato, A., Broda, A., Borroni, E., Cirillo, D., & Drobniewski, F. (2016). MIRU-VNTR genotyping of *Mycobacterium tuberculosis* strains using Qiaxcel technology: a multicentre evaluation study. *Plos One, 11*(3).

Orsi, R. H., den Bakker, H. C., & Wiedmann, M. (2011). *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. *International Journal of Medical Microbiology, 301*(2), 79-96.

Pagotto, F., Ng, L. K., Clark, C., & Farber, J. (2006). Canadian listeriosis reference service. *Foodborne Pathogens and Disease, 3*(1), 132-137.

Paoli, G. C., Bhunia, A. K., & Bayles, D. O. (2005). *Listeria monocytogenes*. In P. M. Fratamico, A. K. Bhunia & J. L. Smith (Eds.), *Foodborne pathogens. Microbiology and molecular biology*. Norfolk, UK: Caister Academic Press.

Ragon, M., Wirth, T., Hollandt, F., Lavenir, R., Lecuit, M., Le Monnier, A., & Brisse, S. (2008). A new perspective on *Listeria monocytogenes* evolution. *Plos Pathogens, 4*(9), e1000146.

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. [Article]. *Journal of Clinical Microbiology, 41*(2), 757-762.

Saleh-Lakha, S., Allen, V. G., Li, J., Pagotto, F., Odumeru, J., Taboada, E., Chen, S. (2013). Subtyping of a large collection of historical *Listeria monocytogenes* strains from Ontario, Canada, by an improved multilocus variable-number

tandem-repeat analysis (MLVA). *Applied and Environmental Microbiology, 79*(20), 6472-6480.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Griffin, P. M. (2011). Foodborne illness acquired in the United States-Major pathogens. *Emerging Infectious Diseases, 17*(1), 7-15.

Sperry, K. E. V., Kathariou, S., Edwards, J. S., & Wolf, L. A. (2008). Multiple-locus variable-number tandem-repeat analysis as a tool for subtyping *Listeria monocytogenes* strains. *Journal of Clinical Microbiology, 46*(4), 1435-1450.

Sperry, K. E. V., Kathariou, S., Edwards, J. S., & Wolf, L. A. (2009). Multiple-locus variable-number tandem-repeat analysis as a tool for subtyping *Listeria monocytogenes* strains (vol 46, pg 1435, 2008). *Journal of Clinical Microbiology, 47*(6), 1984-1984.

Sumrall, E., Klumpp, J., Shen, Y., & Loessner, M. J. (2016). Genome sequences of five nonvirulent *Listeria monocytogenes* serovar 4 strains. *Genome announcements, 4*(2), e00179-00116

Swaminathan, B., & Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. *Microbes and Infection 9*, 1236-1243.

Takahashi, H., Ohshima, C., Nakagawa, M., Thanatsang, K., Phraephaisarn, C., Chaturongkasumrit, Y., Kimura, B. (2014). Development of new multilocus variable number of tandem repeat analysis (MLVA) for *Listeria innocua* and its application in a food processing plant. *Plos One, 9*(9).

Thévenot, D., Delignette-Muller, M. L., Christieans, S., Leroy, S., Kodjo, A., & Vernozy-Rozand, C. (2006). Serological and molecular ecology of *Listeria monocytogenes* isolates collected from 13 French pork meat salting-curing plants and their products. *International Journal of Food Microbiology, 112*(2), 153-161.

Tompkin, R. B. (2002). Control of *Listeria monocytogenes* in the food-processing environment. *Journal of Food Protection, 65*(4), 709-725.

van Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N. K., Esgem. (2007). Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clinical Microbiology and Infection, 13*, 1-46.

Zhang, W., Jayarao, B. M., & Knabel, S. J. (2004). Multi-virulence-locus sequence typing of *Listeria monocytogenes*. *Applied and Environmental Microbiology, 70*(2), 913-920.

Figure Captions

Fig.1. Minimum spanning tree analysis of the 113 isolates of *L*. *monocytogenes* based on MLVA data. Each circle corresponds to one MT and the size of the circle reflects the number of strains within that type. Numbers inside each circle represent the ST of the isolates. The connection lines between circles differ based on the number of different alleles between the corresponding MTs; bold lines correspond to one different allele, plain lines to two alleles, dashed lines to three alleles and doted lines to 4 or more alleles. Grey zone surrounding some circles indicate that these types belong to the same clonal complex. Control strains were also included in the analysis and they are indicated in the figure.

Fig.2. UPGMA dendogram of the 96 Isolates collected from meat-processing plants and either from the food-contact surfaces sampled or the meat products elaborated in each plant. Characteristics of each isolates including serotype, MT (MT), sequence type (ST), source and meat-processing plant of isolation are indicated. Some isolates presenting the same characteristics are grouped together and number of isolates (N) are indicated.

[a] MT, MLVA Type.

[b] Source: FCS, Food-contact surface. When indicated (clean), food-contact surfaces were sampled prior processing but after cleaning and disinfection; RMP, Raw meat product; RTE, Ready-to-eat meat product.

Figure

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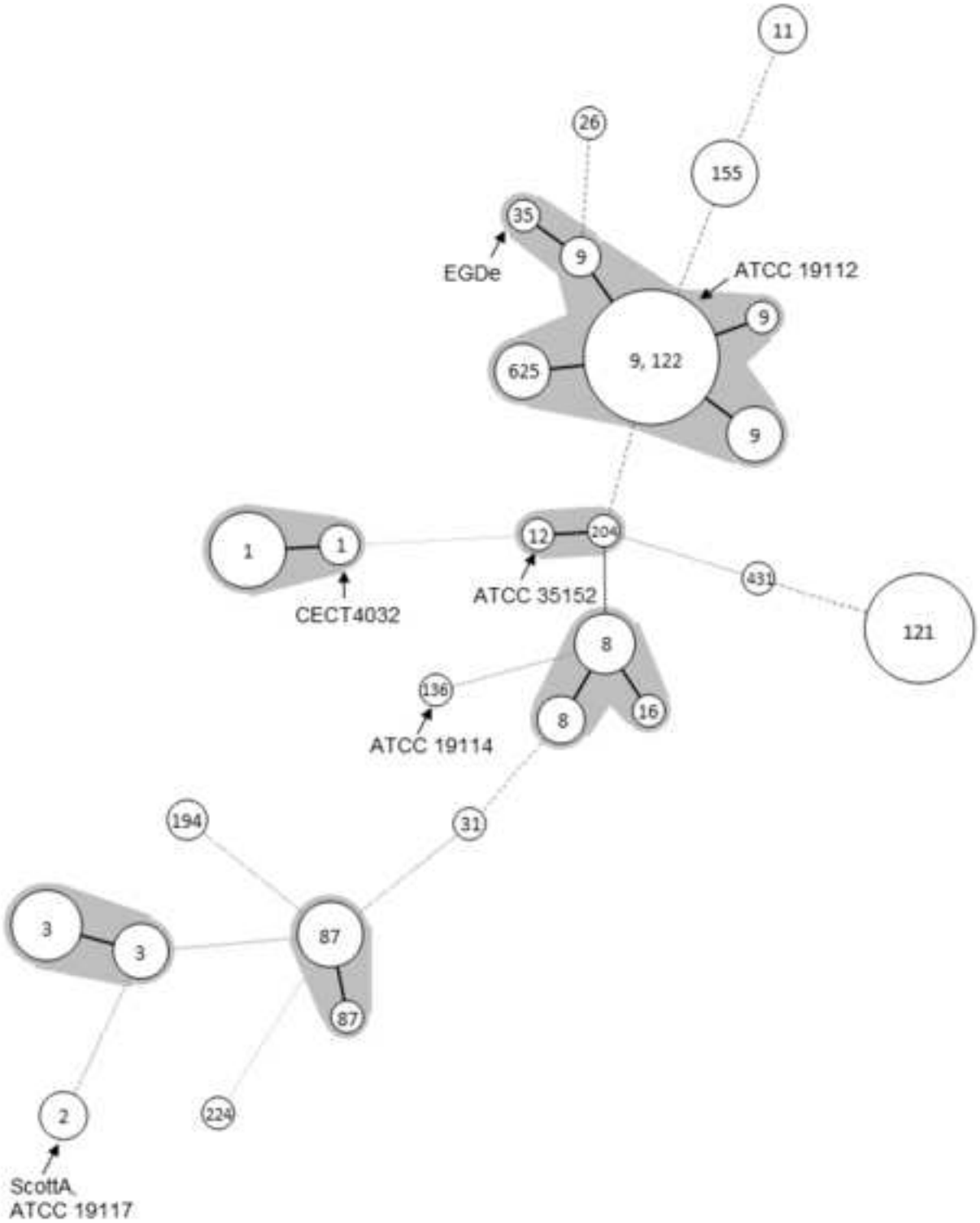
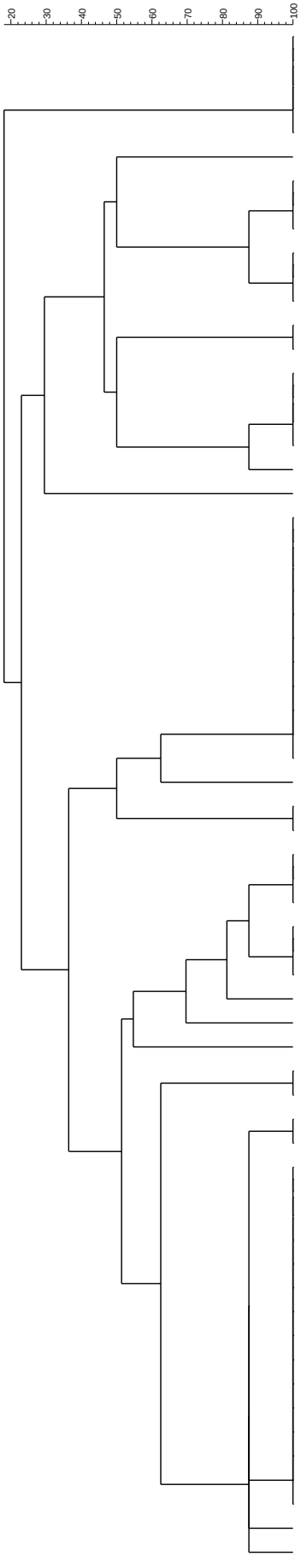


Figure



MLVA profile									Sero-type	MT [a]	ST	Source[b]	Plant
Lm-2	Lm-8	Lm-10	Lm-11	Lm-3	Lm-15	Lm-23	Lm-32					
15	4	5	6	1	3	33	18	4b	3	1	FCS	F
15	4	5	6	1	3	33	18	4b	3	1	RMP	C
15	4	5	6	1	3	33	18	4b	3	1	RMP, N=3	Q
15	4	5	6	1	3	33	18	4b	3	1	RMP, N=2	Q
15	4	5	6	1	3	33	18	4b	3	1	FCS (clean), N=3	Q
15	3	3	5	1	5	23	17	4b	13	2	RTE	K
16	3	5	5	9	5	29	17	1/2b	4	3	FCS	J
16	3	5	5	9	5	29	17	1/2b	4	3	RTE, N=3	R
16	3	5	5	9	5	29	17	1/2b	4	3	RTE, N=3	J
16	3	5	5	8	5	29	17	1/2b	8	3	RTE	P
16	3	5	5	8	5	29	17	1/2b	8	3	RTE	J
16	3	5	5	8	5	29	17	1/2b	8	3	RTE	I
18	4	5	5	3	4	29	17	4b	14	194	FCS	M
18	4	5	5	3	4	29	17	4b	14	194	RTE	M
16	3	7	5	3	4	35	17	1/2b	5	87	FCS, N=2	O
16	3	7	5	3	4	35	17	1/2b	5	87	FCS	A
16	3	7	5	3	4	35	17	1/2b	5	87	RTE	F
16	3	7	5	3	4	35	17	1/2b	5	87	RMP	G
15	3	7	5	3	4	35	17	1/2b	26	87	RTE, N=2	F
17	3	6	4	2	4	-2	17	1/2b	20	224	FCS	P
15	3	5	4	1	4	37	14	1/2a	2	121	RTE	M
15	3	5	4	1	4	37	14	1/2a	2	121	FCS, N=2	R
15	3	5	4	1	4	37	14	1/2a	2	121	FCS	B
15	3	5	4	1	4	37	14	1/2a	2	121	RTE, N=2	E
15	3	5	4	1	4	37	14	1/2a	2	121	FCS	E
15	3	5	4	1	4	37	14	1/2a	2	121	FCS, N=2	I
15	3	5	4	1	4	37	14	1/2a	2	121	RTE, N=3	I
15	3	5	4	1	4	37	14	1/2a	2	121	RTE	B
15	3	5	4	1	4	37	14	1/2a	2	121	RTE	J
15	3	5	4	1	4	37	14	1/2a	2	121	RTE	A
15	3	5	4	1	4	37	14	1/2a	2	121	FCS (clean)	H
17	3	5	4	1	6	18	14	1/2a	23	431	FCS	P
16	3	5	1	1	2	-2	14	1/2a	12	11	FCS, N=2	L
16	3	5	1	1	2	-2	14	1/2a	12	11	RTE	L
16	3	5	-2	5	2	41	13	1/2a	11	8	FCS	K
16	3	5	-2	5	2	41	13	1/2a	11	8	RTE	K
16	3	5	-2	5	2	41	13	1/2a	11	8	FCS	F
17	3	5	-2	5	2	41	13	1/2a	7	8	RTE	P
17	3	5	-2	5	2	41	13	1/2a	7	8	FCS	K
17	3	5	-2	5	2	41	13	1/2a	7	8	RTE	N
17	3	5	-2	4	2	41	13	1/2a	21	16	RTE	E
17	3	5	-2	1	2	20	13	1/2a	22	204	RTE	O
16	3	5	-2	3	4	10	13	1/2a	24	31	FCS	L
16	3	5	4	1	2	29	13	1/2a	6	155	RTE, N=3	R
16	3	5	4	1	2	29	13	1/2a	6	155	FCS (clean), N=2	H
19	3	5	4	5	2	20	13	1/2c	10	625	FCS, N=3	J
19	3	5	4	5	2	20	13	1/2c	10	625	FCS	E
19	3	5	4	6	2	20	13	1/2c	1	9	RTE, N=2	M
19	3	5	4	6	2	20	13	1/2c	1	9	FCS	R
19	3	5	4	6	2	20	13	1/2c	1	9	RTE, N=3	N
19	3	5	4	6	2	20	13	1/2c	1	9	FCS	F
19	3	5	4	6	2	20	13	1/2c	1	9	FCS	B
19	3	5	4	6	2	20	13	1/2c	1	9	RTE	K
19	3	5	4	6	2	20	13	1/2c	1	9	FCS	N
19	3	5	4	6	2	20	13	1/2c	1	9	RTE, N=4	B
19	3	5	4	6	2	20	13	1/2c	1	9	RTE	J
19	3	5	4	6	2	20	13	1/2c	1	9	RTE	E
19	3	5	4	6	2	20	13	1/2c	1	9	RTE, N=2	R
19	3	5	4	6	2	20	13	1/2c	1	9	RTE, N=2	A
19	3	5	4	6	2	20	13	1/2c	1	9	RMP	G
19	3	5	4	6	2	20	13	1/2c	1	9	RMP	C
19	3	5	4	6	2	20	13	1/2c	1	9	RTE	D
19	3	5	4	9	2	20	13	1/2c	9	9	RTE, N=4	L
19	3	5	4	8	2	20	13	1/2c	17	9	RTE	I

Table

Table 1. Parameters for the calculation of VNTR loci copy numbers with the ABI 3730xl platform.

VNTR locus	Offset	Repeat size (bp)	Min value	Max Value	Tolerance	Reaction group /fluorescent dye
Lm-2	291	6	11	20	3	R1 / VIC
Lm-8	185	15	3	4	7	R1/ NED
Lm-10	316	6	3	9	3	R1/ 6-FAM
Lm-11	100	12	1	6	6	R1/ 6-FAM
Lm-3	200	9	1	9	4	R2/ VIC
Lm-15	318	12	1	7	6	R2/ 6-FAM
Lm-23	169	3	15	42	1.5	R2/ NED
Lm-32	83	6	10	21	3	R2/ 6-FAM

Table

Table 2. Fragment size ranges obtained with ABI 3037xl and QIAxcel Advanced System.

Locus	Copy No.	ABI 3037xl				QIAxcel Advanced System				
		Fragment size range (bp)		Mean	SD[a]	Fragment size range (bp)		Mean	SD	No of isolates
		Min	Max			Min	Max			
Lm-2	11	355.4	355.4	-	-	351	351	351.0	-	1
	15	380.8	381.2	381.0	0.14	368	373	371.1	1.55	32
	16	386.8	387.3	387.0	0.18	374	378	375.3	1.11	30
	17	392.8	394.9	393.5	0.54	380	383	381.3	1.02	9
	18	398.9	399.6	399.4	0.32	384	388	386.7	1.41	6
	19	405.7	405.8	405.8	0.03	390	394	391.3	1.15	35
Lm-8	3	231.7	233.0	232.3	0.26	226	231	228.2	1.14	101
	4	247.1	248.2	247.4	0.32	242	246	243.9	1.04	12
Lm-10	3	334.4	334.4	334.4	0.00	332	332	332.0	0.00	3
	5	344.4	347.9	346.7	0.46	337	344	342.3	0.94	102
	6	349.3	349.3	349.3	-	345	345	344.9	-	1
	7	357.3	357.3	357.3	0.03	352	355	353.3	1.04	7
Lm-11	-2	.	.	.	.	.	.	.	.	13
	1	109.4	109.4	109.4	0.01	109	109	109.0	0.11	3
	4	147.4	150.7	147.6	0.42	143	148	144.8	0.76	64
	5	163.0	163.4	163.1	0.11	158	161	159.9	1.00	25
	6	175.0	175.5	175.2	0.15	170	172	171.5	0.95	8
Lm-3	-2	-	-	-	-	-	-	-	-	1
	1	209.6	211.3	209.9	0.32	206	212	209.4	1.36	42
	2	219.2	220.2	219.7	0.67	215	216	215.5	0.70	2
	3	228.4	230.1	228.9	0.54	225	227	225.6	0.72	10
	4	237.9	239.2	238.6	0.92	233	233	233.0	0.00	2
	5	246.8	248.4	247.2	0.60	241	244	242.7	1.05	12
	6	255.6	257.7	256.1	0.65	250	254	252.0	0.98	28
	8	272.7	274.2	273.0	0.58	267	271	269.2	1.62	5
	9	280.6	281.0	280.8	0.18	276	279	278.0	0.98	11
Lm-15	2	342.2	342.8	342.5	0.13	333	339	334.9	1.18	59
	3	353.6	354.8	354.3	0.30	345	350	346.7	1.81	11
	4	364.6	365.3	364.8	0.32	356	360	357.9	1.12	28
	5	376.3	376.5	376.4	0.06	372	374	373.0	0.83	14
	6	387.2	387.2	387.2	.	380	380	379.9	-	1
Lm-23	-2	-	-	-	-	-	-	-	-	4

	10	198.8	198.8	198.8	-	201	201	200.9	-	1
	18	222.7	222.7	222.7	-	224	224	223.9	-	1
	20	228.3	229.9	228.6	0.49	229	232	230.2	0.63	40
	23	236.7	236.9	236.8	0.08	235	237	236.5	0.99	4
	29	252.7	255.9	254.2	0.92	253	255	253.6	0.72	19
	33	265.9	267.2	266.4	0.54	265	267	265.8	0.85	10
	35	271.1	272.3	271.4	0.52	271	271	270.6	0.15	7
	37	280.0	281.0	280.3	0.45	282	283	282.3	0.49	17
	41	290.1	291.2	290.7	0.45	293	294	293.7	0.38	9
	42	295.1	295.1	295.1	.	297	297	297.0	-	1
Lm-32	-2	-	-	-	-	-	-	-	-	1
	13	160.7	161.0	160.8	0.08	163	165	163.7	0.64	57
	14	166.2	166.4	166.3	0.06	169	171	169.7	0.58	21
	17	183.7	183.9	183.8	0.06	186	188	187.2	0.75	24
	18	189.7	189.8	189.7	0.03	192	195	193.5	0.88	10

[a] Standard deviation

Table

Table 3. MLVA subtyping of *L. monocytogenes* isolates in relation with serotypes and sequence types (ST).

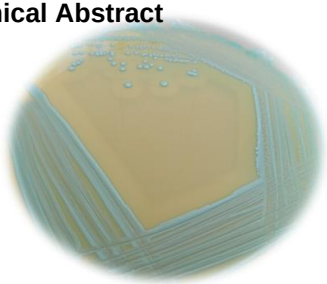
Serotype	Simpson's index (SID)		ST	MLVA Type	MLVA profile[a]	N[b]
	MLST	MLVA				
1/2a	0.778	0.796	431	23	17-3-5-4-1-6-18-14	1
			11	12	16-3-5-1-1-2-NA-14	3
			121	2	15-3-5-4-1-4-37-14	17
			155	6	16-3-5-4-1-2-29-13	6
			204	22	17-3-5-NA-1-2-20-13	1
			26	19	18-3-5-4-2-3-23-13	1
			31	24	16-3-5-NA-3-4-10-13	1
			12	27	15-3-5-NA-1-2-20-13	1
			16	21	17-3-5-NA-4-2-41-13	1
			8	11	16-3-5-NA-5-2-41-13	3
				7	17-3-5-NA-5-2-41-13	5
			35	18	18-3-5-4-4-2-20-13	1
1/2b	0.556	0.754	224	20	17-3-6-4-2-4-NA-17	1
			3	8	16-3-5-5-8-5-29-17	4
				4	16-3-5-5-9-5-29-17	7
			87	26	15-3-7-5-3-4-35-17	1
				5	16-3-7-5-3-4-35-17	6
1/2c	0.246	0.493	122	1	19-3-5-4-6-2-20-13	1
			625	10	19-3-5-4-5-2-20-13	4
			9	1	19-3-5-4-6-2-20-13	25
				9	19-3-5-4-9-2-20-13	4
				15	18-3-5-4-6-2-20-13	2
				17	19-3-5-4-8-2-20-13	1
4b	0.513	0.659	1[C]	16	15-4-5-5-1-3-33-18	2
				3	15-4-5-6-1-3-33-18	8
			2	13	15-3-3-5-1-5-23-17	2
			194	14	18-4-5-5-3-4-29-17	2
4a			136	42	11-3-5-NA-NA-2-42-NA	1
4d			2	5	15-3-3-5-1-5-23-17	1

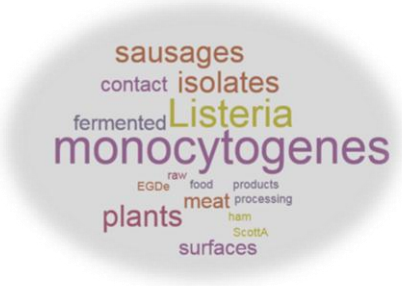
[a] MLVA allele profile was presented in an allelic string as follows: Lm-2/Lm-8/Lm-10/Lm-11/Lm-3/Lm-15/Lm-23/Lm-32. NA denotes a null allele at this locus

[b] Number of isolates that share the same MLVA type.

[C] The ST of one isolate showing MT16 (CECT4032) was not determined, therefore it has not been considered to calculate SID value for MLST in serotype 4b.

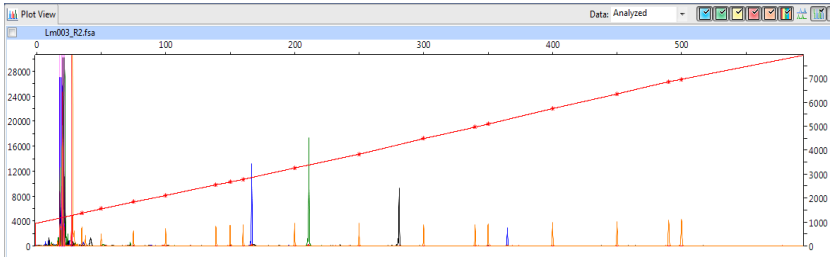
***Graphical Abstract**





L. *monocytogenes* isolates

MLVA Typing using two platforms



1) Reference method: ABI 3730xl DNA analyzer (Life Technologies)

2) QIAxcel Advanced System (Qiagen)