Molecular typing and discrimination of *Listeria monocytogenes* associated with production of food of animal origin in Slovakia

ZUZANA REŠKOVÁ – ADRIANA VÉGHOVÁ – JANA MINAROVIČOVÁ – MICHAL ANDREZÁL – ALEXANDRA BURDOVÁ – HANA DRAHOVSKÁ – EVA KACLÍKOVÁ

Summary

Listeria monocytogenes is an important foodborne pathogen ubiquitous in environment with a potential to colonize food production facilities for long periods. In the present study, diversity of *L. monocytogenes* isolates from two facilities processing food of animal origin in Slovakia was evaluated using several molecular typing methods. Fifty-five isolates clustered by pulsed-field gel electrophoresis (PFGE) to 15 pulsotypes in our previous studies were subjected to multiple locus variable-number tandem repeat analysis (MLVA) resulting in strain discrimination into 16 MLVA profiles. For these *L. monocytogenes* isolates, MLVA showed a slightly higher discriminatory power compared to PFGE when one PFGE cluster was divided into two MLVA clusters. Multi-locus sequence typing (MLST) based on whole genome sequencing (WGS) separated 35 *L. monocytogenes* isolates into 13 sequence types (ST) and 16 groups covering isolates with less than 10 allelic differences in core genome (cg) MLST analysis, five of them containing more than one isolate. PFGE and MLVA allowed reliable primary discrimination of *L. monocytogenes* isolates. WGS data allowed the comparison of genome diversity required for confirmation of persistent *L. monocytogenes*.

Keywords

Listeria monocytogenes; typing; pulsed-field gel electrophoresis; multiple locus variable-number tandem repeat analysis; whole genome sequencing

Listeria monocytogenes is a food-borne pathogen responsible for listeriosis, which continues to be one of the food-borne infections with the highest number of fatal cases in EU [1] with high case-fatality rates [2]. *L. monocytogenes* is widely present in the environment including soil, water, vegetation and animals. Contamination with *L. monocytogenes* can occur at any stage of the food chain. *L. monocytogenes* is recognized as a public health issue and a serious challenge for the food industry [3].

Molecular typing is an effective tool to identify relatedness among isolates and, in relation to food safety, to trace sources, transmission routes and persistent contamination in the food production environment. To determine the genetic variability of *L. monocytogenes* isolates, several typing methods with variable discrimination power have been developed [4]. Serotyping is the traditional method providing the primary level of discrimination of isolates [5]. Molecular serogrouping by multiplex PCR allows differentiation of five main molecular serogroups [6, 7]. High reproducibility and discriminatory power of macrorestriction coupled to pulsed-field gel electrophoresis (PFGE) was proven for *L. monocytogenes* typing during many years of use as "gold standard" [4, 8, 9]. However, this method is considered a laborious and time-consuming technique with limited data portability [10].

Nowadays, sequence-based typing methods provide unambiguous and portable data that can be useful not only for typing purposes. Multi-locus sequence typing (MLST) resulted in sequence types (ST) determination based on sequences of seven housekeeping genes [11] is another widely

Zuzana Rešková, Adriana Véghová, Jana Minarovičová, Eva Kaclíková, Food Research Institute, National Agricultural and Food Centre, Priemyselná 4, 82475 Bratislava, Slovakia.
Michal Andrezál, Alexandra Burdová, Hana Drahovská, Faculty of Natural Sciences, Science Park, Comenius University in Bratislava, Ilkovičova 6, 84215 Bratislava, Slovakia.

Correspondence author:
Eva Kaclíková, eva.kaclikova@nppc.sk

used typing approach providing an easy and unambiguous inter-laboratory exchange of data through public databases. Multivirulence-locus sequence typing (MVLST) has also been used as a sequence-based approach for *L. monocytogenes* genotyping [12]. Nevertheless, these techniques generally show a limited discriminatory power [11]. Potentially useful complementary approach is multiple locus variable-number tandem repeat (VNTR) analysis (MLVA). MLVA schemes are constructed on the basis of the variability of the number of tandem repeats at specific loci in bacterial genomes. Several different MLVA schemes have been developed for *L. monocytogenes* typing [13–15]. Individual typing methods are characterized by different discriminatory power, different degree of reproducibility, labour and financial demands, which predetermine their choice for a certain purpose.

However, confirmation of the isolate persistence requires more powerful techniques comparing the diversity across the whole genome. Whole genome sequencing (WGS) provides complete genetic information of strains, which can be evaluated by several typing approaches. Core genome (cg) MLST scheme based on 1 701 target genes [16] or 1 748 core genome loci [17] can accurately identify genetic distances and closely related isolates. A genomic cluster is defined as a group of isolates, differing from each other by ≤ 10 alleles, according to the previously established cluster threshold [3, 16].

The aim of this study was to evaluate the suitability of MLVA for use as a *L. monocytogenes* typing technique for surveillance and routine control of food products and food processing environments. For this purpose, genetic variability of *L. monocytogenes* isolated during 2010 to 2021 from a meat-processing facility and from a ewes' milk producing farm was determined using MLVA, PFGE, whole genome sequencing-based 7-loci MLST and cgMLST typing.

MATERIALS AND METHODS

L. monocytogenes isolates

A total of 55 *L. monocytogenes* isolates included in the study were characterized in our previous studies [9, 18]. Out of this number, 35 strains were isolated from a meat-processing facility over a four-year period from November 2010 to December 2014 [9] and 20 strains were isolated from an ewes' milk production farm over a 1.5-year period from August 2019 to January 2021 [18]. All isolates were confirmed as *L. monocytogenes* by the species-specific TaqMan real-time PCR targeted to actA gene [19] and characterized by molecular serotyping [7] and PFGE [20].

Multiple locus variable-number tandem repeat analysis

MLVA was performed by a modified method of CHENAL-FRANCISQUE et al. [15]. DNA samples were prepared by chaotropic solid-phase extraction using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) using a protocol for Gram-positive bacteria. Each bacterial culture grown in tryptone soya broth (Merck, Darmstadt, Germany) at 37 °C during 24 h was suspended in 1 ml of deionized water and centrifuged at 10 000 ×*g* for 10 min. The sediment was resuspended in 180 μl of lysis buffer (20 mmol·l^{-1} Tris-HCl pH 8.0; 2 mmol·l^{-1} sodium EDTA; 1.2% Triton X-100; 20 mg·ml^{-1} lysozyme; all chemicals from Merck) and subjected to DNA isolation. The extracted DNA was quantified by QubitT 4 fluorometer with dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Fragments of eight VNTR loci (JLR1, JLR2, LisTr1317, LisTR1869, LisTR881, LMV1, LMV6 and LMV9) were amplified by two multiplex PCR runs with five and three primer pairs, respectively (Tab. 1). Amplification was performed in 25 μl volume using Kapa Taq HotStart PCR kit (Kapa Biosystems, Potters Bar, United Kingdom), primers (Tab. 1), 0,5 mmol·l^{-1} dNTPs, 1.5 mmol·l^{-1} MgCl2, 1 U polymerase and 3 μl of purified DNA. PCR conditions were as follows: initial step of denaturation 2 min at 95 °C followed by 35 cycles of 15 s at 95 °C, 30 s at 55 °C and 60 s at 72 °C and final polymerization for 7 min at 72 °C. PCR products were separated and visualized by flow-through electrophoresis in QIAxcel Advanced instrument equipped with a QIAxcel DNA High Resolution gel cartridge (both from Qiagen). MLVA fragment sizes were determined by Screengel software (Qiagen), 5 bp was set as a threshold for allele differentiation and profile similarity levels were calculated using Edmonds algorithm in GrapeTree software (Warwick, Warwickshire, United Kingdom). Cluster analysis was performed by unweighted pair group method with arithmetic mean (UPGMA) in Molecular Evolutionary Genetics Analysis software (Mega) version X (Pennsylvania State university, Pennsylvania, USA) [21]. The scale bar in dendrogram represents the number of different fragments.

Whole genome sequencing

Total DNA was extracted from individual *L.*

Tab. 1. Primers used for MLVA typing [15].

Primer	Sequence 5' → 3'	Concentration [μmol·l^{-1}]	Multiplex PCR
JLR1_F	GCG CTA TAA CCT GAG GAA AGC	0.20	5 loci
JLR1_R	GTC TTA ATC CAT GCA GAT GGA AC	0.20	
JLR2_F	CCT TCC AGA GAA AGA CAA AAC AG	0.10	
JLR2_R	RCT AAT CCA CCA GCA AAT AGC	0.10	
LisTR1317_F	TGA TTT ACA AAA AGC TTT GCC	0.10	
LisTR1317_R	ACT TGG CAC TTC TGG TTT A	0.10	
LisTR1869_F	CCG CGC TAT AAC CTG AGG AAA GC	0.15	
LisTR1869_R	CTG AAA TCA TTG CAA TCA GAT GCA CC	0.15	
LisTR881_F	TGT AAA TAA AGC TGG TAC GTA C	0.10	
LisTR881_R	GTA TGT TGC TTG TTA TCA ACT AC	0.10	
LMV1_F	CGT ATT GTG CGC CAG AAG TA	0.10	3 loci
LMV1_R	MAR CAA CRC AAC AAC AAA CAG	0.10	
LMV6_F	AAA AGC CCC RAT TGG ATA	0.10	
LMV6_R	CTC GCT GTT TTC TGW TTT CTT AGG	0.10	
LMV9_F	AAC GGT KRC KGA TTT ACT TC	0.30	
LMV9_R	CTT GGY GTC GAG GCA TTT A	0.30	

monocytogenes strains using HigherPurity Bacterial Genomic DNA Isolation Kit (Canvax Biotech, Córdoba, Spain) following the manufacturer's protocol for G$^+$ bacteria. Libraries for high throughput sequencing were prepared by using Nextera XT DNA Library Preparation Kit (Illumina, San Diego, California, USA). Paired-end sequencing with 2×150 bp reads was carried out on NextSeq system or with 2×300 bp on MiSeq system (both Illumina). De novo assembly was performed by SPAdes (Center for Algorithmic Biotechnology, St. Petersburg, Russia) [22]. The obtained contigs were filtered to sequences longer than 500 bp with coverage higher than 20. Assemblies were annotated with Rapid Annotation using Subsystem Technology (RAST; University of Chicago, Chicago, Illinois, USA) [23]. The seven loci MLST sequence types and cgMLST were determined using *L. monocytogenes* BIGSdb-Pasteur database for *Listeria monocytogenes* (Oxford University, Oxford, United Kingdom) [17]. GrapeTree was used for visualization of strain clusters with the numbers next to lines representing allelic differences [24]. Sequenced genomes were deposited in Listeria MLST database under accession numbers 81067-81076 and in GenBank NCBI (National Library of Medicine, Bethesda, USA) as Bioproject PRJNA897729.

RESULTS AND DISCUSSION

Molecular serogroups

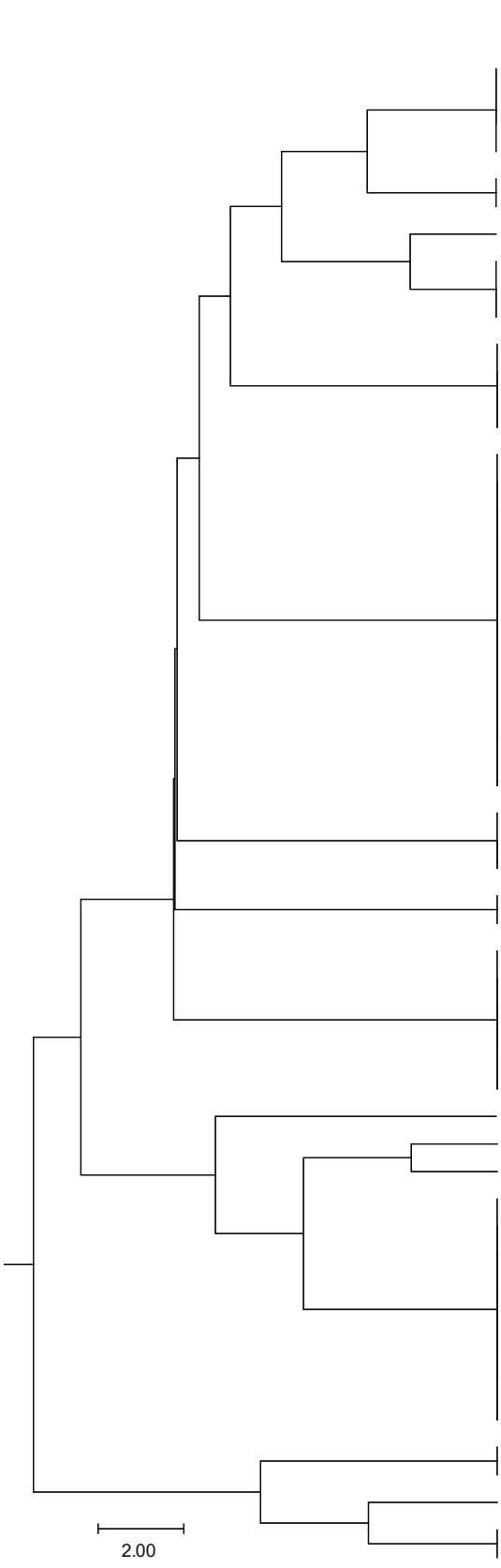
Molecular serotyping by multiplex PCR classified 35 *L. monocytogenes* from the meat-processing facility isolated in our previous study into four serogroups (IIa, IIc, IIb and IVb) [9]. All 20 *L. monocytogenes* isolates from ewes' milk production farm were classified into one serogroup (IIa) [18]. Overall molecular serotyping results of *L. monocytogenes* isolates used in the study are presented in Fig. 1.

Pulsed-field gel electrophoresis

Altogether, 55 *L. monocytogenes* isolates analysed in this study were clustered to 15 pulsotypes. The 35 *L. monocytogenes* isolates from meat-processing facility were divided into nine pulsotypes [9]. PFGE profiling of 20 *L. monocytogenes* isolates from ewes' milk production farm resulted in discrimination into six pulsotypes [18].

Multi-locus variable number tandem repeat analysis

MLVA analysis based on eight VNTR loci clustered the 55 analysed *L. monocytogenes* isolates to 16 MLVA types. Fragment sizes from two multiplex PCR analysis (five and three loci) are presented in Tab. 2. The resulting MLVA types (A to P) together with molecular characteristics (molecular serogroup, pulsotype, ST, CC) of *L. monocytogenes*



Isolate	MLVA	PFGE	Molecular serogroup	ST	CC
S/30/10	A	1	IIc		
S/69/04	A	1	IIc	9	9
S/69/09	A	1	IIc		
S/87/14/2	A	1	IIc		
121/1B	B	NS5	IIa	451	11
124/1B	B	NS5	IIa		
120/5	C	NS4	IIa	91	14
S/82/14	D	6	IIa		
S/75/15	D	6	IIa	8	8
S/76/06/2	D	6	IIa		
S/82/20	E	1	IIc		
S/76/11	E	1	IIc		
S/74/13	E	1	IIc		
S/75/11	E	1	IIc	9	9
70-21	F	NS1	IIa	14	14
127/1	F	NS1	IIa	14	14
9044-20	F	NS1	IIa	14	14
124/1A	F	NS1	IIa	14	14
121/2	F	NS1	IIa	14	14
2224-20	F	NS1	IIa	14	14
119/10	F	NS1	IIa	14	14
117/9	F	NS1	IIa	14	14
116/8	F	NS1	IIa	14	14
115/8	F	NS1	IIa	14	14
114/22	F	NS1	IIa	14	14
9611-19	F	NS1	IIa	14	14
9612-19	F	NS1	IIa		
S/87/1/2	G	18	IIa	121	121
S/74/04	G	18	IIa		
S/87/1/1	G	18	IIa	121	121
121/1F	H	NS6	IIa	394	415
9531-20	H	NS6	IIa	394	415
S/82/07	I	9	IVb		
S/80/01	I	9	IVb		
S/76/15/2	I	9	IVb		
S/74/18	I	9	IVb	2	2
S/69/06	I	9	IVb	2	2
S/74/08	I	9	IVb		
S/76/07	J	11	IIc	9	9
117/4	K	NS3	IIa	21	21
9614-19	L	NS2	IIa	2833	29
S/86/07	M	2	IIa	14	14
S/80/3	M	2	IIa		
S/76/06/1	M	2	IIa		
S/75/13	M	2	IIa	14	14
S/75/12	M	2	IIa	14	14
S/74/12	M	2	IIa		
S/69/08	M	2	IIa	14	14
S/37/05	M	2	IIa	14	14
S/54/01	M	2	IIa	14	14
S/86/8	N	22	IVb		
S/87/12/2	N	22	IVb	6	6
S/86/11	O	17	IVb	194	315
S/75/17	P	15	IIb		
S/76/15/1	P	15	IIb	195	195

Fig. 1. Dendrogram of MLVA cluster analysis of *L. monocytogenes* isolates.

MLVA – multiple locus variable-number tandem repeat analysis, PFGE – pulsed-field gel electrophoresis, ST – sequence type, CC – clonal complex.

Tab. 2. Fragment sizes from MLVA analyses.

Isolate	Allele sizes of 5 loci [bp]	Allele sizes of 3 loci [bp]
S/30/10	175 – 240 – 289 – 349 – 447	241 – 407 – 532
S/69/04	175 – 239 – 289 – 347 – 445	241 – 406 – 532
S/69/09	174 – 240 – 289 – 347 – 445	240 – 406 – 531
S/87/14/2	174 – 239 – 288 – 346 – 442	240 – 404 – 528
S/74/13	176 – 241 – 290 – 350 – 448	257 – 429 – 533
S/75/11	175 – 240 – 289 – 347 – 444	257 – 428 – 532
S/76/11	175 – 241 – 290 – 349 – 446	256 – 428 – 532
S/82/20	175 – 239 – 289 – 345 – 443	256 – 427 – 531
S/37/05	180 – 238 – 251 – 395 – 483	225 – 397 – 532
S/54/01	182 – 240 – 252 – 398 – 487	225 – 398 – 532
S/69/08	181 – 239 – 252 – 396 – 484	224 – 396 – 530
S/74/12	181 – 239 – 251 – 396 – 484	225 – 397 – 531
S/75/12	182 – 240 – 253 – 398 – 486	225 – 399 – 532
S/75/13	182 – 241 – 253 – 398 – 486	226 – 399 – 532
S/76/06/1	182 – 240 – 253 – 399 – 486	225 – 398 – 532
S/80/3	182 – 240 – 252 – 398 – 487	225 – 397 – 532
S/86/07	182 – 240 – 252 – 396 – 484	224 – 395 – 528
S/75/15	175 – 240 – 283 – 435 – 514	240 – 405 – 528
S/76/06/2	174 – 239 – 283 – 434 – 513	240 – 405 – 528
S/82/14	175 – 240 – 283 – 435 – 514	240 – 405 – 528
S/69/06	199 – 224 – 252 – 355 – 453	257 – 389 – 561
S/74/08	199 – 224 – 252 – 356 – 455	256 – 388 – 559
S/74/18	200 – 225 – 253 – 357 – 456	257 – 389 – 563
S/76/15/2	200 – 225 – 253 – 356 – 453	257 – 389 –563
S/80/01	199 – 224 – 252 – 356 – 455	257 – 389 –562
S/82/07	200 – 224 – 252 – 355 – 453	256 – 387 – 559
S/76/07	174 – 239 – 288 – 346 – 444	256 – 419 – 530
S/75/17	200 – 234 – 265 – 381 – 474	271 – 394 – 559
S/76/15/1	200 – 234 – 264 – 380 – 474	272 – 394 – 560
S/86/11	200 – 242 – 264 – 381 – 472	256 – 410 – 579
S/74/04	180 – 251 – 263 – 418 – 503	239 – 393 – 529
S/87/1/1	181 – 252 – 264 – 420 – 504	240 – 393 – 529
S/87/1/2	181 – 252 – 264 – 419 – 503	240 – 393 – 528
S/86/8	200 – 225 – 253 – 428 – 512	284 – 367 – 552
S/87/12/2	200 – 225 – 253 – 428 – 511	285 – 366 – 553
9611-19	178 – 237 – 249 – 581 –685	223 – 396 – 530
9612-19	178 – 237 – 249 – 582 – 684	222 – 394 – 527
114/22	179 – 238 – 250 – 582 – 686	223 – 396 – 530
115/8	179 – 238 – 249 – 583 – 685	223 – 395 – 529
116/8	179 – 238 – 250 – 583 – 686	223 – 395 – 529
117/9	179 – 239 – 249 – 581 – 685	222 – 395 – 527
119/10	179 – 238 – 250 – 582 – 684	222 – 394 – 528
2224	178 – 237 – 248 – 583 – 684	222 – 394 – 526
121/2	179 – 238 – 250 – 584 – 685	224 – 398 – 531
124/1A	179 – 238 – 249 – 584 – 685	224 – 397 – 530
9044-20	178 – 237 – 249 – 581 – 687	223 – 396 – 529
127/1	178 – 237 – 248 – 582 – 684	222 – 394 – 526
70-21	178 – 237 – 248 – 583 – 684	222 – 394 – 526
9614-19	174 – 227 – 275 – 363 – 463	285 – 397 – 534
117/4	174 – 227 – 268 – 363 – 461	241 – 397 – 533
120/5	175 – 240 – 283 – 364 – 462	241 – 405 – 533
121/1B	174 – 240 – 289 – 356 – 456	240 – 420 – 530
124/1B	174 – 240 – 289 – 356 – 456	241 – 421 – 529
121/1F	170 – 238 – 250 – 420 – 508	237 – 408 – 529
9531-20	171 – 238 – 250 – 420 – 509	237 – 408 – 529

isolates are summarized in Fig. 1. Thirty-five *L. monocytogenes* isolates from the meat-processing facility of nine pulsotypes [9] were clustered into 10 MLVA types, when PFGE cluster 1 (IIc) was divided into two MLVA clusters. Twenty *L. monocytogenes* isolates from ewes' milk production farm were differentiated to six MLVA types in correlation with six pulsotypes (NS 1 – NS 6) [18].

The modification of the method originally developed by CHENAL-FRANCISQUE et al. [15] used in our study was based on two multiplex PCR for eight selected loci (five and three) using QIAxcel Advanced electrophoretic system for PCR fragment analysis. Compared to the original method, this method was faster, less labourious and also cheaper, as it did not require fluorescently labelled primers and sequencer or capillary electrophoresis. Compared to conventional gel electrophoresis, the use of an analyser in this study ensured higher accuracy and reliability of fragment size determination, as well as practical applicability by reducing time and labour intensity.

Whole genome sequencing

High coverage draft genome sequences were received for 35 *L. monocytogenes* isolates covering all identified pulsotypes and MLVA types. From the WGS data, clonal complexes (CC) [11], conventional sequence types (ST) based on the seven housekeeping genes and cgMLST based on the scheme containing 1748 core genome loci [17] were extracted. The isolates were clustered to 13 MLST-ST assigned to 12 CC. Based on cgMLST with set limit less than 10 loci difference [16], the analysed strains were divided into 16 clusters. Six isolates from the meat-processing facility and 12 strains from the milk farm belonged to ST14 and in both cases we observed clonality of the isolates but certain differences between isolates from different environments (Fig. 2).

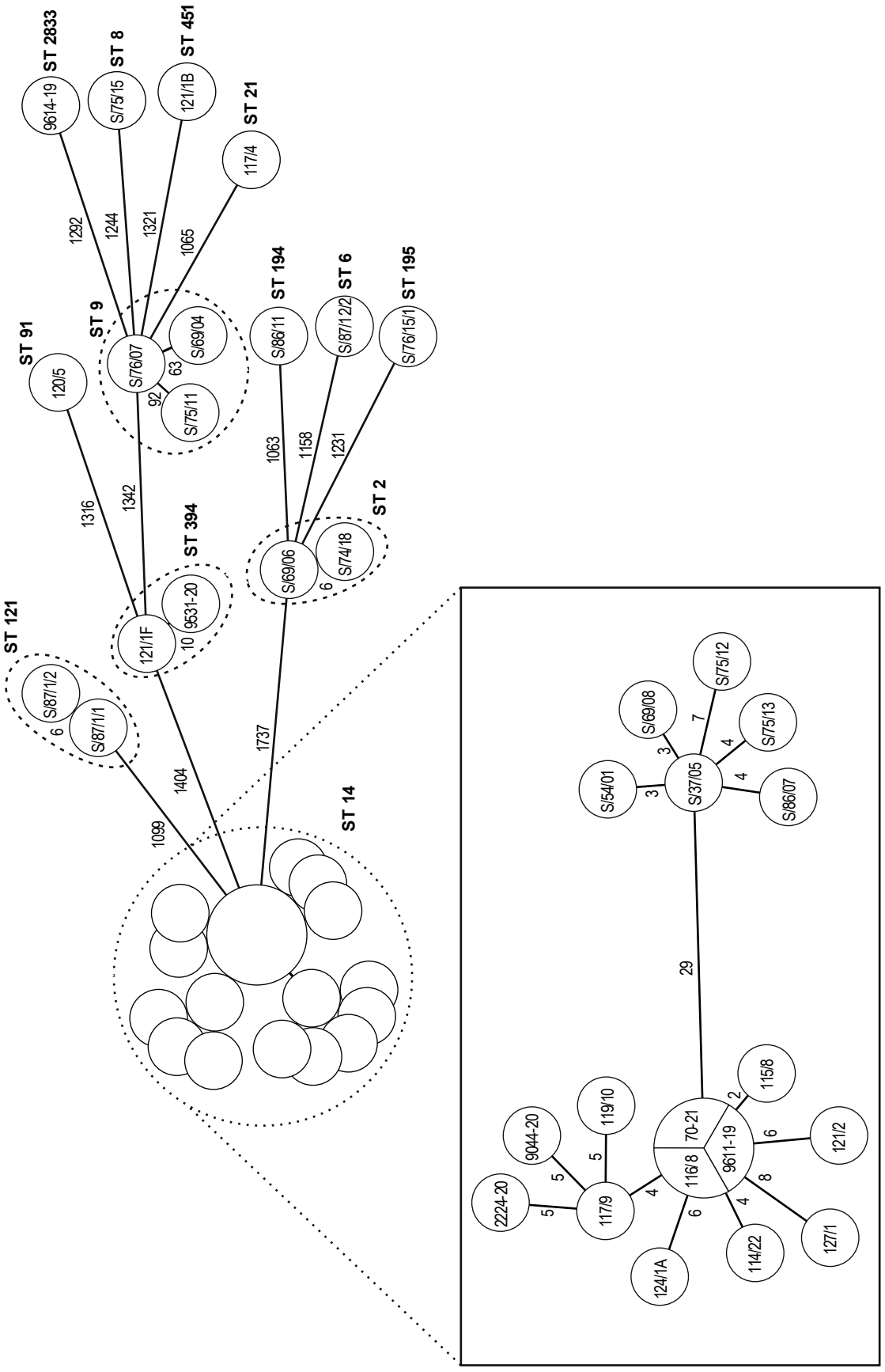


Fig. 2. Dendrogram of *Listeria monocytogenes* strains representing particular sequence types based on core genome multilocus sequence typing.
ST – sequence type.

Consequently, these isolates represented a single persistent contaminant of each food production environment. Likewise, two isolates of each ST2, ST121 and ST394 had similar cgST profiles differing in less than 10 loci representing clusters of related strains.

Discrimination power and application potential of used typing methods

In total, 55 *L. monocytogenes* isolates were clustered to four molecular serogroups, 15 pulsotypes and 16 MLVA types (Tab. 2, Fig. 1). Selected 35 sequenced strains were included in 12 CC, 13 MLST-ST (Fig. 1) and 16 cgMLST profiles including five clusters based on the threshold clonality of 10 different loci (Fig. 2).

In this study, MLVA proved to be slightly more discriminatory than PFGE, when eight isolates of pulsotype 1 (ST 9) were divided to two MLVA types (A and E). The remaining 14 MLVA types were in 100% correlation with 14 pulsotypes. Three ST9/CC9 isolates were divided to two pulsotypes (1 and 11) and three MLVA types (A, E and J; Fig. 1) in accordance with cgMLST clustering (Fig. 2). According to these results, MLVA had the same discriminatory power as cgMLST clustering based on the set threshold clonality of 10 different loci.

Several previous evaluation studies of MLVA had concluded that the method provided better discrimination than PFGE [14, 25], where PFGE was performed with a single enzyme and thus based on a less discriminatory implementation of PFGE. Sperry et al. [26] demonstrated a lower discrimination of MLVA than ApaI/AscI PFGE. On the other hand, high discrimination was achieved by MLVA based on nine loci for solely unrelated strains originating from food and from the environment [27]. In our study, successful MLVA discrimination of *L. monocytogenes* isolates was achieved compared to PFGE, regardless the use of one or two restriction enzymes, when PFGE profiling for isolates from meat-processing facility (including pulsotype 1 divided to two MLVA types) was performed using both restriction enzymes [9].

MLVA subtyping scheme developed by Chenal-Francisque et al. [15] based on 11 loci had a lower discriminatory power than PFGE, except for some clones. It was shown that the discrimination of MLVA is highly dependent on the *L. monocytogenes* clone and should be useful for discrimination of strains belonging to CC4 or CC9 [15], which is consistent with our findings related to CC9 isolates. Given its good discriminatory power and high throughput, MLVA with QIAxcel Advanced electrophoretic system for fragment sizing with two multiplex PCR runs using unlabelled primers can be considered a rapid, reliable, high-throughput and low-cost alternative to typing methods for surveillance and control of *L. monocytogenes* in the food-processing industry.

CONCLUSION

To trace the sources and routes of *L. monocytogenes* in the production chain, using of appropriate typing methods is very important. Compared to the workload as well as the financial burden of PFGE, which was the gold standard in the identification of *L. monocytogenes* for years, MLVA, due to its high reproducibility and high throughput could represent a very attractive first-line screening method for *L. monocytogenes* typing. Subsequently, isolates with identical MLVA profiles should be subjected to cgMLST analysis to determine similarity and confirm persistent contamination. Results of our study showed that MLVA based on eight VNTR loci coupled to high resolution electrophoresis proved to be an effective, comparatively fast and inexpensive method. A two-step MLVA-WGS strategy could significantly lighten the workload and would position MLVA as an important tool for determination of clonality of isolates for tracing of *L. monocytogenes* contamination in food products and food processing environment.

Acknowledgements

This work was supported by the Operational Program Integrated Infrastructure within the project Demand-driven research for the sustainable and innovative foods, Drive4SIFood, 313011V336, co-financed by the European Regional Development Fund.

REFERENCES

1. The European Union One Health 2021 Zoonoses Report. EFSA Journal, *20*, 2022, article e07666. DOI: 10.2903/j.efsa.2022.7666.
2. Farber, J. M. – Zwietering, M. – Wiedmann, M. – Schaffner, D. – Hedberg, C. W. – Harrison, M. A. – Hartnett, E. – Chapman, B. – Donnell, C. W. – Goodburn, K. E. – Gummalla, S.: Alternative approaches to the risk management of *Listeria monocytogenes* in low risk foods. Food Control, *123*, 2021, article 107601. DOI: 10.1016/j.foodcont.2020.107601.
3. Cabal, A. – Pietzka, A. – Huhulescu, S. – Allerberger, F. – Ruppitsch, W. – Schmid, D.: Isolate-based surveillance of *Listeria monocytogenes* by whole genome sequencing in Austria. Frontiers in Microbiology, *10*, 2019, 2282. DOI: 10.3389/fmicb.2019.02282.

4. Belias, A. – Sullivan, G. – Wiedmann, M., – Ivanek, R.: Factors that contribute to persistent Listeria in food processing facilities and relevant interventions: A rapid review. Food Control, *133*, 2021, article 108579. DOI: 10.1016/j.foodcont.2021.108579.
5. Orsi, R. H. – den Bakker, H. C. – Wiedmann, M.: *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. International Journal of Medical Microbiology, *301*, 2011, pp. 79–96. DOI: 10.1016/j.ijmm.2010.05.002.
6. Doumith, M. – Buchrieser, C. – Glaser, P. – Jacquet, C. – Martin, P.: Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. Journal of Clinical Microbiology, *42*, 2004, pp. 3819–3822. DOI: 10.1128/JCM.42.8.3819-3822.2004.
7. Kérouanton, A. – Marault, M. – Petit, L. – Grout, L. – Dao, T. T. – Brisabois, A.: Evaluation of a multiplex PCR assay as an alternative method for *Listeria monocytogenes* serotyping. Journal of Microbiological Methods, *80*, 2010, pp. 134–137. DOI: 10.1016/j.mimet.2009.11.008.
8. Latorre, A. A. – Van Kessel, J. A. S. – Karns, J. S. – Zurakowski, M. J. – Pradhan, A. K. – Boor, K. J. – Adolph, E. – Sukhnanand, S. – Schukken, Y. H.: Increased in vitro adherence and on-farm persistence of predominant and persistent *Listeria monocytogenes* strains in the milking system. Applied and Environmental Microbiology, *77*, 2011, pp. 3676–3684. DOI: 10.1128/AEM.02441-10.
9. Véghová, A. – Minarovičová, J. – Koreňová, J. – Drahovská, H. – Kaclíková, E.: Prevalence and tracing of persistent *Listeria monocytogenes* strains in meat processing facility production chain. Journal of Food Safety, *37*, 2016, article e12315. DOI: 10.1111/jfs.12315.
10. Martín, B. – Bover-Cid, S. – Aymerich, T.: MLVA subtyping of *Listeria monocytogenes* isolates from meat products and meat processing plants. Food Research International, *106*, 2018, pp. 225–232. DOI: 10.1016/j.foodres.2017.12.052.
11. Ragon, M. – Wirth, T. – Hollandt, F. – Lavenir, R. – Lecuit, M. – Le Monier, A. – Brisse, S.: A new perspective on *Listeria monocytogenes* evolution. PLoS Pathogens, *4*, 2008, article e1000146. DOI: 10.1371/journal.ppat.1000146.
12. Chen, Y. – Zhang, W. – Knabel, S. J.: Multi-virulence-locus sequence typing identifies single nucleotide polymorphisms which differentiate epidemic clones and outbreak strains of *Listeria monocytogenes*. Journal of Clinical Microbiology, *45*, 2007, pp. 835–846. DOI: 10.1128/JCM.01575-06.
13. Murphy, M. – Corcoran, D. – Buckley, J. F. – O'Mahony, M. – Whyte, P. – Fanning, S.: 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*, 2007, pp. 187–194. DOI: 10.1016/j.ijfoodmicro.2006.10.022.
14. Lindstedt, B. A. – Tham, W. – Danielsson-Tham, M. L. – Vardund, T. – Helmersson, S. – Kapperud, G.: 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*, 2008, pp. 141–148. DOI: 10.1016/j.mimet.2007.11.012.
15. Chenal-Francisque, V. – Diancourt, L. – Cantinelli, T. – Passet, V. – Tran-Hykes, C. – Bracq-Dieye, H. – Leclercq, A. – Pourcel, C. – Lecuit, M. – Brisse, S.: 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*, 2013, pp. 1868–1880. DOI: 10.1128/JCM.00606-13.
16. Ruppitsch, W. – Pietzka, A. – Prior, K. – Bletz, S. – Fernandez, H. L. – Allerberger, F. – Harmsen, D. – Mellmann, A.: Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. Journal of Clinical Microbiology, *5*, 2015, pp. 2869–2876. DOI: 10.1128/JCM.01193-15.
17. Moura, A. – Criscuolo, A. – Pouseel, H. – Maury, M. M. – Leclercq, A. – Tarr, C. – Bjorkman, J. T. – Dallman, T. – Reimer, A. – Enouf, V. – Larsonneur, E. – Carleton, H. – Bracq-Dieye, H. – Katz, L. S. – Jones, L. – Touchon, M. – Tourdjman, M. – Walker, M. – Stroika, S. – Cantinelli, T. – Chenal-Francisque, V. – Kucerova, Z. – Rocha, E. P. C. – Nadon, C. – Grant, K. – Nielsen, E. M. – Pot, B. – Gerner-Schmidt, P. – Lecuit, M. – Brisse, S.: Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. Nature Microbiology, *2*, 2016, article 16185. DOI: 10.1038/nmicrobiol.2016.185.
18. Minarovičová, J. – Véghová, A. – Kubicová, Z. – Andrezál, M. – Drahovská, H. – Kaclíková, E.: Tracing of persistent *Listeria monocytogenes* contamination in ewe's milk farm. Letters in Applied Microbiology, *76*, 2023, article ovad006. DOI: 10.1093/lambio/ovad006.
19. Oravcová, K. – Kaclíková, E. – Krascsenicsová, K. – Pangallo, D. – Brežná, B. – Siekel, P. – Kuchta, T.: Detection and quantification of *Listeria monocytogenes* by 5'-nuclease polymerase chain reaction targeting the *actA* gene. Letters in Applied Microbiology, *42*, 2006, pp. 15–18. DOI: 10.1111/j.1472-765X.2005.01793.x.
20. PNL04. Standard operating procedure for PulseNet PFGE of *Listeria monocytogenes*. Atlanta : Centers for Disease Control and Prevention, last updated July 2017. <https://www.cdc.gov/pulsenet/pdf/listeria-pfge-protocol-508c.pdf>
21. Kumar, S. – Stecher, G. – Li, M. – Knyaz, C. – Tamura, K.: MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, *35*, 2018, pp. 1547–1549. DOI: 10.1093/molbev/msy096.
22. Bankevich, A. – Nurk, S. – Antipov, D. – Gurevich, A. A. – Dvorkin, M. – Kulikov, A. S. – Lesin, V. M. – Nikolenko, S. I. – Pham, S. –

Prjibelski, A. D. – Pyshkin, A. V. – Sirotkin, A. V. – Vyahhi, N. – Tesler, G. – Alekseyev, M. A. – Pevzner, P. A.: SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology, *19*, 2012, pp. 455–477. DOI: 10.1089/cmb.2012.0021.

23. Aziz, R. K. – Bartels, D. – Best, A. – DeJongh, M. – Disz, T. – Edwards, R. A. – Formsma, K. – Gerdes, S. – Glass, E. M. – Kubal, M. – Meyer, F. – Olsen, G. J. – Olson, R. – Osterman, A. L. – Overbeek, R. A. – McNeil, L. K. – Paermann, D. – Paczian, T. – Parello, B. – Pusch, G. D. – Reich, C. – Stevens, R. – Vassieva, O. – Vonstein, V. – Wilke, A. – Vonstein, V. – Zagnitko, O.: The RAST Server: Rapid annotations using subsystems technology. BMC Genomics, *9*, 2008, article 75. DOI: 10.1186/1471-2164-9-75.
24. Zhou, Z. – Alikhan, N. F. – Sergeant, M. J. – Luhmann, N. – Vaz, C. – Francisco, A. P. – Carrico, J. A. – Achtman, M.: GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Research, *28*, 2018, pp. 1395–1404. DOI: 10.1101/gr.232397.117.
25. Miya, S. – Kimura, B. – Sato, M. – Takahashi, H. – Ishikawa, T. – Suda, T. – Takakura, C. – Fujii, T. – Wiedmann, M.: Development of a multilocus variable number of tandem repeat typing method for *Listeria monocytogenes* serotype 4b strains. International Journal of Food Microbiology, *124*, 2008, pp. 239–249. DOI: 10.1016/j.ijfoodmicro.2008.03.023.
26. Sperry, K. E. – Kathariou, S. – Edwards, J. S. – Wolf, L. A.: Multiple-locus variable-number tandem-repeat analysis as a tool for subtyping *Listeria monocytogenes* strains. Journal of Clinical Microbiology, *46*, 2008, pp. 1435–1450. DOI: 10.1128/JCM.02207-07.
27. Li, X. – Huang, B. – Eglezos, S. – Graham, T. – Blair, B. – Bates, J.: Identification of an optimized panel of variable number tandem-repeat (VNTR) loci for *Listeria monocytogenes* typing. Diagnostic Microbiology and Infection Disease, *75*, 2013, pp. 203–206. DOI: 10.1016/j.diagmicrobio.2012.11.007.

Received 21 February 2023; 1st revised 30 March 2023; accepted 31 March 2023; published online 3 May 2023.