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Research Note

Multiple-Locus Variable-Number Tandem Repeat Analysis Genotypes of *Listeria monocytogenes* Isolated from Farms, Abattoirs, and Retail in Gauteng Province, South Africa

JAMES GANA,[1,2] NOMAKORINTE GCEBE,[3] RIAN PIERNEEF,[4] REBONE MOERANE,[1] AND ABIODUN A. ADESIYUN *https://orcid.org/0000-0001-9470-9421*[1,5]*

[1]*Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa;* [2]*Department of Agricultural Education, Federal College of Education, Kontagora, Niger State, Nigeria (ORCID: https: orcid.org/0000-0001-9415-5297[J.G.]);* [3]*Bacteriology Department, Onderstepoort Veterinary Research, Agricultural Research Council, South Africa;* [4]*Agricultural Research Council-Biotechnology Platform, 100 Old Soutpan Road, Onderstepoort, Pretoria 0110, South Africa; and* [5]*Department of Paraclinical Sciences, School of Veterinary Medicine, The University of West Indies, St. Augustine, Trinidad and Tobago*

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ABSTRACT

The use of multiple-locus variable-number analysis (MLVA) of tandem repeats (TRs) for subtyping *Listeria monocytogenes* has proven to be reliable and fast. This study determined the MLVA genotypes of 60 isolates of *L. monocytogenes* recovered from cattle farms, abattoirs, and retail outlets in Gauteng province, South Africa. The distribution of the 60 *L. monocytogenes* isolates analyzed by type of sample was as follows: raw beef (28, 46.7%), ready-to-eat beef products (9, 15.0%), beef carcass swabs (9, 15.0%), cattle environment (6, 10.0%), and cattle feces (8, 13.3%). The serogroups of the isolates were determined using PCR and the MLVA genotypes based on six selected loci. The frequency of the 60 serogroups detected was as follows: 1/2a-3a (IIa) (27, 45.0%); 4b-4d-4e (1Vb) (24, 40.0%); 1/2c-3c (IIc) (8, 13.3%); and 1/2b-3b (IIb) (1, 1.7%). MLVA successfully clustered genetically related isolates and differentiated nonrelated isolates, irrespective of their sources, sample types, and serogroups, as demonstrated by 16 MLVA pattern types detected. For serogroup 4b-4d-4e (IVb), there was no variation in TRs LM-TR2, LM-TR4, and LM-TR6, which each contained only one allele (02, 00, and 93, respectively). However, across the sources and sample types of isolates, there was variation in serogroup 4b-4d-4e (IVb): LM-TR1 contained 00, 03, and 05; LM-TR3 contained 14, 20, and 22; and LM-TR5 contained 14, 21, and 25. Similar patterns of variation in the TRs were detected in the other serogroups (1/2a-3a, 1/2b-3b, and 1/2c-3c). BioNumeric data analysis identified at least five types in Gauteng province. MLVA epidemiologically clustered the related isolates and differentiated unrelated isolates.

HIGHLIGHTS

- MLVA genotypes of *L. monocytogenes* in South Africa were investigated.
- Serogroups detected were 1/2a-3a (IIa), 4b-4d-4e (1Vb), 1/2c-3c (IIc), and 1/2b-3b (IIb).
- Sixteen MLVA pattern types and four serogroups were detected.
- MLVA type I was predominant in serogroups 1/2a-3a (IIa), 1/2b-3b (IIb), and 1/2b-3c (IIc).
- MLVA genotyping of *L. monocytogenes* is an important molecular epidemiological tool.

Key words: Gauteng; *Listeria monocytogenes*; Multiple-locus variable-number analysis; Serogroup; South Africa; Tandem repeat

Listeria monocytogenes is the causative agent of foodborne listeriosis and was first described by Murray and coworkers in 1926 when they isolated the bacterium from the livers of clinically sick rabbits and guinea pigs *(29)*. The major clinical sign of listeriosis is gastroenteritis, varying from mild to severe, which has been reported in veterinarians, farmers, and abattoir workers *(10, 31)*.

Different phenotypic and molecular methods have been used to classify *L. monocytogenes* during diagnostic (humans, foods, and the environment) and epidemiological investigations of listeriosis outbreaks. These include serotyping (phenotypic and molecular assays), multiplex PCR *(8),* multiple-locus variable-number analysis (MLVA) *(28),* pulsed-field gel electrophoresis *(39,44),* multilocus sequence typing *(45),* and whole genome sequencing *(13),* among others. These assays have advantages and disadvantages and have different discriminatory powers in their application *(1)*.

The serogroups and serovars of *L. monocytogenes* commonly detected in foods and environmental samples include 4b-4d-4e, 1/2a-3a, and 1/2b-3b-7 *(24)* and 1/2b, 4b,

* Author for correspondence. Tel: 012 529 8013, 240-513-9618; E-mail: Abiodun.adesiyun@up.ac.za.

1/2a, 3b, 4d, 4e *(47),* among others. These serogroups were reported to be of public health and epidemiological relevance because they are commonly associated with human listeriosis; they vary by geographical locations and sample type *(24, 50)*. Although human listeriosis outbreaks are mostly caused by serogroups 4b-4d-4c and 1/2b-3b-7 *(18),* sporadic cases caused by the 1/2a-3a or 1/2c-3c serogroups have been reported *(42)*.

MLVA is a PCR-based method that can be used to differentiate strains of a microorganism and group them by their genetic relationships. This method detects the number of tandem repeats (TRs) at a specific locus in the genome of a bacterium. These may vary because of DNA polymerase enzyme slippage during replication, and these differences can be detected using PCR primers designed to anneal the flanking regions *(16)*. Murphy et al. *(28)* were the first to develop an MLVA protocol to genotype *L. monocytogenes*. MLVA is also applied as a next-generation genotyping protocol to subtype other foodborne microorganisms, such as *Escherichia coli* O157*, Staphylococcus aureus,* and *Salmonella (20, 27, 37)*. TRs of a genome are found in stable regions, which are not associated with plasmids and other mobile genetic elements. The MLVA approach is considered a low-cost and easy method that provides easily interpretable and rapid results with high discriminatory power *(20, 41)*. MLVA is the second major genotyping tool, after pulsed-field gel electrophoresis, used in the PulseNet network and was primarily used in public health surveillance and outbreak investigation of *Salmonella* in Europe *(34)*.

The advantages of MLVA are that it is simple, easy, and fast; it does not require use of sophisticated electrophoresis apparatus in the laboratory *(37)*. One disadvantage is that MLVA is not a universal method because primers must be designed that target the pathogen to be investigated. Another disadvantage of MLVA is that its results cannot be compared directly with those from other laboratories because the amplicon banding patterns, in some cases, are monitored by convectional electrophoresis on agarose gel; this does not reveal the actual numbers of repeats in the amplicon obtained, making it impossible to determine the pattern of the bands corresponding with the PCR target *(37)*. Currently no comprehensive MLVA database for *L. monocytogenes* is available for comparison to existing MLVA patterns, for example, if the polymorphism between two strains is restricted to a single locus *(5)*.

During the first human listeriosis outbreak reported in South Africa, over 9 months between August 1977 and April 1988, more than 14 cases occurred in Johannesburg *(14)*. Since then, sporadic cases have been documented annually in South Africa. However, between 2017 and 2018, the country experienced the world's largest outbreak of human listeriosis across all nine provinces in the country *(2)*. The major source of *L. monocytogenes* transmission has been attributed to the consumption of contaminated ready-to-eat food products *(9, 19)*. *L. monocytogenes* has been isolated from various raw and ready-to-eat foods and from environmental samples such as water, equipment swabs, and soil *(12, 26)*. Isolates of *L. monocytogenes* need to be properly characterized to accurately diagnose the infection, understand the epidemiology of infection, investigate outbreaks, and effectively prevent and minimize the transmission of *Listeria* through the food chain and other routes *(5)*.

To date, there has been no published report on the application of MLVA on *L. monocytogenes* from any source in the beef production system (cattle farms, beef abattoirs, and retail outlets) or any other sources in South Africa. Because MLVA has been documented to have several advantages over whole genome sequencing *(37),* its use and performance as an alternative must be investigated in South Africa.

Therefore, the objectives of this study were to determine the MLVA genotypes of *L. monocytogenes* strains isolated from cattle farms, beef abattoirs, and retail outlets in Gauteng province, South Africa, using agarose gel electrophoresis for amplicon sizing. The study was also designed to evaluate MLVA data using the unweighted pair group method with arithmetic mean (UPGMA) to calculate genetic distances between the genotypes and to produce dendrograms and minimum spanning trees. Finally, MLVA data generated in the study were assessed to potentially serve as a basis for future molecular epidemiological studies applying MLVA typing.

MATERIALS AND METHODS

Sources, isolation, and identification of *L. monocytogenes*. Sixty isolates ($n = 60$) of *L. monocytogenes* were originally obtained from various beef and beef products, cattle farms, and red meat abattoirs in Gauteng province, as shown in Supplemental Appendix 1. All 60 isolates of *L. monocytogenes* were unique, that is, not part of the food chain from farm to retail markets. All the isolates were previously identified as *L. monocytogenes* using bacteriological methods *(15, 40)* and multiplex PCR assays *(8)*. The isolates of *L. monocytogenes* were maintained at –20°C for further analysis.

Extraction of DNA from isolates of *L. monocytogenes*. The DNA that was used for the PCR assays for serogrouping and MLVA was extracted from individual colonies, as described by Madoroba et al. *(21)*. Before DNA extraction, the preserved *L. monocytogenes* isolates were revived by inoculation into brain heart infusion broth followed by overnight incubation at 35°C. A loopful from the incubated broth was used to inoculate Brilliance *Listeria* agar plates, followed by incubation at 35°C for 48 h. Thereafter, 200 μL of sterile distilled water was aliquoted into 2-mL tubes and each was inoculated with a loopful of bacterial culture harvested from Brilliance *Listeria* agar plates. The bacterial suspension was vortexed for 10 s and heated at 95°C for 10 min, followed by cooling at room temperature and centrifugation at 15,493 × *g* for 5 min. The supernatants were transferred into sterile 2-mL microcentrifuge tubes, and the debris was discarded. The crude supernatants were stored at –20°C and were subsequently used as DNA templates in the PCR assays for serogrouping and MLVA profiling.

Determination of the serogroups of *L. monocytogenes* isolates. The PCR assay method described by Doumith et al. *(8)* was used to characterize *L. monocytogenes* serogroups. The five primers used to classify the strains into serogroups are shown in Appendix S2. Multiplex PCR was used to classify *L. monocytogenes* strains into serogroups that target the five fragments of *L.*

TABLE 1. *Summary of the 60* L. monocytogenes *isolates based on the number of alleles found at each locus according to their serovars*

	No. of alleles (in parentheses) by serovars of *L. monocytogenes*[a]			
TR locus	IIa	IIb	IIc	IVb
LM-TR1	00, 03 (2)	00 (1)	00, 03 (2)	00, 03, 05 (3)
LM-TR2	02 (1)	02 (1)	02 (1)	02 (1)
LM-TR3	11, 20, 22 (3)	22 (1)	20, 22 (2)	14, 20, 22 (3)
LM-TR4	03 (1)	03 (1)	03 (1)	00 (1)
LM-TR5	14, 16, 21, 22 (4)	21 (1)	16, 21, 22 (3)	14, 21, 25 (3)
LM-TR6	04, 06, 08, 89, 93 (5)	04 (1)	04 (1)	93 (1)

[a] Allele at each locus for a given serogroup.

monocytogenes, namely, *imo1118, imo0737, orf2110, orf2819,* and *prs* (specific for *Listeria* spp.). PCR assay was prepared as follows: 12.5 μL of 2× RED *Taq* master mix (Ampliqon, Odense, Denmark), 3.5 μL of nuclease-free water, 5 μL of DNA template, and 4 μL of 20 μM primer mix for PCR assay. The PCR conditions used were as follows: initial denaturation step at 94°C for 3 min; 35 cycles of 94°C for 0.40 min, 53°C for 1.15 min, and 72°C for 1.15 min; and one final cycle at 72°C for 7 min in a thermocycler (Lasec, Cape Town, South Africa). A 20-μL aliquot of each PCR product was subjected to electrophoresis on 3% ethidium bromide–stained agarose gel for 3 h at 100 V. *L. monocytogenes* ATCC 19111 was used as a positive control, *Campylobacter fetus* ATCC 27373 as a negative control, and water as a blank, for validation of the zero grouping assays with PCR. A 100-bp molecular weight marker (Thermo Fisher Scientific, Waltham, MA) was used as a standard to estimate the amplicon sizes.

MLVA subtyping of *L. monocytogenes* isolates. The MLVA procedure was conducted as a singleplex using the six primer pairs proposed by Murphy et al. *(28)* shown in Appendix S3. PCR assay was prepared as follows: 12.5 μL of 2× RED *Taq* master mix (Ampliqon), 3.5 μL of nuclease-free water, 5 μL of DNA template, and 4 μL of 20 μM primer mix. Thermal cycling consisted of 35 cycles of 94°C for 45 s; the annealing temperature of 54°C for 45 s for all primers except LM-TR 6, where the annealing temperature was adjusted to 52 and 72°C (singleplex assays) for 60 s; with a final extension at 72°C for 5 min (a modification of the cycling conditions by Noller et al. *(32)*). The PCR products (5 μL) were subjected to electrophoresis on 3% ethidium bromide–stained agarose gel for 3 h at 100 V. Amplicon sizes for each marker were estimated using O'RangeRuler 20-bp DNA ladder (Thermo Fisher Scientific). A gel documentation system (Vacutec, Roodepoort, South Africa) was used to capture the amplicon sizes. *L. monocytogenes* ATCC 19111 was used as a positive control and water as a blank.

The TR copy numbers in each sample were determined for each marker by extrapolating fragment sizes and TR sequence size as described by Murphy et al. *(28)*. Allele numbers were assigned for each amplified DNA from each isolate based on the estimated number of TRs.

The number of TRs was rounded down to whole numbers. MLVA types were assigned for samples analyzed in this study based on sequence number (MLVA allele string) representing the number of TRs for each locus and sample (TR1-TR-2, TR-3, TR-4, TR-5, TR-6) because there is no international database for MLVA genotypes.

Data analysis. UPGMA was used to generate dendrograms and minimum spanning trees and to calculate genetic distances among the genotypes. First, distance matrices were calculated using the "daisy" function with the "gower" parameter specified to determine Gower distances with the R package "cluster" *(22)*. Thereafter, UPGMA trees were constructed and visualized with "ggtree" *(49)*. Minimum spanning trees were calculated using the "ape" package *(33)* with the "mst" function and were visualized using "igraph" *(6)* and "ggnetwork" *(3)*. R version 4.0.2 *(42)* was used for the construction of dendrograms and minimum spanning trees.

RESULTS

Detection of serogroups of *L. monocytogenes*. Of the 60 isolates of *L. monocytogenes* recovered from retail outlets, abattoirs, and farms assessed in the current study, the frequency of detection of the serogroups was as follows: 1/2a-3a (IIa) (27, 45.0%); 4b-4d-4e (IVb) (24, 40.0%); 1/2c-3c (IIc) (8, 13.3%); and 1/2b-3b (IIb) (1, 1.7%) ($P < 0.0001$).

MLVA of *L. monocytogenes* isolates. MLVA successfully discriminated among *L. monocytogenes* isolates by region, sample type or class, and serogroup; the method's discriminatory power is demonstrated by the UPGMA dendrogram (Fig. 1 and Table 1) and minimum spanning tree (Fig. 2). A total of 16 MLVA patterns were detected among the four serogroups. The predominant MLVA pattern was type I (14, 23.3%), which consisted of isolates from serogroups IIa, IIb, and IIc; this indicates they are closely related in terms of MLVA despite being in different serogroups (Fig. 2 and Table 2). The unique MLVA types were III (7, 11.7%), IV (4, 6.7%), V (4, 6.7%), VI (3, 5.0%), IX (3, 5.0%), and XI (2, 3.5%), which all were isolates belonging to the same serogroup, IVb. This indicates that although these *L. monocytogenes* isolates belonged to the same serogroup, they were genetically unrelated in terms of TR copy numbers (Figs. 1 and 2). Of interest is that all the isolates in the second most predominant MLVA type, type II (9, 15%), belonged to serogroup IIa. The isolates in MLVA types VII and VIII shared the same serogroups, IIa and IIc, in each of the MLVA types. All the isolates in serogroup IVb in this study do not cluster with other serogroups detected and belonged to MLVA types III, IV, V, VI, IX, XI, and XVI (Table 2).

All *L. monocytogenes* isolates are classified as belonging to MLVA types II, III, IV, VI, VII, VIII, IX, XI, XII, XIII, and XIV, originating from the retail outlets only (Table 2); in contrast, the isolates in MLVA types I, V,

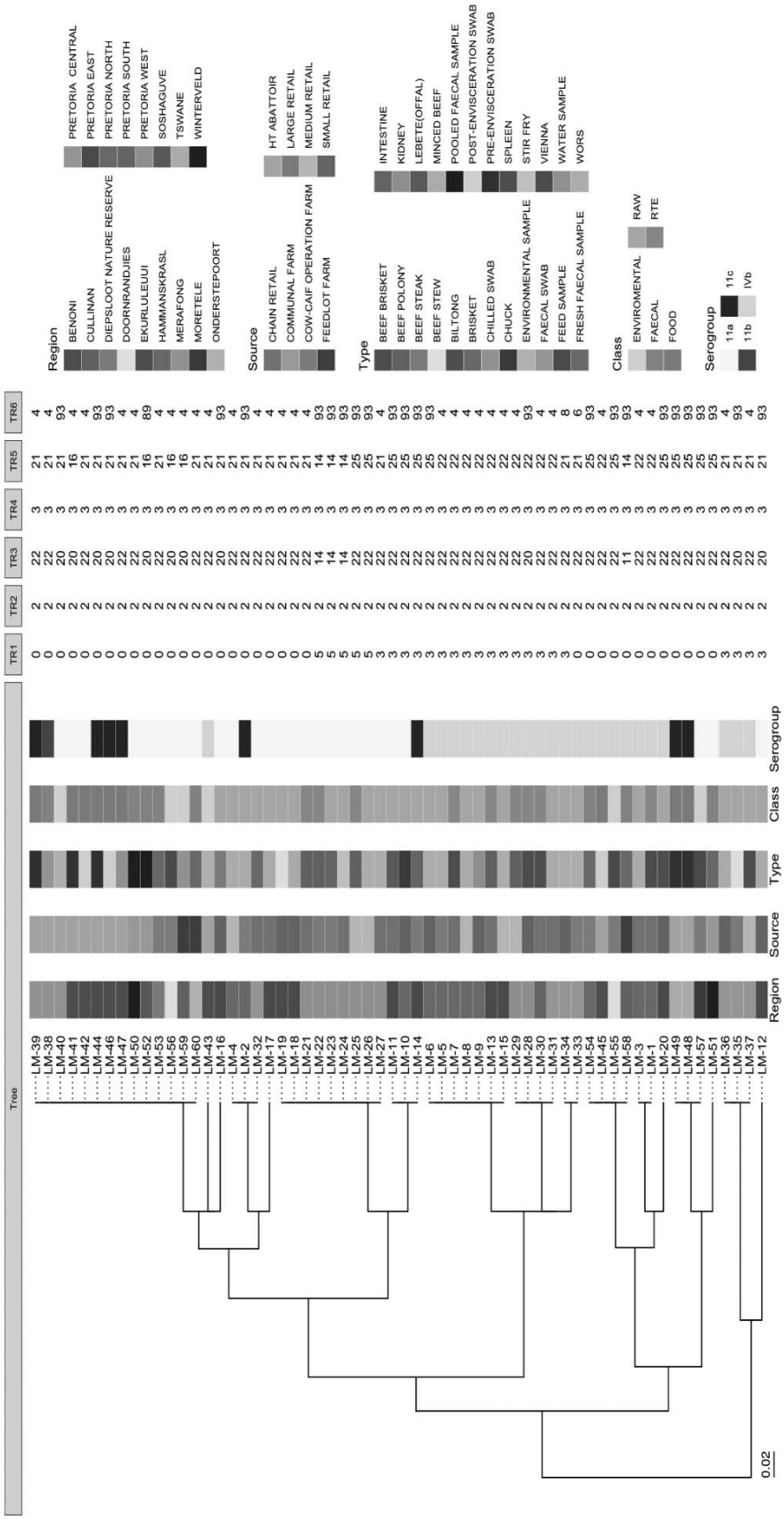

FIGURE 1. *UPGMA dendrogram of the 60 isolates of* L. monocytogenes *collected from retail outlets, abattoirs, and farms in Gauteng province, South Africa. Characteristics of each isolate include serogroup, source, region/area, class, and type of sample.*

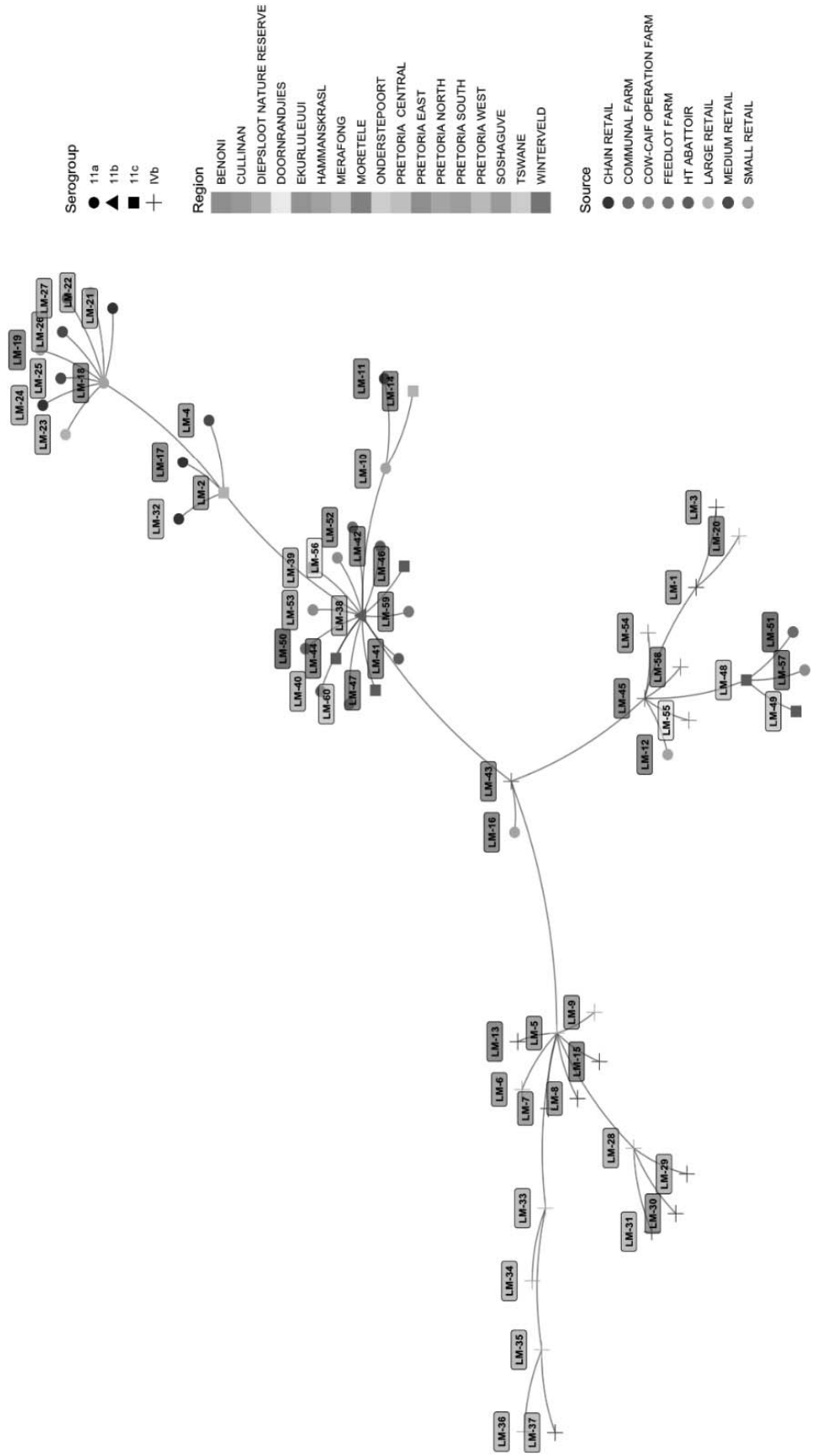

FIGURE 2. *Minimum spanning tree showing the relationship between the isolates of* L. monocytogenes *from Gauteng province retail outlets, abattoirs, and farms by geographical location showing closely related isolates according to the source of isolates.*

TABLE 2. *Clusters of* L. monocytogenes *identified by MLVA subtyping*[a]

MLVA pattern type[b]	% isolates belonging to MLVA pattern	MLVA allele string[c]	Source[d]	Sample type[e]	Serogroup[f]
I (14)	23.3	00-02-22-03-21-04	Abattoir (8), farm (6)	Carcass swab (6), recal (5), environmental (3)	IIa (8), IIc (5), IIb (1)
II (9)	15.0	03-02-22-03-22-04	Retail outlet (9)	Raw beef (6), RTE beef (3)	IIa (9)
III (7)	11.7	00-02-22-03-25-93	Retail outlet (7)	Raw beef (5), RTE beef (2)	IVb (7)
IV (4)	6.7	03-02-22-03-25-93	Retail outlet (4)	RTE beef (3), raw beef (1)	IVb (4)
V (4)	6.7	00-02-20-03-21-93	Abattoir (1), farm (3)	Fecal (2), environmental (1), carcass swab (1)	IVb (4)
VI (3)	5.0	03-02-20-03-21-93	Retail outlet (3)	Raw beef (2), RTE beef (1)	IVb (3)
VII (3)	5.0	03-02-22-03-21-04	Retail outlet (3)	Raw beef (3)	IIa (2), IIc (1)
VIII (3)	5.0	00-02-22-03-22-04	Retail outlet (3)	Raw beef (3)	IIa (2), IIc (1)
IX (3)	5.0	05-02-14-03-14-93	Retail outlet (3)	Raw beef (3)	IVb (3)
X (3)	5.0	00-02-20-03-16-04	Abattoir (2), farm (1)	Carcass swab (2), environmental (1)	IIc (2), IIa (1)
XI (2)	3.3	05-02-22-03-25-93	Retail outlet (2)	Raw beef (2)	IVb (2)
XII (1)	1.7	00-02-11-03-14-93	Retail outlet	Raw beef	IIa
XIII (1)	1.7	00-02-22-03-21-06	Retail outlet	Raw beef	IIa
Xiv (1)	1.7	03-02-22-03-21-08	Retail outlet	Raw beef	IIa
XV (1)	1.7	00-02-20-03-16-89	Farm	Fecal	IIa
XVI (1)	1.7	00-02-22-03-21-93	Abattoir	Environmental	IVb

[a] Six loci, $n = 60$. RTE, ready-to-eat.
[b] Numbers in parentheses are the numbers of isolates that shared the same MLVA allele pattern type.
[c] MLVA allele strings are denoted in the order [LM-TR1]-[LM-TR2]-[LM-TR3]-[LM-TR4]-[LM-TR5]-[LM-TR6].
[d] Number of isolates that shared the same MLVA allele pattern by source of sample: retail outlets (chain, large, medium, and small); farms (communal, cow-calf, and feedlots operations); abattoirs (high throughput HT).
[e] Number of isolates that shared the same MLVA allele pattern type by sample type: raw beef (briskets, minced, chucks, intestine, kidney, etc.); RTE beef ("Polony," Vienna, and "Biltong"); carcass swab (preevisceration swab, postevisceration swab, chilled swab); fecal (perineal [fecal] swab, fresh fecal, pooled fecal); environmental (feed and water).
[f] Number of isolates that shared the same MLVA allele pattern type by serogroup: IIa (1/2a-3a), IIb (1/2b-3b), IIc (1/2c-3c), IVb (4b-4d-4e).

and X were from different sources (abattoirs and farms). As for sample types, all the isolates of *L. monocytogenes* in MLVA types VII, VIII, IX, XI, XII, XIII, and XIV were from the same sample type (raw beef). The isolates in MLVA types II, III, IV, and VI shared the same sample type, raw and ready-to-eat beef, whereas MLVA types I, V, and X came from carcass swab, fecal, and environmental samples. The isolates in MLVA types XV and XVI were recovered from fecal and environmental samples, respectively (Table 2).

Most of the isolates, (14, 23.3%) (LM40, LM44, LM50, LM53, LM39, LM47, LM60, LM41, LM59, LM46, LM42, LM52, LM49, and LM56) from abattoirs and farms formed the same clusters irrespective of their serogroups (Fig. 2), which suggests that most of the isolates from both sources were genetically related.

DISCUSSION

To our knowledge, this is the first report of the application of MLVA to subtyping *L. monocytogenes* in South Africa. MLVA has been used to subtype the following pathogens in South Africa: *Mycoplasma pneumonia (4), Salmonella* Enteritidis *(30),* and *Clostridium difficile (17)*. However, subtyping of *L. monocytogenes* by MLVA has previously been conducted in countries such as Ireland *(7),* Sweden *(20),* Japan *(31),* Spain *(23),* and the United States *(27)*. MLVA used in this study was able to produce quality results based on genetic relatedness among the *L. monocytogenes* isolates, irrespective of their serogroups when compared with the subtyping of *L. monocytogenes* described by Doumith et al. *(8)*. MLVA targets highly variable genomic regions, with the disadvantage that the markers may be unstable and may instantly change during passage in infected hosts, natural environments, or in the laboratory *(46)*. In our study, a total of 16 MLVA patterns were detected, a finding supported by Saleh-Lakha et al. *(38),* who detected a total of 11 MLVA patterns. The authors mentioned that the high frequency of patterns generated by MLVA would potentially lead to different MLVA patterns for epidemiologically linked isolates, thus complicating surveillance and epidemiological investigations.

Human listeriosis outbreaks are predominantly caused by *L. monocytogenes* serogroups 4b-4d-4e and 1/2b-3b-7. Sporadic cases are associated with serogroups 1/2a-3a and 1/2c-3c *(43, 47),* which were the serogroups detected in the current study. In this study, MLVA clustered isolates irrespective of their serogroups, sources, and sample types; it also differentiated samples of the same serogroup, source, and sample type, demonstrating relatedness and distinction among these isolates. These findings agree with the report of Chen et al. *(5)* that MLVA clustered related isolates together and differentiated the nonrelated isolates of various serogroups.

Recording identical MLVA types in this study, irrespective of serogroups, source, and sample types of the isolates, indicates that MLVA generally groups epidemiologically related isolates into the same MLVA type. This agrees with the findings of Miya et al. *(27),* who reported that identical MLVA patterns were found among food isolates obtained from three different plants, providing initial support that MLVA can group epidemiologically related isolates irrespective of the source and sample type.

In our study, in serogroup IVb no variation was detected in the TR numbers in LM-TR2, LM-TR4, and LM-TR6, but there was variation in LM-TR1, LM-TR3, and LM-TR5 across the source and sample type. Similar TRs were recorded in the other serogroups (IIa, IIb, IIc) (Fig. 1 and Table 1). These findings agree with those of Roberts et al. *(36)* that serogroup 1Vb isolates of *L. monocytogenes* are genetically distinct within their lineage and have several unique genetic characteristics.

Also note that nine isolates of *L. monocytogenes* (LM24, LM19, LM23, LM25, LM18, LM22, LM21, LM18, and LM26), which originated from retail outlets and belonged to the same serogroup (1/2a-3a), were in the same cluster. However, isolates of *L. monocytogenes* (LM3, LM20, LM37, LM36, and LM35) of the same serogroup were isolated from retail outlets that belonged to a different cluster (Fig. 2). This observation may be due to differences in sample type and geographical origin of the isolates (Appendix S1), presenting a unique genetic pattern among other isolates based on their origin. This is not supported by other reports *(7, 48)* that groups of similar serogroups and serotypes frequently dominate and persist in the same sources and geographical location.

In our study, we did not detect an absence TR in any of the 60 *L. monocytogenes,* as Murphy et al. *(28)* found for the LM-TR6 locus in serotype 4b. However, we found a null TR in some of LM-TR1 within the serogroups tested (Table 1). This agrees with the findings of Lindstedt et al. *(20)* who also detected null in all their loci except in the LMV9 locus, which had more than null TR in serogroup 1/2b-3b than in the other serogroups tested.

Based on the serogroups and MLVA genotypes detected in our study, there is a likelihood of potential cross-contamination of samples by *L. monocytogenes* isolates from retail outlets, farms, and abattoirs, with possible spillover to human consumers of contaminated foods. This is in line with published reports *(11, 24, 25, 35)* that cattle feces, beef, and beef products, feeds including silage, and farm environments are potential sources of *L. monocytogenes*. Note that our study detected MLVA-related isolates of *L. monocytogenes,* although in some cases belonging to different serogroups, in samples from cattle farms, abattoirs, and retail outlets.

In conclusion, our study shows that MLVA can be applied routinely to genotype *L. monocytogenes* to identify contamination sources, because it is rapid and reliable. It permits the tracking of genetically related and nonrelated *L. monocytogenes* isolates irrespective of their serogroups and sources. MLVA subtyping is an efficient tool to improve food biosecurity, monitoring, and surveillance of human and animal listeriosis. Among the serogroups detected in this study, 4b-4d-4e and 1/2b-3b-7 cause human listeriosis outbreaks and were responsible for the last outbreak in South Africa *(24)*. MLVA was able to cluster the genetically related isolates and differentiate the isolates that were not genetically related, irrespective of their serogroups, demonstrating distinctions among the isolates based on their sources and sample types. We have detected 16 *L. monocytogenes* MLVA types from animals, meat and meat product sources, sample types, and geographical origins that also belonged to different serogroups. Because there are no published data on MLVA genotypes of *L. monocytogenes* in South Africa, this study forms a basis for future molecular epidemiological studies using MLVA in the country.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/JFP-22-081.s1

REFERENCES

1. Adzitey, F., N. Huda, and G. R. R. Ali. 2013. Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. *3 Biotech*. 3:97–107. https://doi.org/10.1007/s13205-012-0074-4
2. Allam, M., N. Tau, S. I. Smouse, P. S. Mtshali, F. Mnyameni, Z. T. Khumalo, A. Ismail, N. Govender, J. Thomas, and A. M. Smith. 2018. Whole-genome sequences of *Listeria monocytogenes* sequence type 6 isolates associated with a large foodborne outbreak in South Africa, 2017 to 2018. *Genome Announc*. 6:e00538-18. https://doi.org/10.1128/genomeA.00538-18
3. Briatte, F. 2021. Ggnetwork: geometries to plot networks with "ggplot2." 2016. R package version 0.5.1. 27–29. Available at: https://github.com/briatte/ggnetwork. Accessed 5 October 2021.
4. Carrim, M., N. Wolter, A. J. Benitez, S. Tempia, M. du Plessis, S. Walaza, F. Moosa, M. H. Diaz, B. J. Wolff, F. K. Treurnicht, and O. Hellferscee. 2018. Epidemiology and molecular identification and characterization of *Mycoplasma pneumoniae,* South Africa, 2012–2015. *Emerg. Infect. Dis*. 24:506–513. https://doi.org/10.3201/eid2403.162052
5. Chen, S., J. Li, S. Saleh-Lakha, V. Allen, and J. Odumeru. 2011. Multiple-locus variable number of tandem repeat analysis (MLVA) of *Listeria monocytogenes* directly in food samples. *Int. J. Food Microbiol*. 148:8–14. https://doi.org/10.1016/j.ijfoodmicro.2011.04.014
6. Csardi, G., and T. Nepusz. 2006. The igraph software package for complex network research. *Int. J. Complex Syst*. 1695:1–9.
7. Dass, S. C., E. J. Cummins, and A. Abu-Ghannam. 2011. Prevalence and typing of *Listeria monocytogenes* strain in retail vacuum-packed cold-smoked salmon in the Republic of Ireland. *J. Food Saf*. 31:21–27. https://doi.org/10.1111/j.1745-4565.2010.00260.x
8. Doumith, M., B. Carmen, G. Philippe, J. Christine, and M. Paul. 2014. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol*. 42:3819–3822. https://doi.org/10.1128/JCM.42.8.3819-3822.2004
9. Evans, M. R., B. Swaminathan, L. M. Graves, E. Altermann, T. R. Klaenhammer, R. C. Fink, S. Kernodle, and S. Kathariou. 2004. Genetic markers unique to *Listeria monocytogenes* serotype 4b differentiate epidemic clone II (hot dog outbreak strains) from other

lineages. *Appl. Environ. Microbiol*. 70:2383–2390. https://doi.org/10.1128/AEM.70.4.2383-2390.2004

10. Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes,* a food-borne pathogen. *Microbiol. Rev.* 55:476–511. https://doi.org/10.1128/mr.55.3.476-511.1991
11. Fenlon, D. R. 1999. *Listeria monocytogenes* in the natural environment, p. 21–38. *In* E. T. Ryser and E. H. Marth (ed.), *Listeria,* listeriosis, and food safety, 2nd ed. Marcel Decker, New York.
12. Gombas, D. E., Y. Chen, R. S. Clavero, and V. N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. *J. Food Prot*. 66:559–569. https://doi.org/10.4315/0362-028X-66.4.559
13. Hyden, P., A. Pietzka, A. Lennkh, A. Murer, B. Springer, M. Blaschitz, A. Indra, S. Huhulescu, F. Allerberger, W. Ruppitsch, and C. W. Sensen. 2016. Whole genome sequence-based serogrouping of *Listeria monocytogenes* isolates. *J. Biotechnol*. 235:181–186. https://doi.org/10.1016/j.jbiotec.2016.06.005
14. Jacbos, M. R., H. Stein, A. Buqwane, A. Dubb, F. Segal, L. Rabinowitz, U. Ellis, I. Freiman, M. Witcomb, and V. Vallabh. 1978. Epidemic listeriosis report of 14 cases detected in 9 months. *S. Afr. Med. J*. 10:389–392.
15. Jamali, H., B. Radmehr, and K. L. Thong. 2013. Prevalence, characterization, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk tanks. *Food Control* 34:121–125. https://doi.org/10.1016/j.foodcont.2013.04.023
16. Keim, P., L. B. Price, A. M. Klevytska, K. L. Smith, J. M. Schupp, R. Okinaka, P. J. Jackson, and M. E. Hugh-Jones. 2000. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J. Bacteriol*. 182:2928–2936. https://doi.org/10.1128/JB.182.10.2928-2936.2000
17. Kullin, B., J. Wojno, V. Abratt, and S. J. Reid. 2017. Toxin A-negative B-positive ribotype 017 *Clostridium difficile* is the dominant strain type in patients with diarrhoea attending tuberculosis hospitals in Cape Town, South Africa. *Eur. J. Clin. Microbiol. Infect. Dis*. 36:163–175. https://doi.org/10.1007/s10096-016-2790-x
18. Laksanalamai, P., B. Huang, J. Sabo, L. S. Burall, S. Zhao, J. Bates, and A. R. Datta. 2014. Genomic characterization of novel *Listeria monocytogenes* serotype 4b variant strains. *PLoS One* 9(2):e89024. https://doi.org/10.1371/journal.pone.0089024
19. Lappi, V. R., J. Thimothe, K. K. Nightingale, K. M. Gall, V. N. Scott, and M. Wiedmann, M. 2004. Longitudinal studies on *Listeria* in smoked fish plants: impact of intervention strategies on contamination patterns. *J. Food Prot*. 67:2500–2514. https://doi.org/10.4315/0362-028X-67.11.2500
20. Lindstedt, B. A., W. Tham, M. L. Danielsson-Tham, T. Vardund, S. Helmersson, and G. Kapperud. 2008. Multiple-locus variable-number tandem-repeats analysis of *Listeria monocytogenes* using multicolour capillary electrophoresis and comparison with pulsed-field gel electrophoresis typing. *J. Microbiol. Methods* 72:141–148. https://doi.org/10.1016/j.mimet.2007.11.012
21. Madoroba, E., A. K. Gelaw, and D. Kapeta. 2016. *Salmonella* contamination, serovars, and antimicrobial resistance profiles of cattle slaughtered in South Africa. *Onderstepoort J. Vet. Res*. 83:1–8. https://hdl.handle.net/10520/EJC189737
22. Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, K. Hornik, M. Studer, P. Roudier, J. Gonzalez, K. Kozlowski, and E. Schubert. 2019. Finding groups in data: cluster analysis extended Rousseeuw et al. R package, version 2. Wiley-Interscience, Hoboken, NJ.
23. Martín, B., S. Bover-Cid, and T. Aymerich. 2018. MLVA subtyping of *Listeria monocytogene*s isolates from meat products and meat processing plants. *Food Res. Int*. 106:225–232. https://doi.org/10.1016/j.foodres.2017.12.052
24. Matle, I., K. R. Mbatha, O. Lentsoane, K. Magwedere, L. Morey, and E. Madoroba. 2019. Occurrence, serotypes, and characteristics of *Listeria monocytogenes* in meat and meat products in South Africa between 2014 and 2016. *J. Food Saf.* 39:e12629. https://doi.org/10.1111/jfs.12629
25. Mena, C., G. Almeida, L. Carneiro, P. Teixeira, T. Hogg, and P. A. Gibbs. 2004. Incidence of *Listeria monocytogenes* in different food products commercialized in Portugal. *Food Microbiol*. 21:213–216. https://doi.org/10.1016/S0740-0020(03)00057-1
26. Miettinen, H., and G. Wirtanen. 2006. Ecology of *Listeria* spp. in a fish farm and molecular typing of *Listeria monocytogenes* from fish farming and processing companies. *Int. J. Food Microbiol*. 112:138–146. https://doi.org/10.1016/j.ijfoodmicro.2006.06.016
27. Miya, S., B. Kimura, M. Sato, H. Takahashi, T. Ishikawa, T. Suda, C. Takakura, T. Fujii, and M. Wiedmann, 2008. Development of a multilocus variable-number of tandem repeats typing method for *Listeria monocytogenes* serotype 4b strains. *Int. J. Food Microbiol*. 124:239–249. https://doi.org/10.1016/j.ijfoodmicro.2008.03.023
28. Murphy, M., D. Corcoran, J. F. Buckley, M. O'Mahony, P. Whyte, and S. Fanning. 2007. Development and application of a multiple-locus variable number of tandem repeat analysis (MLVA) to subtype a collection of *Listeria monocytogenes*. *Int. J. Food Microbiol*. 115:187–194. https://doi.org/10.1016/j.ijfoodmicro.2006.10.022
29. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed *bacillus Bacterium monocytogenes* (n. sp.). *J. Pathol. Bacteriol*. 29:407–439. https://doi.org/10.1002/path.1700290409
30. Muvhali, M., A. M. Smith, A. M. Rakgantso, and K. H. Keddy. 2017. Investigation of *Salmonella* Enteritidis outbreaks in South Africa using multi-locus variable-number tandem-repeats analysis, 2013–2015. *BMC Infect. Dis*. 17:1–9. https://doi.org/10.1186/s12879-017-2751-8
31. Nayak, D. N., C. V. Savalia, I. H. Kalyani, R. Kumar, and D. P. Kshirsagar. 2015. Isolation, identification, and characterization of *Listeria* spp. from various animal origin foods. *Vet. World* 8:695–701. https://doi.org/10.14202/vetworld.2015.695-701
32. Noller, A. C., M. C. McEllistrem, A. G. Pacheco, D. J. Boxrud, and L. H. Harrison. 2003. Multilocus variable-number tandem repeat analysis distinguishes outbreak and sporadic *Escherichia coli* O157:H7 isolates. *J. Clin. Microbiol*. 12:5389–5397. https://doi.org/10.1128/JCM.41.12.5389-5397.2003
33. Paradis, E., and K. Schliep. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–528. https://doi.org/10.1093/bioinformatics /bty633
34. PulseNet. 2020. Molecular typing for MLVA. Available at: https://pulsenetinternational.org/protocols/mlva/. Accessed 5 October 2021.
35. Raschle, S., R. Stephan, M. J. Stevens, N. Cernela, K. Zurfluh, F. Muchaamba, and M. Nüesch-Inderbinen. 2021. Environmental dissemination of pathogenic *Listeria monocytogenes* inflowing surface waters in Switzerland. *Sci. Rep*. 11:1–11.
36. Roberts, A., K. Nightingale, G. Jeffers, E. Fortes, J. M. Kongo, and M. Wiedmann. 2006. Genetic and phenotypic characterization of *Listeria monocytogenes* lineage III. *Microbiology* 152:685–693. https://doi.org/10.1099/mic.0.28503-0
37. Sabat, A. J., A. Budimir, D. Nashev, R. Sá-Leão, J. M. Van Dijl, F. Laurent, and ESCMID Study Group of Epidemiological Markers (ESGEM). 2013. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Euro Surveill*. 18:20380.
38. Saleh-Lakha, S., V. G. Allen, J. Li, F. Pagotto, J. Odumeru, E. Taboada, M. Lombos, K. C. Tabing, B. Blais, D. Ogunremi, and G. Downing. 2013. Subtyping of a large collection of historical *Listeria monocytogenes* strains from Ontario, Canada, by an improved multilocus variable-number tandem-repeat analysis (MLVA). *Appl. Environ. Microbiol*. 79:6472–6480. https://doi.org/10.1128/AEM.00759-13
39. Schwartz, D. C., and C. R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed-field gradient gel electrophoresis. *Cell* 37:67–75. https://doi.org/10.1016/0092-8674(84)90301-5
40. Soumet, C., G. Ermel, P. Fach, and P. Colin. 1994. Evaluation of different DNA extraction procedures for the detection of *Salmonella* from chicken products by polymerase chain reaction. *Lett. Appl. Microbiol*. 19:294–298. https://doi.org/10.1111/j.1472-765X.1994.tb00458.x
41. Sperry, K. E. V., S. Kathariou, J. S. Edwards, and L. A. Wolf. 2008. Multiple-locus variable-number tandem-repeat analysis as a tool for

subtyping *Listeria monocytogenes* strains. *J. Clin. Microbiol*. 46:1435–1450. https://doi.org/10.1128/JCM.02207-07

42. Team R Core. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at: http://www.R-project.org.
43. Todd, E. C. D., and S. Notermans. 2011. Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. *Food Control* 22:1484–1490. https://doi.org/10.1016/j.foodcont.2010.07.021
44. Trindade, P. A., J. A. McCulloch, G. A. Oliveira, and E. M. Mamizuka. 2003. Molecular techniques for MRSA typing: current issues and perspectives. *Braz. J. Infect. Dis*. 7:32–43.
45. Urwin, R., and M. C. Maiden. 2003. Multi-locus sequence typing: a tool for global epidemiology. *Trends Microbiol*. 11:479–487. https://doi.org/10.1016/j.tim.2003 .08.006
46. Van Belkum, A., S. Scherer, L. Van Alphen, and H. Verbrugh. 1998. Short-sequence DNA repeats in prokaryotic genomes. *Microbiol. Mol. Biol. Rev.* 62:275–293. https://doi.org/10.1128/MMBR.62.2.275-293.1998
47. Vitas, A. I. 2004. Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *Int. J. Food Microbiol*. 90:349–356. https://doi.org/10.1016/S0168-1605(03)00314-3
48. Wulff, G., L. Gram, P. Ahrens, and B. F. Vogel. 2006. One group of genetically similar *Listeria monocytogenes* strains frequently dominates and persists in several fish slaughter-and smokehouses. *Appl. Environ. Microbiol*. 72:4313–4322. https://doi.org/10.1128/AEM.02288-05
49. Yu, G., D. K. Smith, H. Zhu, Y. Guan, and T. T. Y. Lam. 2017. gtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol. Evol*. 8:28–36. https://doi.org/10.1111/2041-210X.12628
50. Zhang, Y., E. Yeh, G. Hall, J. Cripe, A. A. Bhagwat, and J. Meng. 2007. Characterization of *Listeria monocytogenes* isolated from retail foods. *Int. J. Food Microbiol*. 113:47–53. https://doi.org/10.1016/j.ijfoodmicro.2006.07.010