Abstract: The genus Listeria consists of a closely related group of Gram-positive bacteria that commonly occur in the environment and demonstrate varied pathogenic potential. Of the 10 species identified to date, L. monocytogenes is a facultative intracellular pathogen of both humans and animals, L. ivanovii mainly infects ungulates (e.g., sheep and cattle), while other species (L. innocua, L. seeligeri, L. welshimeri, L. grayi, L. marthii, L. rocourtiae, L. fleischmannii and L. weihenstephanensis) are essentially saprophyes. Within the species of L. monocytogenes, several serovars (e.g., 4b, 1/2a, 1/2b and 1/2c) are highly pathogenic and account for a majority of clinical isolations. Due to their close morphological, biological, biochemical and genetic similarities, laboratory identification of pathogenic and nonpathogenic Listeria organisms is technically challenging. With the development and application of various molecular approaches, accurate and rapid discrimination of pathogenic and nonpathogenic Listeria organisms, as well as pathogenic and nonpathogenic L. monocytogenes strains, has become possible.

Keywords: Listeria, identification, pathogenic, nonpathogenic, lineage, serovar, epidemic clone
Introduction

Listeria was first described by E.G.D. Murray in 1926 in Cambridge, England, who referred to the causative agent for monocytosis in laboratory rodents as Bacterium monocytogenes. In 1927, a bacterium causing mortality in gerbils was identified in Johannesburg, South Africa, and named Listerella hepatolytica in honor of Joseph Lister, a surgeon who pioneered antiseptic surgery. With the realization that Bacterium monocytogenes and Listerella hepatolytica were in fact the identical bacterium and that the name Listerella had been already taken for a slime mold and a protozoan, the organism was renamed Listeria monocytogenes in 1940. In addition to L. monocytogenes, other species (L. ivanovii, L. innocua, L. seeligeri, L. welshimeri, L. grayi, L. marthii, L. rocourtiae, L. fleischmannii and L. weihenstephanensis) have since been identified within the genus.

Although L. monocytogenes was implicated in human disease from the late 1920s, it was not until 1979 that the link of this bacterium to serious foodborne listeriosis in humans was established. In immunocompetent individuals, L. monocytogenes tends to cause gastrointestinal symptoms that are transient in nature and often disappear within a short period. In the immunocompromised individuals such as pregnant women, neonates, and the elderly, L. monocytogenes infection may lead to severe clinical diseases, with abortion and death being usual outcomes.

Considering their close morphological and biological similarities and their varied pathogenicity, it is important that pathogenic and nonpathogenic Listeria species/L. monocytogenes serovars/strains are correctly identified. Over the years, a number of phenotypic procedures have been developed and used for identification and differentiation of Listeria organisms. However, given their variable performance and slow turnover, phenotypic tests for Listeria diagnosis have been largely superseded by molecular approaches. The purpose of this article is to provide an update on the utility of molecular techniques for the improved determination of pathogenic and nonpathogenic listeriae.

Listeria Classification

The genus Listeria covers a group of Gram-positive, non-spore-forming, rod-shaped bacteria of 0.4–0.5 µm × 1–1.5 µm in size and between 36–39% in G + C content. Taxonomically, the genus Listeria is classified in the family Listeriaceae, order Bacillales, class Bacilli, phylum Firmicutes, domain Bacteria, kingdom Prokaryotae. Apart from Listeria, the only other genus in the Listeriaceae family is Brochothrix. To date, 10 species are recognized within the genus: L. monocytogenes, L. ivanovii (previously known as L. monocytogenes serotype 5), L. seeligeri, L. innocua, L. welshimeri, L. grayi, L. marthii, L. rocourtiae, L. fleischmannii and L. weihenstephanensis. Of these, L. monocytogenes is a facultative intracellular pathogen of both humans and animals, L. ivanovii primarily infects ungulates (eg, sheep and cattle), and the other 8 species are free-living saprophytes. Nonetheless, non-monocytogenes Listeria species including L. ivanovii, L. innocua, L. welshimeri, and L. grayi have been occasionally implicated in human clinical cases, mainly in individuals with suppressed immune functions and/or underlying illnesses. Since L. monocytogenes strains display notable variations in virulence, attempts have been made to develop and use laboratory procedures to differentiate pathogenic from nonpathogenic strains, and to monitor the strains involved in the listeriosis outbreaks. Serotyping on the basis of immunological reactions between listerial somatic (O)/flagellar (H) antigens and specific antibodies represents an early approach to identifying and tracking Listeria bacteria. Using this approach, Listeria is separated into at least 16 serovars, including 13 for L. monocytogenes (serovars 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7), 1 for L. ivanovii (serovar 5), 3 for L. innocua (serovars 1/2b, 6a and 6b), 3 for L. welshimeri (serovars 1/2b, 6a and 6b), 6 for L. seeligeri (serovars 1/2a, 1/2b, 3b, 4a, 4b, 4c and 6b), and 1 for L. grayi (serovar Grayi). The determination of L. monocytogenes serovars has clinical implications, as serovar 4b strains have been shown to cause endemic human listeriosis, and serovars 1/2a, 1/2b and 1/2c are responsible for sporadic listeriosis in humans. Indeed, according to a French study conducted in 2006, L. monocytogenes serovars 4b, 1/2a, 1/2b and 1/2c account for over 98% isolations from clinical cases of human listeriosis, with serovar 4b alone causing 49% of Listeria-related endemic foodborne diseases (Table 1). Similarly, in experimental mouse models, L. monocytogenes serovars 4b, 1/2a, 1/2b and 1/2c show a heightened infectivity through intragastric inoculation. However,
Table 1. *L. monocytogenes* serovars causing human listeriosis.*

| Serotype | No. of isolates (%) | Tendency to cause |
|----------|---------------------|-------------------|
| 4b       | 294/603 (49%)       | CNS infections > M/N diseases > Bacteremia |
| 1/2a     | 163/603 (27%)       | Bacteremia > M/N diseases > CNS infections |
| 1/2b     | 120/603 (20%)       | M/N diseases > Bacteraemia > CNS infections |
| 1/2c     | 22/603 (4%)         | Bacteremia > CNS infections > M/N diseases |
| 3a/3b    | 4/603 (<1%)         | Bacteremia |

*Adapted from Goulet et al., which was based on the analysis of 603 *L. monocytogenes* isolates from 603 French patients during 2001–2003.

**Abbreviations:** M/N diseases, maternal-neonatal diseases; CNS infections, central nerve system infections.

all *L. monocytogenes* serovars except 4a are capable of inducing mouse mortality via intraperitoneal route.36–39 In light of the extensive antigenic sharing among *Listeria* serovars (e.g., serovars 1/2a and 3a both contain H antigens A and B; serovars 1/2c and 3c both possess H antigens B and D; serovars 1/2b, 3b, 4a, 4b, 4c, 4d, 5, 6a, 6b and 7 all have H antigens A, B, and C; serovars 1/2a, 1/2b, 1/2c, 3a, 3b and 3c all share O antigen II; serovars 4a, 4ab, 4b, 4c, 4d, 4e, 5, 6a and 6b all have O antigen V), serotyping lacks desired specificity.40,41 As a consequence, genotyping techniques have been developed to improve the identification and epidemiological tracking of *Listeria* bacteria.42 This has facilitated the separation of *L. monocytogenes* bacteria into 4 genetic lineages (I–IV) (Table 2).43–50 While lineage I encompasses serovars 1/2b, 3b, 4b, 4d and 4e; lineage II covers serovars 1/2a, 1/2c, 3a and 3c; lineage III includes serovars 4a and 4c. In addition, lineage III has been further distinguished into subgroup IIIA (containing typical rhamnose-positive avirulent serovar 4a and virulent serovar 4c strains), subgroup IIIC (consisting of atypical rhamnose-negative virulent serovar 4c strains), and subgroup IIIB (which is now known as lineage IV) (covering atypical rhamnose-negative, virulent non-serovar 4a and non-serovar 4c, as well as serovar 7 strains).51

**Genus-Specific Identification**

Being small, Gram-positive rods, listeriae resemble other Gram-positive bacteria such as streptococci and corynebacteria morphologically. To differentiate the genus *Listeria* from other bacterial genera, a batch of biochemical tests has been traditionally employed.32,52,53 Recent application of molecular techniques has simplified the genus-specific identification

Table 2. Characteristics of *L. monocytogenes* lineages I–IV.*

| Lineage | Serovar | Rhamnose activity | PCR reactivity |
|---------|--------|------------------|---------------|
| I       | 1/2b   | + + + + + + + + | inlA lmo0733 lmo2672 inlJ inlC** lmo1134 ORF2819 ORF2110 lmo0737 lmo1118 |
|         | 3b     | + + + + + + + + |               |
|         | 4b     | + + + + + + + + |               |
|         | 4d     | + + + + + + + + |               |
|         | 4e     | + + + + + + + |               |
| II      | 1/2a   | + + + + + + + + |               |
|         | 1/2c   | + + + + + + + + |               |
|         | 3a     | + + + + + + + + |               |
|         | 3c     | + + + + + + + + |               |
| IIIA    | 4a     | + + + + + + + + |               |
|         | 4c     | + + + + + + + + |               |
| IIIC    | 4c     | + + + + + + + + |               |
| IV (IIIB) | 7 and unusual | - + + + + | inlA lmo0733 lmo2672 inlJ inlC** lmo1134 ORF2819 ORF2110 lmo0737 lmo1118 |
|         | 4a,4b,4c | - + + + + |               |

*Summarized from Liu et al.; Doumith et al.; Roberts et al.

**inlC** is also found in some *L. ivanovii* strains.
of listeriae, with the following gene targets being commonly exploited:

(i) the house-keeping genes prs and ldh flanking the prs-prfA-plcA-hly-mpl-actA-plcB-ortX-ortF-ortB-ortA-ldh cluster, which consists of the well known 9.6 kb PrfA-regulated virulence gene cluster (or Listeria pathogenicity island 1, LIPI-1). While the ldh gene codes for lactate dehydrogenase ( ~ 310 amino acids), the prs gene encodes phosphoribosyl pyrophosphate synthetase (318 amino acids). Additionally, the underlying gene encoding VclB (Lmo0209/Lin0289), a conserved protein of unknown function, is also found in all Listeria species and can be used for Listeria determination.

(ii) the 23S rRNA-16S rRNA locus (consisting of about 1500 and 2500 bp, respectively), which is a highly conserved gene region encoding ribosomal RNA molecules (rRNA). The key functions of rRNA are to decode messenger RNA (mRNA) into amino acids and to interact with the transfer RNA (tRNA) during translation by providing petidyltransferase activity. Because of its conserved nature, the 23S rRNA-16S rRNA locus offers a valuable target for phylogenetic analysis. Paillard et al. employed primers S2F and S2R to generate an 890 bp fragment from the 5’ end of Listeria rRNA 23S gene. Subsequent digestion of this fragment with restriction enzyme XmnI enabled distinction of L. monocytogenes, L. ivanovii and L. seeligeri (forming 770 and 120 bp bands) from L. innocua, L. welshimeri and L. grayi (forming 650, 120 and 120 bp bands). Moreover, digestion of the 890 bp fragment with restriction enzyme CfoI facilitated differentiation of L. ivanovii, L. seeligeri and L. grayi (forming 600, 170 and 120 bp bands) from L. monocytogenes, L. innocua and L. welshimeri (forming 470, 170, 130 and 120 bp bands).

(iii) the iap gene. This gene encodes the “invasion-associated protein” (IAP, also known as P60 reflecting its molecular size of 60 kDa), which is involved in host cell invasion by pathogenic listeriae and acts in all Listeria species as a murein hydrolyase necessary for proper cell division. The iap gene has been successfully incorporated in PCR for Listeria genus specific detection.

An added benefit of incorporating a genus-specific primer set in a PCR assay for listerial identification lies in the fact that it also functions as an internal control for the assay.

Species-Specific Identification

Correct identification of Listeria species is critical for effective control and prevention of listeriosis. Previously, phenotype-based methods (such as biochemical and serological tests) have been employed for the specification of Listeria bacteria. In view of their superior sensitivity and specificity over the phenotypic methods, molecular techniques have been widely adopted in clinical and research laboratories for discrimination between pathogenic and nonpathogenic Listeria organisms. Evolving from non-amplified procedures (eg., DNA hybridization), molecular detection of Listeria bacteria has increasingly moved towards nucleic acid amplification and real time detection. The identification of a range of gene targets has further enhanced the appeal and versatility of molecular procedures for Listeria species-specific determination. While several shared genes such as 16S and 23S rRNA genes, their intergenic spacer regions, ssrA gene (which encodes a transfer-messenger RNA or tmRNA), and iap (which encodes invasion associated protein) have proven valuable for identification of all Listeria species, many Listeria species-specific genes have been described. For instance, the following genes targets may be used for specific determination of L. monocytogenes: hly, plcA, plcB, actA, inlA, inlB, lmaA/lmaB, flaA, pepC, clpE, fbp, lmo0733, and lmo2234. Similarly, liv22–228 and smcE have been specifically targeted for L. ivanovii, lse24–315 for L. seeligeri, lin0464 for L. welshimeri, and lgr20–246 for L. grayi.

Lineage Delineation

As L. monocytogenes lineages I (particularly serovars 1/2b and 4b) and II (especially serovars 1/2a and 1/2e) strains are commonly associated with human clinical cases, it is important that they are accurately identified and subtyped. Similar to species-specific identification, 2 major approaches are used for L. monocytogenes lineage delineation and subtyping: phenotypic and genetic. The phenotypic subtyping approach utilizes serotyping,
phage typing, multilocus enzyme electrophoresis (MLEE) and esterase typing techniques. The genetic subtyping approach ranges from pulsed-field gel electrophoresis (PFGE), ribotyping, PCR-based subtyping [e.g., random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), PCR-restriction fragment length polymorphism (PCR-RFLP), repetitive element PCR (REP-PCR)], to DNA sequencing-based subtyping techniques [such as multilocus sequence typing (MLST)]. With its high sensitivity, discriminatory power and reproducibility, the genetic subtyping approach offers a method of choice for the laboratory determination of L. monocytogenes lineages and subtypes. In particular, a combination of 2 or more subtyping techniques helps clarify the ambiguity that can be encountered when a single typing method is used.

Due to their sequence divergences among Listeria serovars, the actA and plcB genes have been often targeted for the determination of L. monocytogenes lineages and genotypes. Analyses of 2 housekeeping genes (ribC and purM) together with 2 virulence genes (actA and inlA) uncovered evidence of a more prevalent recombination in lineage II than in lineage I. Moreover, comparisons of the actA gene sequences of L. seeligeri isolates from different habitats permitted discrimination of 2 different actA subtypes forming 2 phylogenetic lineages.

Another important group of gene targets for Listeria lineage determination is internalin genes. Through sequencing analysis of the ascB-dapE internalin cluster, Chen et al. showed that L. monocytogenes lineage II can be distinguished into 3 distinct sublineages, IIA, IIB, and IIC, with inlGHE, inlGC2DE, and inlC2DE for IIA, IIB, and IIC, respectively. While IIA and IIC displayed a higher frequency of recombination, IIB was more notably affected, leading to high nucleotide diversity. Furthermore, internalin profiling of 13 L. monocytogenes lineage III strains identified 10 internalin types that are clustered in 4 subpopulations IIIA–1, IIIA–2, HIB, and IIC. Whereas lineage IIIA–2 strains had reduced pathogenicity, the other lineage III strains had comparable virulence to lineages I and II. Because of its phylogenetical distinction from other sub-populations, HIB may represent a novel lineage. Similarly, examination of internalin genes of L. innocua resulted in the identification of 4 subgroups within the species.

### Group-Specific Identification

Given the predominance of L. monocytogenes serovars 4b, 1/2a, 1/2c and 1/2b in human clinical isolations, the availability of methods to determine the serotype of a particular strain is vital for its epidemiological tracking and therapeutic monitoring (Table 3). Although conventional serotyping methods have played a valuable role in the tracking of L. monocytogenes isolates involved in listeriosis, they are sometimes unable to correlate serovars directly with species identities, and are expensive to set up and maintain.

### Table 3. L. monocytogenes group-specific gene targets.

| Gene | Specificity | Reference |
|------|-------------|-----------|
| inlJ (IMO2821) | All L. monocytogenes serovars but 4a | Liu et al.36-39 |
| lmo2470 | All L. monocytogenes serovars but 4a and some 4c | Liu et al.36 |
| inC | All L. monocytogenes serovars but 4a and some 4c | Liu et al.36 |
| lmo2672 | All L. monocytogenes serovars but 4a and some 4c | Liu et al.36,37 |
| lmo1134 | All L. monocytogenes serovars but 4a and 4c | Liu et al.36 |
| lmaA | All L. monocytogenes serovars but 4a and 4c | Schafkerkordt and Chakraborty79 |
| lmaB | All L. monocytogenes serovars but 4a and 4c | Schafkerkordt and Chakraborty79 |
| lmo0038 | All L. monocytogenes serovars but 4a and 4c | Chen et al.100 |
| ORF2619 | L. monocytogenes serovars 1b, 2b, 3b, 4b, 4d, 4e and 7 | Doumith et al.54 |
| ORF2110 | L. monocytogenes serovars 4b, 4d and 4e | Doumith et al.54 |
| ORF2372 | L. monocytogenes serovars 4b, 4d and 4e | Zhang and Knabel135 |
| lmo0737 | L. monocytogenes serovars 1b, 2b, 1b, 2b, 3a and 3c | Doumith et al.54 |
| lmo0171 | L. monocytogenes serovars 1b, 2b, 1b, 2b, 3a and 3c | Zhang and Knabel135 |
| lmo1118 | L. monocytogenes serovars 1b, 2b and 3b | Doumith et al.54 |
| Gene region flanking glia-gltB | L. monocytogenes serovars 1b and 3a | Borucki and Call193 |
| flaA | L. monocytogenes serovars 1b and 3a | Borucki and Call193 |
these obvious shortcomings, molecular techniques provide a precise and low-cost alternative for determination of *L. monocytogenes* serovars/groups.\textsuperscript{130,131}

Jinneman and Hill\textsuperscript{132} reported a mismatch amplification mutation fixation assay (MAMA) targeting a 446-bp region within the *hly* gene for rapid screening and characterization of *L. monocytogenes* lineage types I–III. Borucki and Call\textsuperscript{133} utilized primers from an iron transport protein gene, GLT primers (from a 1/2b serotype-specific region flanking the *gltA-gltB* cassette), the MAMA-C PCR primers,\textsuperscript{132} and primers from the *flaA* gene (encoding the *L. monocytogenes* flagellin protein) to identify *L. monocytogenes* serotypes. Doumith et al\textsuperscript{134,135} developed a multiplex PCR that incorporates *L. monocytogenes* *lmo0737* gene primers for recognition of serovars 1/2a, 1/2c, 3a, and 3c; *lmo1118* gene primers for detection of serovars 1/2c and 3c; ORF2819 primers for serovars 1/2b, 3b, 4b, 4d, and 4e; ORF2110 primers for serovars 4b, 4d, and 4e; and *prs* primers as an internal amplification control covering all *L. monocytogenes* serovars. Zhang and Knabel\textsuperscript{136} described a multiplex PCR assay for rapid identification and easily interpretable differentiation of serovars 1/2a and 4b from other serovars of *L. monocytogenes* by simultaneously targeting 2 virulence genes (*inlB* and *inlC*) and 2 serovar-specific genes (ORF2372 and *lmo0171*). Nightingale et al\textsuperscript{137} combined a multiplex PCR with sigB allelic typing to classify the 4 major serovars (i.e., 1/2a, 1/2b, 1/2c, and 4b) into unique genetic subgroups, and to differentiate lineage I serovar 4b isolates from the genetically distinct lineage III serovar 4b isolates. More recently, Kérouanton et al\textsuperscript{137} designed 2 multiplex PCR assays to cluster *L. monocytogenes* strains into 5 molecular serogroups: IIa, IIb, IIc, IVa, and IVb. The first multiplex PCR recognizes *L. monocytogenes* serotypes 1/2a, 1/2c, 1/2b and 4b, together with the *prfA* gene primers for *L. monocytogenes* species confirmation. The second multiplex PCR incorporating the *flaA* gene primers (specific for 1/2a and 3a strains) and *prs* gene primers (specific for *Listeria* genus) resolves a small number of IIa and IIc molecular serogroup strains (consisting of serotypes atypical 1/2a, 3a and 1/2c strains) that give equivocal results in the first multiplex PCR, leading to a total agreement between molecular and conventional serotyping methods.

In addition, by using primers from *inlA* for species-specific recognition, and those from *inlJ* (or *lmo2821*) and *inlC* for virulence determination in a multiplex PCR, *L. monocytogenes* naturally avirulent serovar 4a strains were rapidly differentiated from other serovars that have the potential to cause mouse mortality via the intraperitoneal route.\textsuperscript{37}

**Identification of Epidemic Clones**

Although a variety of *L. monocytogenes* strains have been isolated from environments and foodstuffs, only a limited number of virulent strains are known to cause listeriosis epidemics, particularly of those belonging to serovars 4b, 1/2a, and 1/2b.\textsuperscript{138–141} The term “epidemic clone” refers to a group of genetically related isolates of a common ancestor that are implicated in geographically and temporally unrelated outbreaks.\textsuperscript{142} To date, 5 epidemic clones (ECs) of *L. monocytogenes* (ECI, ECII, ECIII, ECIV, and ECV) have been defined (Table 4).\textsuperscript{60,142–146}

Identification and tracking of *L. monocytogenes* epidemic clones are critical to understanding the long-term transmission of *L. monocytogenes* and to establishing efficient surveillance systems for this pathogen.\textsuperscript{147–149} The methods for the identification of *L. monocytogenes* epidemic clones have evolved over the years from the phenotypic (e.g., serotyping and phage typing) to genotypic methods.\textsuperscript{150–153} The latter include the fragment-based typing methods, which range from (i) restriction digestion-based methods such as ribotyping (RT) and pulsed-field gel electrophoresis (PFGE) and (ii) PCR-based methods such as randomly amplified polymorphic DNA and repetitive sequence-based PCR to (iii) combined amplification-restriction methods such as amplified fragment length polymorphism (at endonuclease restriction or primer annealing sites) and PCR-restriction fragment length polymorphism. This was followed by DNA sequence-based methods such as multilocus sequence typing (MLST) that combines PCR and automated DNA sequencing to analyze slowly diversified house-keeping gene sequences.\textsuperscript{154} More recently, multi-virulence-locus sequence typing (MVLST, targeting virulence genes *prfA, inlB, inlC, dal, clpP*, and *lisR*) was developed to overcome the limited discriminatory power associated with MLST, allowing categorization of *L. monocytogenes* isolates into higher-level groups, such as evolutionary lineages, clonal complexes, and epidemic clones.\textsuperscript{90,155} Indeed, Knabel et al\textsuperscript{145} employed multilocus sequence typing (MLST) and multi-virulence-locus sequence typing (MVLST)
Molecular identification of pathogenic and nonpathogenic listeriae

Table 4. *Listeria monocytogenes* epidemic clones.

| Epidemic clone (EC) | Serovar | Ribotype | MVLST ST (VT)* | Molecular marker | Outbreak involved | Reference |
|---------------------|---------|----------|----------------|------------------|------------------|-----------|
| ECI                 | 4b      | DUP-1038B 20 | LMOF2365_2798 (AATAGAAATAAGCGGAAGTGT/TATTTTCCTGTCGCTTAG) 303 bp | Nova Scotia, 1981; California, 1985; Switzerland, 1983–1987; Denmark, 1985–1987; France, 1992 | Chen and Knabel⁶⁰; Yildirim et al¹⁵¹ |
| ECII                | 4b      | DUP-1044A 19 | LMOh7858_0487.8 to inlA (ATTATGCAAGTGGTACGGA/ATCTGTTTGGCAGACCGTGTC) 889 bp | USA, 1998–1999; USA, 2002 | Chen and Knabel⁶⁰; Evans et al¹³⁸ |
| ECIII               | 1/2a    | DUP-1053A 1 | LMOF6854_2463.4 (TTGCTAATTCTGATGCGTTGG/GCGCTAGGGAATAGTAAAGG) 497 bp | USA, 2000 | Chen and Knabel⁶⁰ |
| ECIV (formerly ECla) | 4b      | DUP-1042B 21 | Reactive with 4b-specific primers (ORF2110), but not with LMOF2365_2798 and LMOh7858_0487primers | Boston, 1979, 1983; UK, 1989 | Chen and Knabel⁶⁰ |
| ECV                 | 1/2a    | 59 | LM5578_2229 (TGTTGAAGGAAGGTGGTG/TCCTTTGCCGCTATTTCGT) 191 bp | Canada, 1988–2000 | Knabel et al¹⁴⁵ |
|                     |         |          | LM5578_2228_30 (CTGTTGTCCTCCTTTGTT/AGCACAGGTTGCTTTGGAC) 982 bp | | |

*Multi-virulence-locus sequence typing (MVLST) sequence types (Virulence Types, VTs) were assigned according to Chen et al.⁶⁰

Sources: Chen Y, Zhang W, and Knabel SJ. *J Clin Microbiol*. 2007;45:835. Knabel SJ, et al. *J Clin Microbiol*. 2012;50:1748.

To identify a predominant clone (clonal complex 8; virulence type 59; proposed epidemic clone 5 [ECV]) belonging to serotype 1/2a that has caused human illness across Canada for more than 2 decades.

To further streamline the identification of *L. monocytogenes* epidemic clones, Chen and Knabel⁶⁰ developed a multiplex PCR assay that facilitated simultaneous detection of *Listeria* genus, *L. monocytogenes* serovar 1/2a and 4b, and *L. monocytogenes* epidemic clones I, II, and III. This multiplex PCR assay offers a powerful tool to screen and subgroup *L. monocytogenes* cultures and significantly reduces the number of isolates that need to be subtyped by more expensive and discriminatory molecular methods, such as PFGE and sequence-based typing.

**Conclusion**

The genus *Listeria* contains 10 closely related Gram-positive bacterial species with ubiquitous distribution. Although a majority of *Listeria* species are nonpathogenic, *L. monocytogenes* is a well known pathogen of both humans and animals, and *L. ivanovii* causes severe diseases in ungulates. For the epidemiological tracking and control of listeriosis outbreaks, it is important to distinguish between pathogenic and nonpathogenic *Listeria* species, as well as between pathogenic and nonpathogenic *L. monocytogenes* strains. While traditional phenotypic methods have contributed to the identification and detection of *Listeria* organisms in the past, they are largely overtaken by new generation molecular techniques that demonstrate superior sensitivity, specificity and speed. It is envisaged that continuing innovations such as microarrays, biosensors, and next generation sequencing will offer promise to further improve the sensitivity, rapidity and specificity of laboratory characterization of *Listeria* genus, species, lineages, serovars and epidemic clones.

**Author Contributions**

Conceived and designed the experiments: DL. Analyzed the data: DL. Wrote the first draft of the manuscript: DL. Contributed to the writing of the manuscript: DL. Agree with manuscript results and conclusions: DL. Jointly developed the structure and arguments for the paper: DL. Made critical revisions and approved final version: DL. All authors reviewed and approved of the final manuscript.
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References
1. McCarthy, SA. Listeria in the Environment. In: Foodborne Listeriosis. Miller AJ, Smith AK, Somkuti GA (Eds). 1990; Elsevier: New York; 25–29.
2. Vazquez-Boland JA, Kahn M, Berche P, et al. Listeria pathogenesis and molecular virulence determinants. Clin Microbiol Rev. 2001;14:584–640.
3. Doganay M. Listeriosis: Clinical presentation. Clin Microbiol Rev. 1995;8(2):230–231.
4. Liu D. Identification, subtyping and virulence determination of Listeria species. Clin Microbiol Rev. 2003;16:584–640.
5. Liu D. Identification, subtyping and virulence determination of Listeria monocytogenes. J Med Microbiol. 2006;55(6):645–659.
6. Graves LM, Helser LO, Steigerwalt AG, et al. Listeria marrhii sp. nov., isolated from the natural environment. Finger Lakes National Forest. Int J Syst Evol Microbiol. 2001;51:173–175.
7. Liu D. Identification. Subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. J Med Microbiol. 2006;55(6):645–659.
8. Bertsch D, Rau J, Eugster MR, et al. Listeria fleischmannii sp. nov., isolated from cheese. Int J Syst Evol Microbiol. 2013;63(2):526–532.
9. den Bakker HC, Manuel CS, Fortes ED, Wiedmann M, Nightingale KK. Genome sequencing identifies Listeria fleischmannii subspp. coloradensis subspp. nov., a novel Listeria fleischmannii subspecies isolated from a ranch in Colorado. Int J Syst Evol Microbiol. 2013, Mar 22. [Epub ahead of print].
10. Lang Halter E, Neuhaus K, Scherer S. Listeria weihenstephanensis sp. nov., isolated from the water plant Lemna trisulca taken from a freshwater pond. Int J Syst Evol Microbiol. 2013;63(2):641–647.
11. Glaser F, Frangeul L, Buchrieser C, et al. Comparative genomics of Listeria species. Science. 2001;294(5543):849–852.
12. Buchrieser C, Rusniok C, Kunst F, Cossart P, Glaser P; Listeria Consor-tium. Comparison of the genome sequences of Listeria monocytogenes and Listeria innocua: clues for evolution and pathogenicity. FEMS Immunol Med Microbiol. 2003;35(3):207–213.
13. Liu D, Ainsworth AJ, Austin FW, Lawrence ML. Characterization of virulence genes reveal new insights into the core genome components of this species. Nucleic Acids Res. 2004;32:2386–2395.
14. Schnid MW, Ng EYW, Lampidis R, et al. Evolutionary history of the genus Listeria and its virulence genes. Syst Appl Microbiol. 2005;28:1–18.
15. Hain T, Steinweg C, Chakraborty T. Comparative and functional genomics of Listeria spp. J Biotechnol. 2006;126(1):37–51.
16. Hain T, Ghar R, Billion A, et al. Comparative genomics and transcriptomics of lineages I, II, and III strains of Listeria monocytogenes. BMC Genomics. 2012;13:144.
17. Steinweg C, Kuenne CT, Billion A, et al. Complete genome sequence of Listeria seeligeri, a nonpathogenic member of the genus Listeria. J Bacteriol. 2010;192:1473–1474.
18. Cummins AJ, Fielding AK, McLauchlin J. Listeria ivanovii infection in a patient with AIDS. J Infect. 1994;28(1):89–91.
19. Lessing MP, Curtis GD, Bowler IC. Listeria ivanovii infection. J Infect. 1994;29(2):230–231.
20. Snapir YM, Vaisbein E, Nussar F. Low virulence but potentially fatal outcome-Listeria ivanovii. Eur J Intern Med. 2006;17(4):286–287.
21. Guillet C, Join-Lambert O, Le Monnier A, et al. Human listeriosis caused by Listeria ivanovii. Emerg Infect Dis. 2010;16(1):136–138.
22. Roccourt J, Hof H, Schrettenbrunner A, Malinverni R, Bille J. [Acute purulent Listeria seelingeri meningitis in an immunocompetent adult]. Schweiz Med Wochenschr. 1986;116(8):248–251. French.
23. Perrin M, Bemer M, Delamare C. Fatal case of Listeria innocua bacteremia. J Clin Microbiol. 2003;41(11):5308–5309.
24. Andre P, Genicot A. First isolation of Listeria welshimeri from human beings. Zentralbl Bakteriol. Parasitenkd. Infektkrankh. Hyg Abt 1 Orig Reihe A. 1987;263:605–606.
25. Todeschini G, Friso S, Lombardi S, Casaril M, Fontana R, Corrocher R. A case of Listeria murrayi/greyi bacteremia in a patient with advanced Hodgkin’s disease. Eur J Clin Microbiol Infect Dis. 1998;17(11):808–810.
26. Rapose A, Lick SD, Ismail N. Listeria grayi bacteremia in a heart transplant recipient. Transplant Infect Dis. 2008;10(6):434–436.
27. Salimnia H, Patel D, Lephart PR, Fairfax MR, Chandrasekar PH. Listeria grayi: vancomycin-resistant, gram-positive rod causing bacteremia in a stem cell transplant recipient. Transplant Infect Dis. 2010;12(6):526–528.
28. Seeliger HPR, Höhne K. Serotyping of Listeria monocytogenes and related species. Methods Microbiol. 1979;13:31–49.
29. Seeliger HPR, Jones, D. Listeria. In: Bergy’s Manual of Systematic Bacteriology, Vol. 2. Sneath PHA, et al. (Eds). 1986; Baltimore: Williams and Wilkins; 1235–1245.
30. Vaneechoutte M, Boerlin P, Tichy HV, Bannerman E, Jager B, Bille J. Comparison of PCR-based DNA fingerprinting techniques for the identification of Listeria species and their use for atypical Listeria isolates. Int J Syst Bacteriol. 1998;48:127–139.
31. Palumbo JD, Borucki MK, Mandrell, RE, Gorski, L. Serotyping of Listeria monocytogenes by enzyme-linked immunosorbent assay and identification of mixed-serotype cultures by colony immunoblotting. J Clin Microbiol. 2003;41:564–571.
32. Gorski L. Phenotypic identification. In: Liu D. (Ed). Handbook of Listeria monocytogenes. 2008; Boca Raton: Taylor and Francis CRC Press; 139–168.
33. Goulet V, Jaquet C, Martin P, Vaillant V, Laurent E, de Valk H. Surveillance of Listeria and its virulence genes. Nucleic Acids Res. 2004;32:2386–2395.
34. Barbour AH, Rampling A, Hormaeche CE. Variation in the infectivity of Listeria monocytogenes strain 4b and 1/2a strains of the food-borne pathogen Listeria monocytogenes reveal new insights into the core genome components of this species. Nucleic Acids Res. 2004;32:2386–2395.
35. Kim SH, Bakko MK, Knowles D, Borucki MK. Oral inoculation of A/J mice with Listeria epidermolytica strain 4b and nonpathogenic Listeria grayi results in fatal Listeria monocyto-genes. FEMS Immunol Med Microbiol. 2005;43:238–245.
36. Liu D, Ainsworth AJ, Austin FW, Lawrence ML. Characterization of virulence genes reveal new insights into the core genome components of this species. Nucleic Acids Res. 2004;32:2386–2395.
37. Liu D, Lawrence ML, Wiedmann M, et al. Listeria monocytogenes subgroups IIA, IIB and III delineate genetically distinct populations with varied virulence potential. J Clin Microbiol. 2006;44:4229–4233.
38. Liu D, Lawrence ML, Ainsworth AJ, Austin FW. A multiplex PCR for species- and virulence-specific determination of Listeria monocytogenes. J Microbiol Methods. 2007;71:133–140.
39. Liu D. Listeria monocytogenes: comparative interpretation of mouse virulence assay. FEMS Microbiol Lett. 2004;233(1):159–164.
40. Schönberg A, Bannerman E, Courtieu AL, et al. Serotyping of 80 strains from the WHO multicentre international typing study of Listeria monocytogenes. Int J Food Microbiol. 1996;32(3):279–287.
41. Liu D, Lawrence M, Gorski L, et al. Listeria monocytogenes serotype 4b strains belonging to subgroups I and III possess distinct molecular features. J Clin Microbiol. 2006;44:214–217.
42. Orsi RH, den Bakker HC, Wiedmann M. Listeria monocytogenes lineages: Genomics, evolution, ecology, and phenotypic characteristics. Int J Med Microbiol. 2011;301(2):79–96.
43. Rasmussen OF, Skouboe P, Dons L, Rossen L, Olsen JE. Listeria monocytogenes exists in at least three evolutionary lines: Evidence from flagellin, invasive associated protein and listeriolysin O genes. Microbiology. 1997;141:2053–2061.
44. Wiedmann M, Bruce LJ, Keating C, Johnson AE, McDonough PL, Batt CA. Ribotypes and virulence gene polymorphisms suggest three distinct Listeria monocytogenes lineages with differences in pathogenic potential. Infect Immun. 1997;65:2707–2716.
45. Kerouanton A, Brisabois A, Denoyer E, et al. Comparison of five typing methods for the epidemiological study of Listeria monocytogenes. Int J Food Microbiol. 1998;43:61–71.
46. Ducey TF, Page B, Usgraft T, et al. A single-nucleotide-polymorphism-based multilocus genotyping assay for subtyping line 1 isolates of Listeria monocytogenes. Appl Environ Microbiol. 2007;73:133–147.
47. Meinersmann RJ, Phillips RW, Wiedmann M, Berrang ME. Multilocus sequence typing of Listeria monocytogenes by use of hypervariable genes reveals clonal and recombination histories of three cultures. Appl Environ Microbiol. 2004;70:2193–2203.
48. Saunders BD, Schukken Y, Kornstein L, et al. Molecular epidemiology and cluster analysis of human listeriosis cases in three U.S. states. J Food Prot. 2006;69(7):1680–1689.
49. Saunders BD, Overdevest J, Fortes E, et al. Diversity of Listeria species in urban and natural environments. Appl Environ Microbiol. 2012;78(12):4420–4433.
50. Tsai YH, Maron SB, McGann P, Nightingale KK, Wiedmann M, Orsi RH. Recombination and positive selection contributed to the evolution of Listeria monocytogenes lineages III and IV, two distinct and well-supported uncommon L. monocytogenes lineages. Infect Genet Evol. 2011;11(8):1881–1890.
51. Ward TJ, Ducey TF, Usgraft T, Dunn KA, Bielawski JP. Multilocus genotyping assays for SNP-based subtyping of Listeria monocytogenes. Appl Environ Microbiol. 2008;74(24):7629–7642.
52. Bille J, Catinel B, Bannerman E, et al. API Listeria, a new and promising one-day system to identify Listeria isolates. Appl Environ Microbiol. 1992;58:1857–1860.
53. Harvey J, Gilmour A. Characterization of Listeria monocytogenes isolates by esterase electrophoresis. Appl Environ Microbiol. 1996;62:1461–1466.
54. Dounith M, Buchrieser C, Glaser P, Jacquet C, Martin P. Differentiation of the major Listeria monocytogenes serovars by multiplex PCR. J Clin Microbiol. 2004;42:3819–3822.
55. Bruce JL, Hubner RJ, Cole EM, McDowell CI, Webster JA. Automated ribotyping using different enzymes to improve discrimination of Listeria monocytogenes isolates, with a particular focus on serotype 4b strains. J Clin Microbiol. 2001;39:3002–3005.
56. Paillard D, Dubois V, Duran R, et al. Rapid identification of Listeria species by using restriction fragment length polymorphism of PCR-amplified 23S rRNA gene fragments. Appl Environ Microbiol. 2003;69:6386–6392.
57. Bubert A, Köhler S, Goebel W. The homologous and heterologous regions within the iap gene allow genus- and species-specific identification of Listeria spp. by polymerase chain reaction. Appl Environ Microbiol. 1992;58:2625–2632.
58. Chen Y, Knabel SJ. Multiplex PCR for simultaneous detection of bacteria of the genus Listeria, Listeria monocytogenes, and major serotypes and epidemic clones of L. monocytogenes. Appl Environ Microbiol. 2007;73(19):6299–6304.
59. Donnelly CW. Historical perspectives on methodology to detect Listeria monocytogenes. J Assoc Off Anal Chem. 1988;71(3):644–646.
60. Rocourt J, Catinel B. Biochemical characterization of species in the genus Listeria. Zentralbl Bakteriol Mikrobiol Hyg A. 1985;260:221–231.
61. Loesnner JM. Improved procedure for bacteriophage typing of Listeria strains and evaluation of new phages. Appl Environ Microbiol. 1991;57:882–884.
62. Aznar R, Alarcón B. On the specificity of PCR detection of Listeria monocytogenes in food: a comparison of published primers. Syst Appl Microbiol. 2002;25:109–119.
63. Huang B, Egelesos H, Héron BA, et al. Comparison of multiplex PCR with conventional biochemical methods for the identification of Listeria spp. isolates from food and clinical samples in Queensland, Australia. J Food Prot. 2007;70:1874–1880.
64. Klinger JD, Johnson A, Croan D, et al. Comparative studies of nucleic acid hybridization assay for Listeria in foods. J Assoc Off Anal Chem. 1988;71:669–673.
65. Köhler S, Leimeister-Wächter M, Chakraborty T, Lottspeich F, Goebel W. The gene coding for protein p60 of Listeria monocytogenes and its use as a specific probe for Listeria monocytogenes. Infect Immun. 1990;58(6):1943–1950.
66. Rattanachaikunsopon P, Phumkhachorn P. Identification of viable Listeria species based on reverse transcription-multiplex PCR (RT-MPCR) and restriction digestion. Biosci Biotechnol Biochem. 2012;76(6):1189–1194.
67. Laksanalamai P, Jackson SA, Mammel MK, Datta AR. High density microarray analysis reveals new insights into genetic footprints of Listeria monocytogenes strains involved in listeriosis outbreaks. PLoS ONE. 2012;7(3)e32896.
68. Bubert A, Hein I, Rauch M, et al. Detection and differentiation of Listeria spp. by a single reaction based multiplex PCR. Appl Environ Microbiol. 1999;65:4688–4692.
69. Wang RF, Cao WW, Johnson MG. 16S rRNA-based probes and polymerase chain reaction method to detect Listeria monocytogenes cells added to foods. Appl Environ Microbiol. 1992;58:2827–2831.
70. Graham T, Golsteyn-Thomas EJ, Gannon VP, Thomas JE. Genus- and species-specific detection of Listeria monocytogenes using polymerase chain reaction assays targeting the 16S/23S intergenic spacer region of the rRNA operon. Can J Microbiol. 1996;42:1155–1161.
71. Graham TA, Golsteyn-Thomas EI, Thomas JE, Gannon VP. Inter- and intraspecies comparison of the 16S–23S rRNA operon intergenic spacer regions of six Listeria spp. Int J Syst Bacteriol. 1997;47:863–869.
72. Manzano M, Cocolin L, Cantoni C, Coni G. Temperature gradient gel electrophoresis of the amplified product of a small 16S rRNA gene fragment for the identification of Listeria species isolated from food. J Food Prot. 2000;63:659–661.
73. Somer L, Kashi, Y. A PCR method based on 16S rRNA sequence for simultaneous detection of the genus Listeria and the species Listeria monocytogenes in food products. J Food Prot. 2003;66:1658–1665.
74. Wang J, Yamada S, Ohashi E. Rapid identification of Listeria species and screening for variants by melting curve and high-resolution melting curve analyses of the intergenic spacer region of the rRNA gene. Can J Microbiol. 2012;58(6):676–682.
75. Jin D, Luo Y, Zhang Z, et al. Rapid molecular identification of Listeria species by use of real-time PCR and high-resolution melting curve analysis. FEMS Microbiol Lett. 2012;334(1):72–80.
76. Furrer B, Candrian U, Hoeeflein C, Luethy J. Detection and identification of Listeria monocytogenes in cooked sausage products and in milk by in vitro amplification of haemolysin gene fragments. J Appl Bacteriol. 1991;70:372–379.
Liu D, Ainsworth AJ, Austin FW, Lawrence ML. Identification of Listeria monocytogenes on frankfurters using oligonucleotide primers targeting the genes encoding internalin AB. J Food Prot. 2003;66:237–241.

Pangalo D, Kaciklova E, Kuchta T, Drahovsky H. Detection of Listeria monocytogenes by polymerase chain reaction oriented to inlB gene. New Microbiol. 2001;24:333–339.

Schäferkordt S, Chakraborty T. Identification, cloning, and characterization of the Lm operon, whose gene products are unique to Listeria monocytogenes. J Bacteriol. 1997;179(8):2707–2716.

Gray DJ, Kroll RG. Polymerase chain reaction amplification of the flaA targets and AmpliFluor technology. J Food Prot. 2004;91:297–304.

Winters DK, Maloney TP, Johnson MG. Rapid detection of Listeria monocytogenes by a PCR assay specific for an aminopeptidase. Mol Cell Probes. 1999;13:127–131.

Gilot P, Content J. Specific identification of Listeria welshimeri and Listeria monocytogenes by PCR assays targeting a gene encoding a fibronectin-binding protein. J Clin Microbiol. 2002;40:698–703.

Liu D, Ainsworth AJ, Austin FW, Lawrence ML. Use of PCR primers derived from a putative transcriptional regulator gene for species-specific identification of Listeria monocytogenes. Int J Food Microbiol. 2004;91:297–304.

Zhang W, Jayarao BM, Knabel SJ. Multi-virulence-locus sequence typing of Listeria monocytogenes. Appl Environ Microbiol. 2004;70(2):913–920.

Liu D, Ainsworth AJ, Austin FW, Lawrence ML. PCR detection of a putative N-acetylMuramidase gene from Listeria ivanovii facilitates its rapid identification. Vet Microbiol. 2004;101:83–89.

Rodríguez-Lázaro D, López-Enríquez L, Hernández M. smcL as a novel discriminatory marker for quantitative detection of Listeria monocytogenes in biological samples. J Appl Microbiol. 2010;109(3):863–872.

Liu D, Lawrence ML, Ainsworth AJ, Austin FW. Species-specific PCR determination of Listeria seeligeri. Res Microbiol. 2004;155:741–746.

Liu D, Ainsworth AJ, Austin FW, Lawrence ML. Identification of Listeria innocua by PCR targeting a putative transcriptional regulator gene. FEMS Microbiol Lett. 2003;203:205–210.

Rodríguez-Lázaro D, Hernández M, Scortti M, Esteve T, Vázquez-Boland JA, Pla M. Quantitative detection of Listeria monocytogenes and Listeria innocua by real-time PCR: assessment of hly, iap, and lin20483 targets and AmpliFluor technology. Appl Environ Microbiol. 2004;70:1366–1377.

Liu D, Ainsworth AJ, Austin FW, Lawrence ML. Identification of a gene encoding a putative phosphotransferase system enzyme IIIBC in Listeria welshimeri and its application for diagnostic PCR. Lett Appl Microbiol. 2004;38:151–157.

Liu D, Lawrence ML, Ainsworth AJ. A novel PCR assay for Listeria welshimeri targeting species-specific transcriptional regulator gene hwe1801. J Rapid Methods Mol Microbiol. 2008;16:154–163.

Liu D, Lawrence ML, Ainsworth AJ, Austin FW. Isolation and PCR amplification of a species-specific, oxidoreductase-coding gene region in Listeria grayi. Can J Microbiol. 2005;51:95–98.

Dounith M, Cazalet C, Simoes N, et al. New aspects regarding evolution and virulence of Listeria monocytogenes revealed by comparative genomics and DNA arrays. Infect Immun. 2004;72:1072–1083.

Chen J, Jiang L, Chen Q, et al. Iml6038 is involved in acid and heat stress responses and specific for Listeria monocytogenes lineages I and II, and Listeria ivanovii. Foodborne Pathog Dis. 2009;6:365–376.

Dunn KA, Bielawski JP, Ward TJ, Urquhart C, Gu H. Reconciling ecological and genomic divergence among lineages of listeria under an "extended mosaic genome concept". Mol Biol Evol. 2009;26(11):2605–2615.

McLauchlin J, Hampton MD, Shah S, Threlfall EJ, Wieneke AA, Curtis GD. Subtyping of Listeria monocytogenes on the basis of plasmid profiles and arsenic and cadmium susceptibility. J Appl Microbiol. 1997;83(3):381–388.

Bibb WF, Schwartz B, Gellin BG, Plkaytis BD, Weaver RE. Analysis of Listeria monocytogenes by multilocus enzyme electrophoresis and application of the method to epidemiologic investigations. Int J Food Microbiol. 1989;8:233–239.

Brosch R, Chen J, Luchansky JB. Pulsed-field fingerprinting of listeriae: identification of genomic divisions for Listeria monocytogenes and their correlation with serovar. Appl Environ Microbiol. 1994;60:2584–2592.

Farber J, Addison C. RAPD typing for distinguishing species and strains in the genus Listeria. J Appl Bacteriol. 1994;77:242–250.

Graves LM, Swaninathan B, Reeves MW, et al. Comparison of ribotyping and multilocus enzyme electrophoresis for subtyping of Listeria monocytogenes isolates. J Clin Microbiol. 1994;32:2936–2943.

O'Donoghue K, Bowker K, McLauchlin J, Reeves DS, Bennett PM, MacGowan AP. Typing of Listeria monocytogenes by random amplified polymorphic DNA (RAPD) analysis. Int J Food Microbiol. 1995;27:245–252.

Wiedmann M, Bruce JL, Knorr R, et al. Ribotype diversity of Listeria monocytogenes strains associated with outbreaks of listeriosis in ruminants. J Clin Microbiol. 1996;34:1086–1090.

Lomonaco S, Nucera D, Parisi A, Normanno G, Bottero MT. Comparison of two AFLP methods and PGE using strains of Listeria monocytogenes isolated from environmental and food samples obtained from Piedmont, Italy. Int J Food Microbiol. 2011;149:177–82.

Jersé B, Gilot P, Gubina M, et al. Typing of Listeria monocytogenes strains by repetitive element sequence-based PCR. J Clin Microbiol. 1999;37(1):103–109.

Ripabelli G, McLauchlin J, Threlfall EJ. Amplified fragment length polymorphism (AFLP) analysis of Listeria monocytogenes. Syst Appl Microbiol. 2000;23:132–136.

Franciosa G, Tartaro S, Wedell-Neergaard C, Aureli P. Characterization of Listeria monocytogenes strains involved in invasive and noninvasive listeriosis outbreaks by PCR-based fingerprinting techniques. Appl Environ Microbiol. 2001;67:1793–1799.

Jeffers GT, Bruce JL, McDonough PL, Scarlett J, Boor KJ, Wiedmann M. Comparative genetic characterization of Listeria monocytogenes isolates from human and animal listeriosis cases. Microbiology. 2001;147:1095–1104.

Guerra MM, Bernardo F, McLauchlin J. Amplified fragment length polymorphism (AFLP) analysis of Listeria monocytogenes. Syst Appl Microbiol. 2002;25:456–461.

Jaradat ZW, Schutze GE, Bhunia AK. Genetic homogeneity among Listeria monocytogenes strains from infected patients and meat products from two geographic locations determined by phenotyping, ribotyping and PCR analysis of virulence genes. Int J Food Microbiol. 2002;76:1–10.

Keto-Timonen RO, Autio TJ, Korkeala HJ. An improved amplified fragment length polymorphism (AFLP) protocol for discrimination of Listeria isolates. Syst Appl Microbiol. 2003;26:236–244.

Zhang C, Zhang M, Ju J, et al. Genome diversification in phylogenetic lineages I and II of Listeria monocytogenes: identification of segments unique to lineage II populations. J Bacteriol. 2003;185(18):5573–5584.

Miya S, Takashashi H, Kamimura C, Nakagawa M, Kuda T, Kimura H. Highly discriminatory typing method for Listeria monocytogenes using polymorphic tandem repeat regions. J Microbiol Methods. 2012;90(3):285–291.

Wiedmann M. Molecular subtyping methods for Listeria monocytogenes. J AOAC Int. 2002;85:524–531.

Ward TJ, Gorski L, Borucki MK, Mandrell RE, Hutchins J, Pupedis K. Intraspecific phylogeny and lineage group identification based on the prfA virulence gene cluster of Listeria monocytogenes. J Biotechnol. 2004;186:4994–5002.
Molecular identification of pathogenic and nonpathogenic listeriae

121. Ward TJ, Usgaard T, Evans P. A targeted multilocus genotyping assay for lineage, serogroup, and epidemic clone typing of Listeria monocytogenes. *Appl Environ Microbiol*. 2010;76(19):6680–6684.

122. Roberts A, Nightingale K, Jeffers G, Fortes E, Kongo JM, Wiedmann M. Genetic and phenotypic characterization of Listeria monocytogenes lineage III. *Microbiology*. 2006;152:685–693.

123. den Bakker HC, Didelet X, Fortes ED, Nightingale KK, Wiedmann M. Lineage specific recombination rates and microevolution in Listeria monocytogenes. *BMC Evol Biol*. 2008;8:277.

124. Müller AA, Schmid MW, Meyer O, Meussdoerffer FG. Listeria seeligeri isolates from food processing environments form two phylogenetic lineages. *Appl Environ Microbiol*. 2010;76(9):3044–3047.

125. Chen J, Fang C, Zhu N, et al. Genetic organization of ascl-dapE internalin cluster serves as a potential marker for Listeria monocytogenes sublineages IA, IB, and IIC. *J Microbiol Biotechnol*. 2012;22(5):575–584.

126. Zhao H, Chen J, Fang C, et al. Deciphering the biodiversity of Listeria monocytogenes lineage III strains by phylpastic approaches. *J Microbiol*. 2011;49(5):759–767.

127. Chen J, Chen Q, Jiang L, et al. Intralining profiling and multilocus sequence typing suggest four Listeria innocua subgroups with different evolutionary distances from Listeria monocytogenes. *BMC Microbiol*. 2010;10:97.

128. Brosch R, Brett M, Catimel B, Luchansky JB, Ojenyi B, Rocourt J. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of Listeria monocytogenes via pulsed-field gel electrophoresis (PFGE). *Int J Food Microbiol*. 1996;32:343–355.

129. Liu D, Lawrence ML, Wiedmann M, et al. Listeria monocytogenes subgroups I/2A, I/2B, I/2C and 4b: toward an international standard. *J Clin Microbiol*. 2003;41(12):5537–5540.

130. Nadon CA, Woodward DL, Young C, Rodgers FG, Wiedmann M. Correlations between molecular subtyping and serotyping of Listeria monocytogenes. *J Clin Microbiol*. 2001;39:2704–2707.

131. Datta AR, Laksanalamai P, Solomotis M. Recent developments in molecular sub-typing of Listeria monocytogenes. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2012, Oct 12. [Epub ahead of print].

132. Jinneman KC, Hill WE. Listeria monocytogenes lineage group classification by MAMA-PCR of the listeriolysin gene. *Curr Microbiol*. 2001;43:129–133.

133. Borucki MK, Call DR. Listeria monocytogenes serotype identification by PCR. *J Clin Microbiol*. 2003;41(12):5537–5540.

134. Doumith M, Jacquet C, Gerner-Smidt P. Multicenter validation of a multiplex PCR assay for differentiating the major Listeria monocytogenes serovars 1/2a, 1/2b, 1/2c and 4b: toward an international standard. *J Food Prot*. 2005;68(12):2648–2650.

135. Zhang W, Knabel SJ. Multiplex PCR assay simplifies serotyping and sequence typing of Listeria monocytogenes associated with human outbreaks. *J Food Prot*. 2005;68(9):1907–1910.

136. Nightingale K, Bovell L, Graczyk A, Wiedmann M. Combined sigB allelic typing and multiplex PCR provide improved discriminatory power and reliability for Listeria monocytogenes molecular serotyping. *J Microbiol Methods*. 2007;68(1):52–59.

137. Kérouanton A, Marault M, Petit L, Grout J, Dao TT, Frisbaos A. Evaluation of a multiplex PCR assay as an alternative method for Listeria monocytogenes serotyping. *J Microbiol Methods*. 2010;80(2):134–137.

138. Evans MR, Swaminathan B, Graves LM, et al. Genetic markers unique to Listeria monocytogenes serotype 4b differentiate epidemic clone II (hot dog outbreak strains) from other lineages. *Appl Environ Microbiol*. 2004;70(4):2383–2390.

139. Cheng Y, Seletzyk RM, Kathariou S. Genomic divisions/lineages, epidemic clones, and population structure. In: Liu D (Ed). *Handbook of Listeria monocytogenes*. 2008; Boca Raton: CRC Press; 337–357.

140. Chenal-Francisque V, Lopez J, Cantinelli T, et al. Worldwide distribution of major clones of Listeria monocytogenes. *Emerg Infect Dis*. 2011;17(6):1110–1112.

141. Kathariou, S. Listeria monocytogenes virulence and pathogenicity, a food safety perspective. *J Food Prot*. 2002;65:1811–1829.

142. Chen Y, Zeng W, Knabel SJ. Multi-virulence-locus sequence typing identifies single nucleotide polymorphisms which differentiate epidemic clones and outbreak strains of Listeria monocytogenes. *J Clin Microbiol*. 2007;45(3):835–846.

143. Chen Y, Knabel SJ. Prophages in Listeria monocytogenes contain single-nucleotide polymorphisms that differentiate outbreak clones within epidemic clones. *J Clin Microbiol*. 2008;46(4):1478–1484.

144. Chen Y, Kumar N, Siddique N. Development and evaluation of a real-time polymerase chain reaction assay targeting iap for the detection of Listeria monocytogenes in select food matrices. *Foodborne Pathog Dis*. 2011;8(10):1063–1069.

145. Knabel SJ, Reimer A, Verghese B, et al. Canadian Public Health Laboratory Network (CPHLN). Sequence typing confirms that a predominant Listeria monocytogenes clone caused human listeriosis cases and outbreaks in Canada from 1988 to 2010. *J Clin Microbiol*. 2012;50(5):1748–1751.

146. Chenal-Francisque V, Diancourt L, Cantinelli T, et al. Optimized Multilocus Variable-Number Tandem-Repeat Analysis Assay and Its Complementarit y with Pulsed-Field Gel Electrophoresis and Multilocus Sequence Typing for Listeria monocytogenes Clone Identification and Surveillance. *J Clin Microbiol*. 2013;51(6):1868–1880.

147. Lomonaco S, Knabel SJ, Dalmasso A, Civera T, Bottero MT. Novel multiplex single nucleotide polymorphism-based method for identifying epidemic clones of Listeria monocytogenes. *Appl Environ Microbiol*. 2011;77(17):6290–6294.

148. Lomonaco S, Patti R, Knabel SJ, Civera T. Detection of virulence-associated genes and epidemic clone markers in Listeria monocytogenes isolates from PDO Gorgonzola cheese. *Int J Food Microbiol*. 2012;160(1):76–79.

149. Lomonaco S, Verghese B, Gerner-Smidt P, et al. Novel epidemic clones of Listeria monocytogenes, United States, 2011. *Emerg Infect Dis*. 2013;19(1):147–150.

150. Piffaretti JC, Kressbach H, Aeschbacher M, et al. Genetic characterization of clones of the bacterium Listeria monocytogenes causing epidemic disease. *Proc Natl Acad Sci USA*. 1989;86(10):3818–3822.

151. Vildirim S, Lin W, Hitchins AD, et al. Epidemic clone I-specific genetic markers in strains of Listeria monocytogenes serotype 4b from foods. *Appl Environ Microbiol*. 2004;70(7):4158–4164. Erratum in: *Appl Environ Microbiol*. 2004;70(12):7581.

152. Franciosa G, Sefcikaro C, Maugliani A, et al. Distribution of epidemic clonal genetic markers among Listeria monocytogenes 4b isolates. *J Food Prot*. 2007;70(3):574–581.

153. Lomonaco S, Chen Y, Knabel SJ. Analysis of additional virulence genes and virulence gene regions in Listeria monocytogenes confirms the epidemiologic relevance of multi-virulence-locus sequence typing. *J Food Prot*. 2008;71(12):2559–2566.

154. den Bakker HC, Fortes ED, Wiedmann M. Multilocus sequence typing of outbreak-associated Listeria monocytogenes isolates to identify epidemic clones. *Foodborne Pathog Dis*. 2010;7(3):257–265.

155. Huang B, Fang N, Dimovski K, Wang X, Hogg G, Bates J. Observation of a new pattern in serogroup-related PCR typing of Listeria monocytogenes 4b isolates. *J Clin Microbiol*. 2011;49(4):426–429.