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REVIEW

Molecular Approaches to the Identification of Pathogenic and Nonpathogenic Listeriae

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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 (eg., 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 saprophytes. 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

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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.[1] In addition to *L. monocytogenes*,[9] 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.[1] 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.[2,3]

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 *Furmicutes,* 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*.[4–9] 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.[10–17] Nonetheless, non-monocytogenes *Listeria* species including *L. ivanovii,*[18–21] *L. seeligeri,*[22] *L. innocua,*[23] *L. welshimeri,*[24] and *L. grayi*[25–27] 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).[28–32] 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).[33] Similarly, in experimental mouse models, *L. monocytogenes* serovars 4b, 1/2a, 1/2b and 1/2c show a heightened infectivity through intragastric inoculation.[34,35] 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[33], 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 and3c 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									
			inlA	*lmo0733*	*lmo2672*	*inlJ*	*inlC***	*lmo1134*	ORF2819	ORF2110	*lmo0737*	*lmo1118*
I	1/2b	+	+	+	+	+	+	+	+	–	–	–
	3b	+	+	+	+	+	+	+	+	–	–	–
	4b	+	+	+	+	+	+	+	+	+	–	–
	4d	+	+	+	+	+	+	+	+	+	–	–
	4e	+	+	+	+	+	+	+	+	+	–	–
II	1/2a	+	+	+	+	+	+	+	–	–	+	–
	1/2c	+	+	+	+	+	+	+	–	–	+	+
	3a	+	+	+	+	+	+	+	–	–	+	–
	3c	+	+	+	+	+	+	+	–	–	+	+
IIIA	4a	+	+	+	–	–	–	–	–	–	–	–
	4c	+	+	+	–	+	–/+	–	–	–	–	–
IIIC	4c	–	+	+	+	+	+	–	–	–	–	–
IV (IIIB)	7 and unusual 4a,4b,4c	–	+	–	+	–	+	–	+	–	–	–

*Summarized from Liu et al[36–39]; Doumith et al[54]; Roberts et al.[122]
***inlC* is also found in some *L. ivanovii* strains.[38]

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-orfX-orfZ-orf B-orfA-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).[54] 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.[30,55–58] Paillard et al[58] 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 *Xmn*I 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 *Cfo*I 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 hydrolase necessary for proper cell division. The *iap* gene has been successfully incorporated in PCR for *Listeria* genus specific detection.[59,60]

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 speciation of *Listeria* bacteria.[61–63] 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.[64,65] Evolving from non-amplified procedures (eg., DNA hybridization), molecular detection of *Listeria* bacteria has increasingly moved towards nucleic acid amplification and real time detection.[66–69]

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,[56,58,59,70–77] many *Listeria* species-specific genes have been described. For instance, the following genes targets may be used for specific determination of *L. monocytogenes*: *hly*,[78,79] *plcA*,[80] *plcB*,[80] *actA*,[81] *inlA*,[82,83] *inlB*,[83,84] *lmaA*/*lmaB*,[85] *flaA*,[86] *pepC*,[87] *clpE*,[81] *fbp*,[88] *lmo0733*,[89] and *lmo2234*[90] Similarly, *liv22–228*[91] and *smcL*[92] have been specifically targeted for *L. ivanovii*, *lse24–315*[93] for *L. seeligeri*, *lin0464*[94] and *lin2483*[95] for *L. innocua*, *fbp*,[88] *lwe7–571*[96] and *lwe1801*[97] for *L. welshimeri*, and *lgr20–246*[98] for *L. grayi*.

Lineage Delineation

As *L. monocytogenes* lineages I (particularly serovars 1/2b and 4b) and II (especially serovars 1/2a and 1/2c) strains are commonly associated with human clinical cases, it is important that they are accurately identified and subtyped.[99–101]

Similar to species-specific identification, 2 major approaches are used for *L. monocytogenes* lineage delineation and subtyping: phenotypic and genetic.[102] The phenotypic subtyping approach utilizes serotyping,

phage typing, multilocus enzyme electrophoresis (MLEE) and esterase typing techniques.[103] 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)].[30,43–45,55,57,104–118] 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.[119–121]

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.[44,113,120,122] 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.[123] 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.[124]

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[125] 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 IIIC. 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.[126] Similarly, examination of internalin genes of *L. innocua* resulted in the identification of 4 subgroups within the species.[127]

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.[40,128,129] Without

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

Gene	Specificity	Reference
inlJ (*lmo2821*)	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]
inlC	All *L. monocytogenes* serovars but 4a and some 4c	Liu et al[38]
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	Schaferkordt and Chakraborty[79]
lmaB	All *L. monocytogenes* serovars but 4a and 4c	Schaferkordt and Chakraborty[79]
lmo0038	All *L. monocytogenes* serovars but 4a and 4c	Chen et al[100]
ORF2819	*L. monocytogenes* serovars 1/2b, 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 Knabel[135]
lmo0737	*L. monocytogenes* serovars 1/2a, 1/2c, 3a and 3c	Doumith et al[54]
lmo0171	*L. monocytogenes* serovars 1/2a, 1/2c, 3a and 3c	Zhang and Knabel[135]
lmo1118	*L. monocytogenes* serovars 1/2c and 3c	Doumith et al[54]
Gene region flanking *gltA-gltB*	*L. monocytogenes* serovars 1/2b and 3b	Borucki and Call[133]
flaA	*L. monocytogenes* serovars 1/2a and 3a	Borucki and Call[133]

these obvious shortcomings, molecular techniques provide a precise and low-cost alternative for determination of *L. monocytogenes* serovars/groups.[130,131]

Jinneman and Hill[132] reported a mismatch amplification mutation 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[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,[132] and primers from the *flaA* gene (encoding the *L. monocytogenes* flagellin protein) to identify *L. monocytogenes* serotypes. Doumith et al[54,134] 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[135] 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[136] 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[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 *prf*A gene primers for *L. monocytogenes* species confirmation. The second multiplex PCR incorporating the *fla*A 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.[37]

Identification of Epidemic Clones

Although a variety of *L. monocytogenes* strains have been isolated from environments and foodstuff, 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.[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.[142] To date, 5 epidemic clones (ECs) of *L. monocytogenes* (ECI, ECII, ECIII, ECIV, and ECV) have been defined (Table 4).[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.[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.[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 housekeeping gene sequences.[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.[90,155] Indeed, Knabel et al[145] employed multilocus sequence typing (MLST) and multi-virulence-locus sequence typing (MVLST)

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/ TTATTTCCTGTCGGCTTAG) 303 bp	Nova Scotia, 1981 California, 1985 Switzerland, 1983–1987 Denmark, 1985–1987 France, 1992	Chen and Knabel[60]; Yildirim et al[151]
ECII	4b	DUP-1044A	19	LMOh7858_0487.8 to *inlA* (ATTATGCCAAGTGGTTACGGA/ ATCTGTTTGCGAGACCGTGTC) 889 bp	USA, 1998–1999 USA, 2002	Chen and Knabel[60]; Evans et al[138]
ECIII	1/2a	DUP-1053A	1	LMOF6854_2463.4 (TTGCTAATTCTGATGCGTTGG/ GCGCTAGGGAATAGTAAAGG) 497 bp	USA, 2000	Chen and Knabel[60]
ECIV (formerly ECIa)	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 Knabel[60]
ECV	1/2a		59	LM5578_2229 (TTGTTGAAGGAAGAGGTGGTC/ TCTTTTCGGCTCATTTTCGT) 191 bp LM5578_2228_30 (CTGGTGTTGCCTCCTTTGTT/ AGCACAGGGTTCCTTTGACA) 982 bp	Canada, 1988–2000	Knabel et al[145]

[#]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 Knabel[60] 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 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 beween 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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As a requirement of publication the author has provided signed confirmation of compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests. Provenance: the authors were invited to submit this paper.

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