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Genomic analysis of *Listeria monocytogenes* diversity over a 10-year period in Uruguay

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Listeria monocytogenes is a globally relevant foodborne pathogen and a major public health concern because of its ability to cause severe invasive disease and persist in food processing environments. This study aimed to characterize the genomic diversity of _L. monocytogenes_ isolates collected in Uruguay from food and clinical cases of listeriosis between 2010 and 2019. The genomes sequences of 142 isolates representatives from a national collection were obtained and used for comparative genomic and phylogenetic analysis along with other 55 genomes from different geographical regions. The isolates belonged to lineages I (88%) and II (12%) and were distributed across 20 clonal complexes. The clonal complexes CC3, CC2, and CC1 were predominant. Notably, CC3 accounted for nearly one-third of the isolates and was evenly distributed between food and clinical sources, contrasting with its relatively low frequency in most international datasets. A novel sequence type (ST2832) and 112 new core genome MLST profiles were identified. The circulation of the rare clonal complex CC517 was detected, with evidence of persistence in food environments and a potential link to a human case. Comparative analysis revealed considerable virulence gene diversity, including specific distribution of LIPI-3 and LIPI-4 among lineages and clonal complexes, and the presence of truncated allelic variants of the _inlA_ gene in food-derived lineage II isolates. Phylogenetic analysis showed strong concordance with MLST-based classification and reveals linkage among isolates form different sources suggesting epidemiological relation between food and human cases of listeriosis. This study provides the first comprehensive genomic overview of _L. monocytogenes_ in Uruguay, revealing the predominance of lineage I isolates from food and clinical sources, a particular high prevalence of CC3 and the local circulation of the rare CC517. The results highlight the importance of whole genome and phylogenetic analysis as molecular epidemiology tools and show the contribution of including isolates from underrepresented regions in global genomic databases.

Keywords *Listeria monocytogenes*, Foodborne pathogens, Molecular epidemiology, Whole-genome sequencing, Genomic surveillance, Core genome MLST, Clonal complexes, Listeria pathogenicity islands (LIPIs)

Listeria monocytogenes is a Gram-positive bacterium responsible for listeriosis, a serious and potentially life-threatening infection in humans and animals. This pathogen is ubiquitous in nature and has been isolated from a wide range of sources, including soil, groundwater, animal and human feces, as well as from environments associated with food processing, distribution and retail. Its remarkable adaptability—characterized by the ability

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to survive under low temperatures, acidic pH, and high salt concentrations—presents significant challenges for control and regulation within the food industry and commercial supply chains[1–4].

L. monocytogenes is transmitted primarily through contaminated food products, including processed meats, dairy products, prepackaged sandwiches, cold-smoked fish, and various fruits and vegetables[3]. Although many cases of listeriosis occur sporadically, foodborne outbreaks are also reported. The clinical spectrum of human listeriosis is broad, ranging from asymptomatic or mild gastrointestinal symptoms to severe invasive disease. In its invasive form, listeriosis typically presents in three major clinical syndromes: pregnancy-associated and neonatal infections, bacteremia, and central nervous system involvement. Individuals at highest risk include pregnant women, neonates, elderly individuals, and immunocompromised individuals[5].

L. monocytogenes is a genetically diverse species comprising four major evolutionary lineages (I–IV), 14 lineage-associated serovars, and over 100 clonal complexes, as defined by multilocus sequence typing (MLST)[3,6–9]. More recently, strain-level differentiation has been further refined via whole-genome and core-genome multilocus sequence typing (wgMLST and cgMLST, respectively)[10,11]. A standardized nomenclature system based on the cgMLST scheme, which includes 1,748 core loci, has been proposed to improve strain classification[11]. Under this scheme, isolates are grouped into sublineages (SL) and cgMLST types (CT) on the basis of thresholds of fewer than 150 and 7 allelic differences, respectively.

Although all *L. monocytogenes* strains possess pathogenic potential, epidemiological and experimental data indicate considerable variation in virulence. Notably, three serotypes—4b, 1/2a, and 1/2b—account for at least 95% of clinical isolates[3,6]. Similarly, clonal complexes (CCs) within lineage I, particularly CC1, CC2, CC4, and CC6, are overrepresented among clinical and outbreak-associated strains[3,12]. These CCs are frequently isolated from otherwise healthy individuals or those with minimal comorbidities and are commonly referred to as "hypervirulent clones". It is hypothesized that hypervirulent strains possess specific attributes, in addition to prolonged in vivo survival, that promote the invasion of organs such as the brain and placenta[12]. In contrast, lineage II CCs, such as CC121 and CC9, are predominantly associated with food- and food-processing environments. When they are implicated in human infections, they are more likely to affect individuals with severe immunosuppression[13,14].

Genomic surveillance of *L. monocytogenes* has become an essential tool for investigating outbreaks, monitoring population dynamics, and evaluating control strategies[15]. Consequently, whole-genome sequencing (WGS) is increasingly adopted as the standard approach for epidemiological investigations in many countries[3]. WGS enables high-resolution characterization of isolates, including the identification of genetic lineages, detection of virulence and antimicrobial resistance genes, and reconstruction of evolutionary relationships among strains.

In Uruguay, surveillance of invasive *L. monocytogenes* infections is conducted through the mandatory notification of meningitis cases and foodborne disease. However, underreporting occurs, particularly in cases of foodborne diseases. To date, no foodborne listeriosis outbreaks have been confirmed in the country. On average, three cases of listeriosis are reported annually in the country[16]. Several previous reports have described the prevalence of *L. monocytogenes* from food sources and their characterization at the serogroup level[17], as well as case reports of listeriosis in humans and animals[18–21]. In this context, the aim of this study was to perform a genomic analysis of *L. monocytogenes* isolates collected in Uruguay over a 10-year period. Specifically, this study sought to characterize the genetic diversity of these isolates, explore their associations with epidemiological events, and elucidate the population structure of *L. monocytogenes* in Uruguay in the context of global strain diversity.

Methods
Bacterial isolates
A total of 142 *L. monocytogenes* isolates were selected from the strain collection maintained in *the Unidad Académica de Bacteriología y Virología* laboratory at the Instituto de Higiene, Facultad de Medicina, Universidad de la República. This collection comprises 476 isolates (food 392, human 56, animal 19, environment 9) obtained between 2010 and 2019 from several national, public, and private laboratories involved in food quality control, as well as human and veterinary clinical microbiology. Overall PCR-based serogroup distribution was 43% for serogroup IIb, 42% for serogroup IV, 13% serogroup IIa and 3% for IIc. The selection method was designed to capture the genetic and epidemiological diversity of the strains, considering their source, PCR-based serogroup, and year of isolation. All available human isolates (n = 52; one per patient) were included, along with a representative subset of isolates from food (n = 78), animal (n = 9), and the environment (n = 3). Detailed information on the origin, year of isolation, and PCR-based serogroup of each strain is provided in Supplementary Table S1.

DNA sequencing and assembly
Genomic DNA was extracted with a DNeasy® Blood & Tissue Kit (QIAGEN) from overnight cultures in brain heart infusion broth and then subjected to WGS via the Illumina MiSeq platform with Nextera XT library prep kits or Illumina NovaSeq 6000 with the TruSeq Nano library kit.

The raw reads were quality trimmed, and the adapters were removed via the Trimmomatic tool (version 0.39) via the following parameters: ILLUMINACLIP [adapter_file]:2:30:10 LEADING:20 TRAILING:20 SLIDINGWINDOW:5:20 AVGQUAL:20[22]. The adapter files used were those included in the Trimmomatic distribution (NexteraPE-PE.fa or TruSeq3-PE-2.fa).

The trimmed reads were then used for genome assembly, which was performed with SPAdes v 3.13.1[23]. The only SPAdes options in this step were –careful and –only assembler.

Determination of PCR-based serogroups, MLST, clonal complexes, lineages, sublineages, and core genome MLST
Draft genome assemblies of *L. monocytogenes* isolates were uploaded to a private project within the BIGSdb-Lm platform[9,11,15,24–28], powered by the BIGSdb software (version 1.31.0). From this platform, PCR-based serogroups[26], seven-locus multilocus sequence typing (MLST) sequence types (STs) and clonal complexes[9], sublineages and 1,748-locus core genome MLST (cgMLST) complex types (CTs)[11] were extracted.

cgMLST allele calling was performed via the BLASTN algorithm[29], which applies a minimum threshold of 70% nucleotide identity and 70% alignment coverage, with a word size of 10.

Phylogenetic analyses
For phylogenetic and comparative genomic analyses, 55 additional *L. monocytogenes* genomes were selected from public databases and previously published regional and international studies[11,28,30–35]. These genomes were specifically chosen on the basis of geographical origin, date of isolation, source, serotype, and clonal complex (CC) to ensure representation of the main CCs identified in the Uruguayan dataset. Detailed information on the selected genomes is available in Supplementary Table S1.

Core genome multiple sequence alignments were generated for the 142 Uruguayan isolates and the 55 comparator genomes via the Snippy pipeline version 4.6.0[36], which processes both sequencing reads and NCBI assemblies. The *L. monocytogenes* strain F2365 (NCBI Nucleotide accession NC_002973; NCBI Assembly accession GCF_000008285.1) was used as the reference genome for read mapping. This strain was chosen because it belongs to serotype 4b, one of the predominant serotypes identified in our dataset.

Genome regions putatively subjected to recombination were detected and removed from the final alignments via Gubbins v.2.2.0[37] with default parameters and a minimum of three base substitutions required to identify recombination. Maximum likelihood phylogenies were obtained from the recombination-filtered alignments via IQ-tree version 2.3.0[38] under the determined best-fit nucleotide substitution model (TVM + F + ASC + R2, as determined by ModelFinder[39] with the Bayesian information criterion) and ultrafast bootstrapping of 1000 replicates[40]. Trees were visualized and annotated with iTOL v7[41].

Comparative genomics analysis: virulence and resistance gene profiles
The presence of virulence and resistance genes was assessed in silico across the *L. monocytogenes* genomes analysed. A total of 85 loci associated with virulence or antimicrobial resistance were screened via the BIGSdb-Lm platform. Gene detection was performed with the GenPresence tool, based on BLASTN[10] with the following parameters: minimum nucleotide identity of 80%, alignment coverage of at least 80%, and a word size of 20.

Statistical analysis
The chi-square test was used to compare proportions, with a significance level set at $p < 0.05$.

Availability of data
The datasets of raw sequencing data (FASTQ files) supporting the conclusions of this article are available in the NCBI Sequence Read Archive (SRA) repository under the project accession numbers PRJNA647899 (Instituto de Higiene; https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA647899) and PRJNA215355 (FDA/CFSAN; https://www.ncbi.nlm.nih.gov/bioproject/215355). The accession numbers corresponding to each individual isolate are listed in Supplementary Table S1.

Results

Genome-based characterization of isolates from Uruguay
All analysed isolates were classified as either lineage I (125 isolates) or lineage II (17 isolates). They were assigned to 34 STs, comprising 20 CCs and one singleton (ST2522). Our analysis identified a novel sequence type, ST2832, as well as 112 previously unreported CTs, designated CT10675 to CT10788 (excluding CT10742), according to the BIGSdb database (Supplementary Table S1).

Figure 1 shows the distribution of strains across STs and PCR-based serogroups. As expected, each PCR-based serogroup encompasses multiple distinct CCs. Most CCs displayed limited ST diversity, typically consisting of one or two STs; however, CC3 exhibited the greatest heterogeneity, comprising eight distinct STs.

Clonal complex distribution by lineage and PCR-based serogroup
The predominant CCs, in decreasing order of frequency, were CC3, CC2, and CC1, which together account for 68% of all the isolates. Within lineage I, the majority of serogroup IVb strains were assigned to CC1 and CC2 (55 of 61 isolates), whereas most serogroup IIb strains were associated with CC3 (41 of 64 isolates). In lineage II, serogroup IIa strains were primarily found in CC8 (8 of 14), whereas all serogroup IIc strains were exclusively assigned to CC9 (3 of 3) (Supplementary Table S1; Fig. 1).

Source distribution of major clonal complexes
As illustrated in Fig. 2, CC3 and CC2 were the most prevalent clonal complexes identified in both clinical and food-derived isolates. CC1 was significantly associated with human clinical cases ($p < 0.05$), as depicted in Figs. 2a and 2b. In contrast, CC2 and CC517 were significantly associated with food sources ($p < 0.05$), whereas CC3 showed a similar frequency among clinical and food isolates (Fig. 2b).

Genetic diversity based on core genome MLST
Core genome MLST analysis of *L. monocytogenes* isolates revealed a total of 118 distinct CTs), 112 of which were novel according to the BIGSdb database. Among these, 104 CTs were unique to individual isolates, whereas the remaining 14 CTs included two or more isolates each (Table 1).

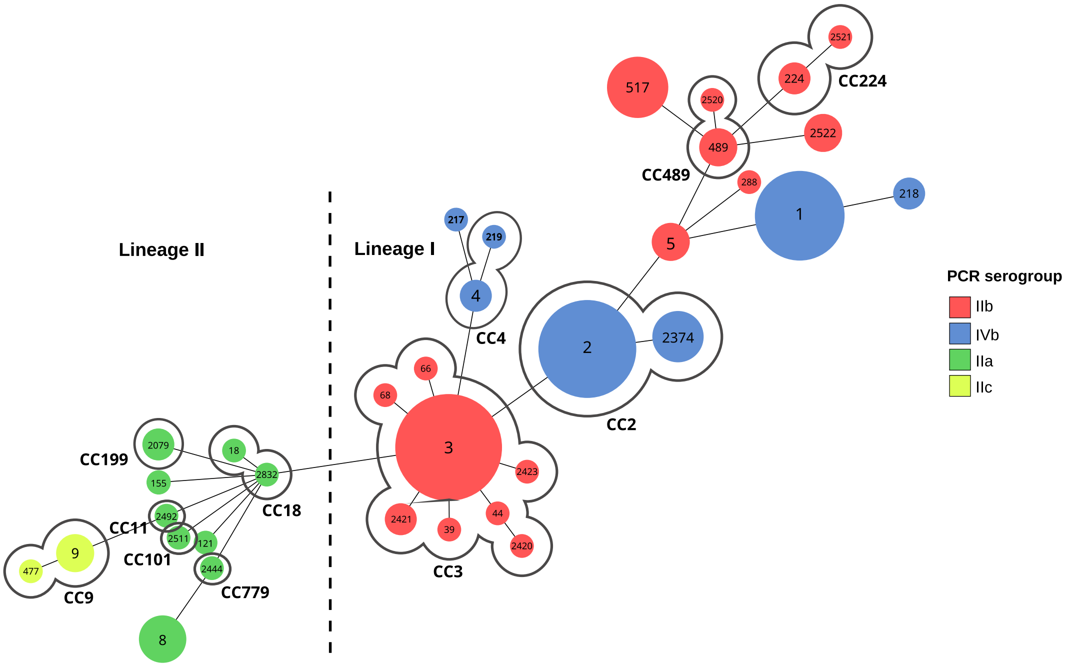

Fig. 1. Minimum spanning tree based on MLST analysis of 142 *Listeria monocytogenes* isolates from Uruguay. Sequence types (STs) are shown within circles; circle size is proportional to the number of isolates. Colours indicate distinct PCR-based serogroups. Clonal complexes (CCs) are outlined when they include more than one ST or when a CC is represented by a single ST whose number does not match the CC designation.

Phylogenetic analysis

The core genome phylogeny of 142 Uruguayan *L. monocytogenes* isolates, together with 55 genomes from diverse geographic regions, is shown in Fig. 3.

Most of the isolates (125/142) belonged to lineage I and were distributed across two main clades corresponding to serogroups IIb and IVb. This lineage exhibited considerable clonal diversity, including clusters corresponding to CC1, CC2, CC3, CC4, and CC517. In particular, CC3 formed a large clade subdivided into two closely related clusters, reflecting its high prevalence in the dataset. In contrast, lineage II was underrepresented, forming smaller clusters, which is consistent with its lower frequency in the population studied.

Most clades included isolates from multiple sources (animal, human, and food), revealing their co-circulation in different environments and suggesting epidemiological links between food and human cases of listeriosis.

As expected, isolates assigned to the same CC consistently grouped together regardless of geographic origin, supporting the concordance between MLST-based classification and whole-genome phylogenetic structure. In general, the CCs presented high genomic similarity.

No clear clustering pattern was observed based on the year of isolation (data not shown). The major CCs in this study, such as CC1, CC2, and CC3, were detected throughout the entire study period, suggesting long-term persistence of these clones in the region. In contrast, CC517 was identified only during later years (2017–2019), suggesting a more recent emergence or detection.

The selected genomes from other countries were interspersed among Uruguayan isolates, reflecting the global distribution and genetic overlap of certain CCs.

Virulence gene profiles

Comparative analysis of virulence genes revealed notable variation among *L. monocytogenes* isolates, with distinct profiles associated with specific lineages and CCs (Fig. 4).

The *Listeria* pathogenicity island 1 (LIPI-1) was universally present across all the isolates analysed in this study. In contrast, the distributions of LIPI-3 and LIPI-4 were variable and restricted to lineage I. Specifically, LIPI-3 was detected in all lineage I isolates except those belonging to CC5, CC2, and CC517. LIPI-4 was identified in CC4, as well as in CC517, ST2522, and CC217.

With respect to internalin genes, most isolates carried the full complement of known internalins, except the *inlG* and *inlL* genes. The *inlG* gene was detected in nearly all lineage II CCs—excluding CC121 and CC101—and in CC517 isolates from lineage I. In contrast, the *inlL* gene was exclusively found in lineage II isolates from CC9, CC18, and CC155.

Additionally, six isolates were found to harbor a truncated *inlA* gene due to premature stop codons (Table 2). All six isolates belonged to lineage II (CC9, CC199, and CC121) and were recovered from food sources.

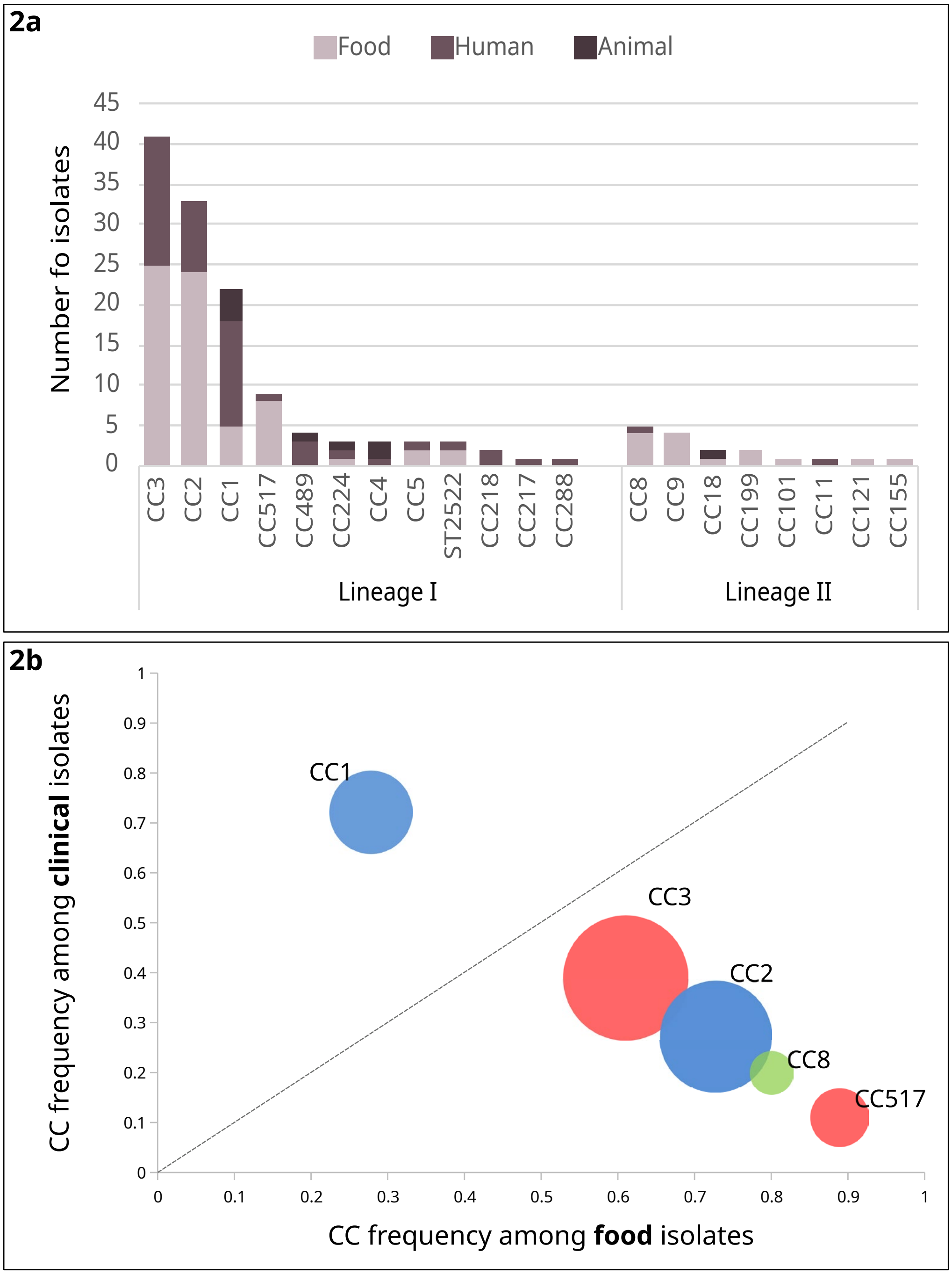

Fig. 2. Prevalence and distribution of *Listeria monocytogenes* clonal complexes by source. (**a**) Distribution of 142 isolates by clonal complex (CC), source (human, food, animal), and lineage. (**b**) Frequencies of predominant CCs (≥ 5 isolates) by source: food (x-axis) and human (y-axis). The circle size is proportional to the number of isolates.

Resistance gene profiles

The intrinsic resistance genes *fosX*, *lin*, *norB*, and *sul* were detected in all the isolates analysed. The aminoglycoside resistance gene *aacA4* was identified in seven CC3 *L. monocytogenes* isolates, including four from human sources and three from food, collected between 2011 and 2018. All *aacA4*-positive isolates had distinct CTs.

With respect to resistance determinants to quaternary ammonium compounds , five isolates carried relevant genetic elements. Four strains belonging to CC1, CC5, and CC199 harbored the *bcrABC* cassette, which confers resistance to benzalkonium chloride. Additionally, the single CC121 isolate carried the *qacH* gene associated with the transposon Tn*6188*, which encodes a small multidrug resistance efflux pump linked to benzalkonium chloride tolerance.

cgMLST type	Clonal complex	Lineage	PCR Serogroup	No. of Isolates	Isolation Year	Source
CT10690	CC1	I	IVb	2	2004–2012	Human
CT271	CC1	I	IVb	2	2014	Human*
CT7621	CC1	I	IVb	4	2018–2019	Human, Food
CT10708	CC2	I	IVb	4	2015–2018	Human, Food
CT10711	CC2	I	IVb	2	2012	Human
CT10737	CC3	I	IIb	3	2011–2016	Human, Food
CT10740	CC3	I	IIb	3	2012–2013	Human, Food
CT10741	CC3	I	IIb	2	2018	Food
CT10749	CC3	I	IIb	4	2012–2013	Human, Food
CT10755	CC3	I	IIb	2	2013–2015	Food
CT10757	CC3	I	IIb	3	2011–2015	Food
CT10695	CC517	I	IIb	2	2019	Human, Food
CT10696	CC517	I	IIb	3	2017–2019	Food
CT10788	CC8	II	IIa	2	2016–2017	Food

Table 1. *Listeria monocytogenes* core genome MLST types that include more than one Uruguayan isolates. *Neonatal listeriosis outbreak.

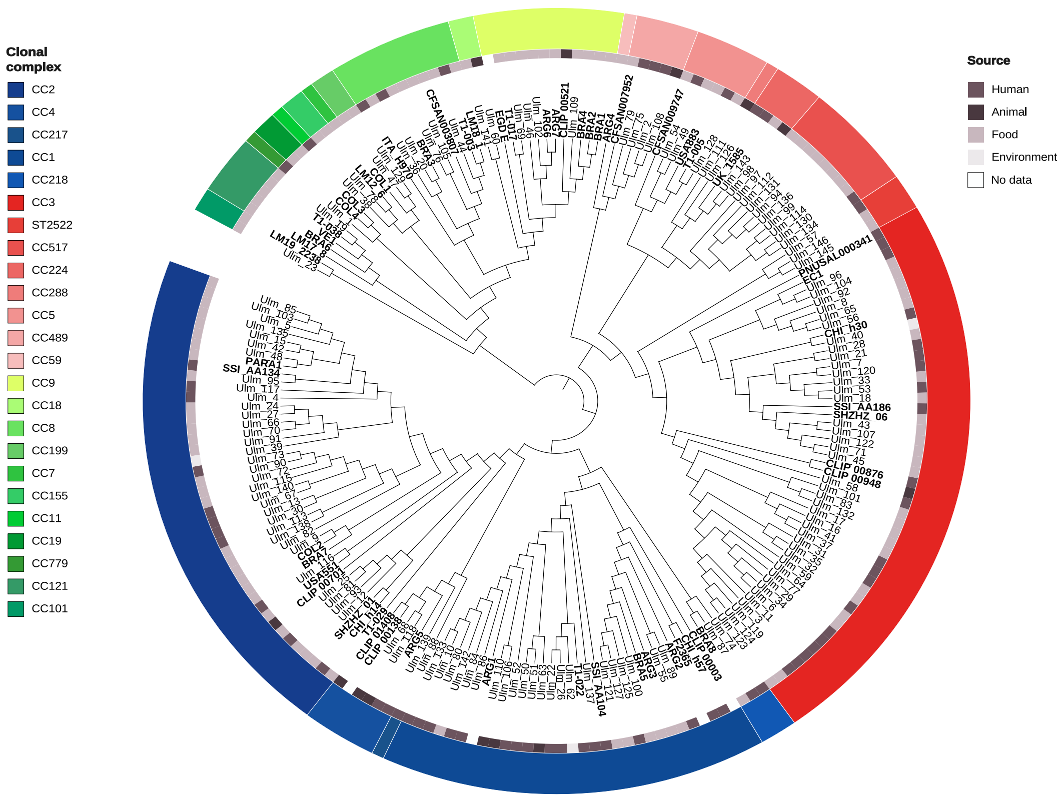

Fig. 3. Maximum-likelihood SNPs phylogenetic tree of *Listeria monocytogenes* genomes from Uruguay and other regions. The inner ring indicates the source of each isolate, as shown in the colour legend. The outer ring shows the CCs and PCR-based serogroups: blue indicates CCs corresponding to serogroup IVb, red to IIb, yellow to IIc, and green to IIa. The non-Uruguayan genomes are labeled in bold face. Branch lengths are disabled for clearer cluster distinction. The genome of the *L. monocytogenes* strain F2365 was used as a reference for SNP alignment.

Discussion

The *L. monocytogenes* genomes analysed in this study correspond to the main lineages I and II, which are the most common globally. Lineages III and IV—considered infrequent worldwide[11]—were not detected in our dataset.

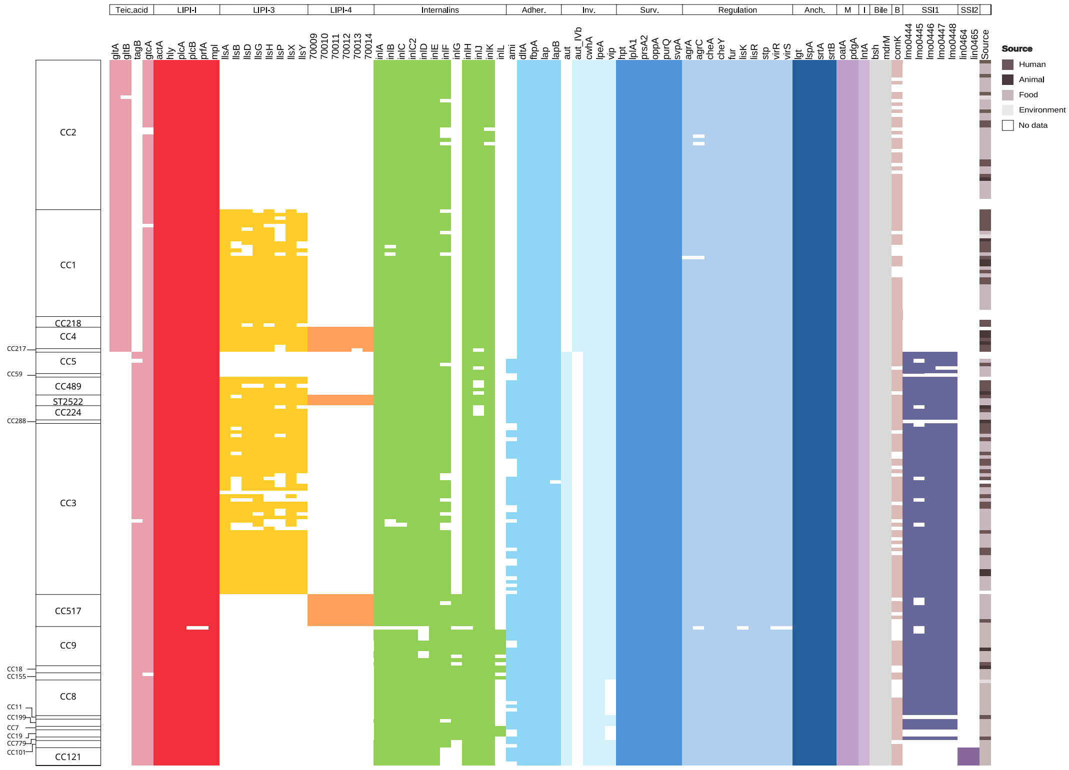

Fig. 4. Presence and distribution of virulence-associated genes among 197 *Listeria monocytogenes* isolates. Gene presence (coloured) or absence (white) is indicated. From left to right, the analysed genes include those involved in teichoic acid biosynthesis (*gltAB*, *tagB*, *gtcA*); genes located in the pathogenicity islands LIPI-1 (*actA*, *hly*, *plcA*, *plcB*, *prfA*, *mpl*), LIPI-3 (*llsAGHXBYDP*), and LIPI-4 (*LM9005581_70009* to *LM9005581_70014*); internalin genes (*inlA*, *inlB*, *inlC*, *inlC2*, *inlD*, *inlE*, *inlF*, *inlG*, *inlH*, *inlJ*, *inlK*, *inlL*); genes associated with adherence (*ami*, *dltA*, *fbpA*, *lap*, *lapB*), invasion (*aut*, *aut_IVb*, *cwhA*, *lpeA*, *vip*), intracellular survival (*hpt*, *lplA1*, *prsA2*, *oppA*, *purQ*, *svpA*), transcriptional and translational regulation (*agrAC*, *cheAY*, *fur*, *lisKR*, *stp*, *virRS*), surface protein anchoring (*lgt*, *lspA*, *srtAB*), peptidoglycan modification (*oatA*, *pdgA*), immune modulation (*lntA*), bile resistance (*bsh*, *mdrM*), and biofilm formation/virulence (*comK*), stress survival islets (SSI) 1 (*lmo0444* to *lmo0448*) and SSI-2 (*lin0464*, *lin0465*). The final columns indicate the sample source (from darkest to lightest: animal, human, food).

inlA Allele (BIGSdb)	Mutation position (nt)	Length truncated InlA (aa)	Lineage	PCR serogroup	CC	Source	N° of isolates
*inlA*_28	12 (deletion A)	8	II	IIa	CC199	Food	2
*inlA*_49	1474 (C → T)	491	II	IIa	CC121	Food	1
*inlA*_44	2054 (G → A)	684	II	IIc	CC9	Food	2
*inlA*_69	976 (G → T)	325	II	IIc	CC9	Food	1

Table 2. Frameshifts and mutations identified in this study leading to premature stop codons (PMSC) in gene *inlA*.

Our findings agree with previously reported associations between clonal complexes and disease. In particular, the high frequency of CC1 among human listeriosis cases is consistent with global trends[11–13,25,42,43]. CC6—commonly reported in clinical cases in Europe and North America[3,13,44]—was absent in our study. All three CC4 isolates identified in our study originated from clinical cases (human or animal), reinforcing the previously reported linkage between this CC and invasive listeriosis[3,13].

A distinctive feature of the *L. monocytogenes* isolates from Uruguay analysed in this study is the predominance of CC3, accounting for approximately one-third of the isolates from both food and clinical sources. This finding contrasts with reports from other regions, where food isolates are predominantly associated with CC9 and CC121, whereas in Uruguay, these clonal complexes are detected at low frequencies[13,14]. CC3 has a low frequency in the BIGSdb database, with only 473 out of 5,327 genomes (8.9%) belonging to this clonal complex (accessed May 6, 2025). In this database, the prevalence of CC3 in South America is similarly low (8.1%), except in Peru, where it accounts for 8 out of 39 isolates (21%). In other countries, such as Mexico, the USA, Canada, France, and China, CC3 rarely exceeds 10% of isolates. However, Australia stands out as a notable outlier, with a CC3 prevalence of 26.4% (BIGSdb, accessed May 2025), which is comparable to that observed in Uruguay. This observation is consistent with previous reports that showed high frequency of CC3 within Australian isolates[45,46]. According to the BIGSdb data, Australian CC3 isolates are derived from both clinical and food sources, particularly dairy products such as cheese. Most Uruguayan food isolates could not be linked to a specific product, as they were recovered from a variety of delicatessen items. Consistent with Maury et al.[13], CC3 in our study showed a balanced distribution between clinical and food sources (Fig. 2), reinforcing its classification as an "intermediate" clonal complex. CC3 has also been associated with outbreaks[47] and is not strongly associated with any specific food product[48]. The similar prevalence of CC3 in Uruguay and Australia may reflect shared ecological or epidemiological factors, such as comparable farming systems or dietary habits—particularly the high consumption of beef, sheep meat, and dairy products. However, further investigation is needed to elucidate the determinants underlying this distinctive geographical distribution.

Another noteworthy finding of our study was the detection of CC517, with nine isolates recovered between 2017 and 2019. These isolates were obtained primarily from food samples, mainly from cured meat products, and from one human case of listeriosis. Notably, the genomes of the human sample and one of the food isolates, both of which were recovered in 2019, shared the same cgMLST profile (CT10695), suggesting a potential epidemiological link (Table 1). Two additional isolates sharing the same cgMLST type (CT10696) were recovered from the same food-processing facility in 2017 and 2019, suggesting the persistence of this strain within the facility. CC517 has rarely been reported in other parts of the world, as reflected in the BIGSdb-Lm database. According to this database, only two CC517 isolates have been previously reported, both from Austria (in 2009 and 2017), originating from compost and from an unknown source (isolate IDs 2138 and 5257; genome sequences not available).

The presence of genetically related *L. monocytogenes* isolates from different geographical regions across phylogenetic clusters supports the notion of repeated international dissemination of specific sublineages, as demonstrated by Moura et al., who reported that certain sublineages undergo multiple geographic dispersal events following their emergence[11].

Whole-genome sequencing provides higher resolution than traditional subtyping methods do, allowing precise differentiation between sporadic and outbreak-related *L. monocytogenes* strains and offering robust phylogenetic evidence to link clinical cases[11,12,44]. Given the difficulties in gathering epidemiological data—due to the pathogen's long incubation period and the severity of the disease—combining WGS with epidemiological investigations enhances the ability to establish links between human listeriosis cases and their sources[3,10,15]. In our study, cgMLST analysis via the 1,748-locus scheme identified 14 distinct CTs among the Uruguayan isolates, nine of which included at least one human isolate, suggesting potential epidemiological links between human and food sources or among human cases. Notably, two isolates classified as CT271 were epidemiologically linked to a neonatal listeriosis outbreak that occurred in Uruguay in 2014. The fact that outbreak-related strains share an identical CT reinforces the value of cgMLST as a high-resolution tool for outbreak investigation and source attribution.

We found the key pathogenicity island LIPI-1 in all isolates, corroborating previous reports[3,11,44,49]. Given its essential role in intracellular growth and cell-to-cell spread, the universal presence of LIPI-1 highlights its fundamental contribution to the pathogenic potential of *L. monocytogenes* across diverse lineages.

The distributions of LIPI-3 and LIPI-4 were variable but exclusively restricted to lineage I isolates. LIPI-3, which encodes the cytolytic peptide listeriolysin S, was detected in all lineage I isolates except those belonging to CC5, CC2, and CC517. LIPI-4, a putative phosphotransferase system linked to clinical infections, was identified in CC4, as previously described[13], as well as in the CC517, ST2522, and CC217 strains in our collection. We did not find an association between the presence of LIPI-4 and central nervous system or maternal–fetal infections, as most of these isolates were obtained from bacteremia cases (3/4); however, this may be due to the low number of human isolates carrying LIPI-4. Despite their close phylogenetic relationships, the CC3 and CC517 isolates clearly differ in their carriage of LIPI-3 and LIPI-4 (see Fig. 3 and 4). This observation supports the previously proposed hypothesis that different clades of *L. monocytogenes* have undergone distinct pathogenicity island loss events, considering that both LIPI-3 and LIPI-4 are also present in non-pathogenic *Listeria* species[50]. The presence of LIPI-3 in CC3 and LIPI-4 in CC517 suggests that these lineages have adapted to different ecological niches, which is consistent with the evolutionary model proposed by den Bakker et al.[51].

The variable presence of the *inlG* and *inlL* genes among the isolates analysed aligns with previous reports. The *inlG* gene has been associated primarily with lineage II and less frequently with some CCs from lineage I, particularly within CC6 isolates[8,49,52]. Although our dataset did not include CC6 isolates, the detection of *inlG* in CC517 suggests a more heterogeneous distribution of this gene within lineage I than previously recognized.

Our results show that the *inlL* gene was restricted to lineage II, specifically in CC9, CC18, CC155, CC7, and CC19, consistent with previous reports. Since InlL is a known adhesin that facilitates *L. monocytogenes* attachment to abiotic surfaces, promotes biofilm formation, and is implicated in mucin binding[53], its lineage- and CC-specific distribution may contribute to differences in ecological adaptation and pathogenic potential across strains[14,54].

Additionally, mutations leading to premature stop codons in the *inlA* gene have been reported globally and are associated with attenuated virulence in mammalian hosts[9,55–59]. In our study, these *inlA* truncations were identified exclusively in lineage II isolates (CC9, CC199, and CC121), all of which were recovered from food sources. This finding is consistent with prior evidence indicating that such hypovirulent variants are predominantly associated with food and environmental isolates, highlighting their relevance in risk assessment and surveillance strategies[55–59].

The uniform presence of genes associated with intrinsic antibiotic resistance in *L. monocytogenes* (i.e., *fosX*, *norB*, *lin*, and *sul*) aligns with our previous observations from a subset of Uruguayan isolates[60] and with broader findings from a large dataset of clinical and food-derived strains[61]. In contrast, acquired antibiotic resistance genes are rarely detected. The *aacA4* gene, encoding an aminoglycoside 6′-N-acetyltransferase, was identified at a low frequency (4.9%) in our study, although its frequency was slightly higher than that reported by Moura et al.[61] in a collection of 5,339 *L. monocytogenes* isolates (0.92%). Although *aacA4* has the potential to confer resistance to aminoglycosides, no phenotypic resistance to streptomycin, gentamicin, kanamycin, amikacin, or tobramycin was observed in the study by Moura et al.[61]. Similarly, Uruguayan *aacA4*-positive isolates were previously tested and shown to be susceptible to gentamicin[60]. This lack of phenotypic expression may be attributed to reduced promoter activity, possibly due to the distance between the promoter region and the gene's start codon[61]. Furthermore, *aacA4* was found to be integrated within prophage regions of the bacterial genome, suggesting a potential phage-mediated horizontal gene transfer mechanism[62]. In our study, the *aac*A4 gene was identified in isolates belonging to CC3, which is consistent with prior findings[61,62].

Interestingly, Scortti et al.[63] demonstrated that although *fosX* is conserved across the *Listeria* genus, its role in fosfomycin resistance is epistatically suppressed in *L. monocytogenes* during host infection. This suppression occurs via activation of the PrfA virulence regulon, which upregulates Hpt permease and increases fosfomycin uptake, ultimately negating the protective effect of FosX[63]. These insights highlight the complex interplay between resistance and virulence, emphasizing that the mere presence of resistance genes does not necessarily result in phenotypic resistance—particularly when expression is modulated by host-specific regulatory networks. Understanding these regulatory interactions is essential for interpreting antimicrobial susceptibility in clinical contexts and may inform future therapeutic strategies aimed at targeting such regulatory pathways during infection.

Moura et al. (2016) described an association between benzalkonium chloride resistance genes and isolates from lineage II, particularly those belonging to CC121, which correlates with the well-documented persistence of this clonal complex in food processing environments[11]. Consistent with their findings, the only CC121 isolate in our study harbored this resistance determinant. However, we could not establish a broader association between lineage II and quaternary ammonium compound resistance in our dataset, likely due to the limited number of lineage II isolates included in the analysis.

The ability of *L. monocytogenes* to survive and adapt to harsh conditions—such as those encountered in food production environments and the human gastrointestinal tract—is facilitated by genes encoded within stress survival islets (SSIs). SSI-1 plays a critical role in tolerance to acid, osmotic, and bile stress encountered in the gastrointestinal tract[64], whereas SSI-2 contributes to resistance against alkaline and oxidative stress, which are common in food processing settings[65]. Both SSI-1 and SSI-2 are located in a hypervariable region between the genes *lmo0443* and *lmo0449*[65]. Our study demonstrated that SSI-1 is widely distributed among lineage II and serogroup IIb lineage I isolates (including CC3, CC5, CC489, CC224, CC288, CC517, and ST2522), which is consistent with previous reports indicating its presence in strains other than serotype 4[64]. SSI-2 was detected in all CC121 isolates analysed, in agreement with prior descriptions[65]. While the precise mechanism underlying this distribution remains unclear, it has been hypothesized that phage-mediated horizontal gene transfer may play a role, potentially influenced by serogroup-specific teichoic acid structures that serve as phage receptors[66].

Conclusions

Overall, this study provides a comprehensive characterization of local *L. monocytogenes* isolates and reveals a distinct population structure. The predominance of lineage I contrasts with global trends where lineage II is often more frequent in food-associated isolates. The high prevalence of CC3, both in food and clinical sources, suggests its ecological success and potential pathogenic relevance. The detection of CC517, a rarely reported clonal complex, along with the identification of several novel cgMLST types and a new MLST sequence type, underscores the need to expand global genomic databases with isolates from underrepresented regions.

Data availability

The datasets of raw sequencing data (FASTQ files) supporting the conclusions of this article are available in the NCBI Sequence Read Archive (SRA) repository under the project accession numbers PRJNA647899 (Instituto de Higiene; https://www.ncbi.nlm.nih.gov/bioproject/?term = PRJNA647899) and PRJNA215355 (FDA/CFSAN ; https://www.ncbi.nlm.nih.gov/bioproject/215,355). The accession numbers corresponding to each individual isolate are listed in Supplementary Table S1.

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Author contributions

MIM and LB participated in isolate selection and DNA extraction for sequencing. BD and MIM performed all bioinformatic analyses. MIM, BD and LB prepared figures and tables and draft the manuscript. MIM, LB, BD, VB, SV, SC, IM, and GV contributed to the interpretation of the results, as well as to the discussion and conclusions. All authors reviewed and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Additional information

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