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Ten years of molecular epidemiology surveillance of *Listeria monocytogenes* in Chile 2008–2017.

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ABSTRACT

Listeria monocytogenes causes severe diseases in humans, including febrile gastroenteritis and systemic infections that has a high mortality despite antibiotic treatment. This pathogen may cause massive outbreaks associated to the consumption of contaminated food products, which highlight its importance in public health. In the last decade, *L. monocytogenes* has emerged as a foodborne pathogen of major importance in Chile. A previous work showed that in Chile during 2008 and 2009, *L. monocytogenes* serotypes 1/2a, 1/2b and 4b were the most frequently identified in food and clinical strains. Here we report the molecular characterization of *L. monocytogenes* strains isolated from 2008 to 2017 in the country. Our results indicate that serotypes 1/2a, 1/2b and 4b continue to be the most commonly found in food products. In addition, we identify persistent and widespread PFGE subtypes. This study reports ten years of epidemiological surveillance of*L. monocytogenes* in Chile.

1. Introduction

Listeria monocytogenes is a Gram-positive, facultative anaerobic pathogen of humans and animals having the ability to survive in diverse environments and inside eukaryotic cell (Ferreira et al., 2014). This pathogen is the causative agent of listeriosis, a food-borne disease that may show up as a febrile gastroenteritis or as an invasive form (systemic infection). While the febrile gastroenteritis is a self-limiting disease mainly affecting immunocompetent individuals, the invasive form of this infection causes severe symptoms, high rates of hospitalization and mortality (20–30%) affecting principally nursing infants, immunocompromised and elderly individuals (Buchanan et al., 2017; Centers for Disease Control and Prevention (CDC), 2013; Thønnings et al., 2016). In pregnant women, listeriosis may present as a mild disease, but it can cause severe clinical outcomes for the fetus, including sepsis, meningitis, and death (Centers for Disease Control and Prevention (CDC), 2013). Although the incidence of listeriosis is low, it is a major cause leading to death related to foodborne diseases in developed countries (Kirk et al., 2015; Scallan et al., 2011; Thomas et al., 2015).

L. monocytogenes grows and survives in different types of habitats, even in adverse conditions, such as dry environments, high salt concentrations (10%, wt/vol), low temperatures (−0.5–9.3 °C) and a wide pH range (4.7–9.2) (Ferreira et al., 2014). Because of its special growth abilities, ubiquity, attachment and biofilm formation on inert surfaces, this bacterium may easily colonize and persist in food processing plants. Therefore, its surveillance, control and elimination constitute a major challenge for the food industry and public health (Autio et al., 2002; Ferreira et al., 2014; Gianfranceschi et al., 2009).

Based on the somatic (O) and flagellar (H) antigens, *L. monocytogenes* is classified into 13 serotypes. Four of them (1/2a, 1/2b, 1/2c y 4b) cause most of cases of human listeriosis (Cossart, 2011). In particular, serotype 4b is linked to the majority of listeriosis outbreaks (Cartwright et al., 2013; Montero et al., 2015).

In 2008, 2009, two massive listeriosis outbreaks occurred in Chile, which were associated to the consumption of soft cheese (Brie and Camembert) and sausages/meat products, respectively (Montero et al., 2015). These outbreaks highlighted the public health importance of this pathogen in the country, and consequently, the Chilean sanitary legislation was modified to implement a more rigorous and comprehensive

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epidemiological surveillance. Since then, a number of studies have investigated the presence of *L. monocytogenes* in food-associated environments and food products distributed throughout the country and reported that a percentage of them were contaminated with this pathogen (Costa et al., 2016; Foerster et al., 2015, 2013; Montero et al., 2015; Saludes et al., 2015; Toledo et al., 2018).

In one of the aforementioned studies, our group analyzed the presence of *L. monocytogenes* in several food products distributed in Chile in 2008 and 2009, and found that serotypes 1/2a, 1/2b and 4b were the most frequently detected (Montero et al., 2015). Even more importantly, by pulsed field gel electrophoresis (PFGE), we were able to demonstrate that some food strains were clonally related to clinical strains which caused sporadic cases and the 2008 listeriosis outbreak. Here we complement our previous results and report the molecular characterization of *L. monocytogenes* strains isolated from 2008 to 2017. This study represents ten years of continuous epidemiological surveillance of this pathogen and reinforce the need to continue implementing food safety regulations to prevent new outbreaks and sporadic cases of listeriosis in the country.

2. Materials and methods

2.1. Strains

Isolation and detection of *L. monocytogenes* strains in food and clinical samples was performed, at the Laboratorio de Salud Pública Ambiental y Laboral de la Seremi de Salud de Santiago, as previously reported (Montero et al., 2015). The epidemiological data of each strain is shown in Table S1. Additionally, the reference strains *L. monocytogenes* ATCC 19115 and *L. ivanovii* ATCC 19119 were used.

2.2. Identification of serotypes and virulence associated genes by PCR

Genomic DNA was obtained growing *L. monocytogenes* strains in trypticase soy agar with yeast extract (TSAYE), followed by suspension and boiling of selected colonies in 150 µl of distilled sterile water for 10 min. Suspensions were then centrifuged at 9000 rpm and 2 µl of the supernatant was used as template for the PCR assays. Serotyping of the strains was performed by multiplex PCR following the protocols described by Doumith et al. (2004) (Doumith et al., 2004). This assay detects the presence of four serotype-specific marker genes (lmo0737, lmo1118, ORF2819, ORF2110) and amplifies *prs*, which is found in all *Listeria* spp. Moreover, as an internal control. *L. monocytogenes* serotypes 1/2a, 1/2b, 1/2c, and 4b were included in each reaction. Isolates were grouped into molecular serogroups based on the presence or absence of each target included in the multiplex PCR assay as detailed previously (Doumith et al., 2004). Molecular serotyping helped to cluster *L. monocytogenes* isolates into five serogroups: IVb (4b-4 ab, 4d-4e); IIa (1/2a-3a); IIb (1/2b-3b-7); IIc (1/2c-3c) and IVa (4a-4c); specifically associated with the following three lineages: I (1/2b-3b-7-4b-4d-4e), II(1/2a-3a-1/2c-3c) and III (4a and 4c) (Doumith et al., 2004; Michelon et al., 2015; Ragon et al., 2008). The virulence associated genes *hly*, *inlA*, *inlB* and *prfA* were detected by PCR using primers and protocols that we described in a previous work (Montero et al., 2015). It is worth mentioning that the detection of *hlyA* was done using two pairs of primers, Hly-F/Hly-R and Hly2-F/Hly2-R, which amplify products of 1100 and 852 bp, respectively (Montero et al., 2015).

2.3. Pulse field gel electrophoresis (PFGE)

The PFGE was performed following the PulseNet protocol for subtyping *L. monocytogenes* (Graves and Swaminathan, 2001). The *ApaI* enzyme was used for macrorestriction of genomic DNA embedded in the agarose plugs (1.2% SeaKem Gold Agarose). Electrophoresis was conducted on a CHEF-DR III BIO-RAD in a 1% Pulse Field Certified Agarose gels (Ultrapure DNA grade agarose) at 6 V/cm and 14 °C during 21 h. Genomic DNA of *Salmonella* serotype Braenderup H9812 was digested with the *XbaI* enzyme and used as a reference size standard. The gels were stained with ethidium bromide (Sigma) and analyzed using the software BioNumerics GelCompar II v. 6.0 (Applied Maths, Sint-Martens-Latem, Belgium). The similarity between fingerprints was determined using Dice's correlation coefficient with a 1% tolerance between band positions. The cluster analysis and generation of dendrograms was performed using the UPGMA clustering algorithm. Pulsotypes and pulsogroups were defined on the basis of similarities of ≥95 and ≥80%, respectively.

2.4. Statistical analysis

Pairwise association between serotypes and virulence associated genes was performed in contingency tables by odds ratios. The statistical significance of these associations was determined using Pearson's chi-squared test or Fisher's exact test (when frequencies were less than 5). When any of the cells values of the contingency table was zero, 0.5 was added (Haldane correction) to all the cells to avoid errors in the statistical test. A p value < 0.05 was considered significant.

3. Results

3.1. Serotyping and detection of virulence genes

A total of 365 *L. monocytogenes* strains were analyzed (Table S1). These strains were isolated from 2008 to 2017 from clinical cases (n = 40) and several food products including cheese (n = 49), cooked meat (n = 8), cooked sausage (n = 9), fresh fish (n = 11), fresh vegetables (n = 2), frozen seafood (n = 39), frozen vegetables (n = 27), ham (n = 5), ice cream (n = 4), mixed food (n = 39), pâté (n = 32), raw meat (n = 52), raw poultry (n = 20), raw sausages (n = 8) and smoked fish (n = 20) (Table 1).

Regarding the prevalence of serotypes, the most common one was 4b (146/365; 40%) followed by 1/2a (114/365; 31,2%), 1/2b (63/365; 17,3%) and 1/2c (30/365; 8,2%). Besides, 12/365 (3,3%) strains were

Table 1
Serotypes, date of isolation and virulence genes content of Listeria monocytogenes strains.

Serotype	No. of strains (%)	Clinical strains	Food strains *	Year of isolation from 2008 to 2017. No of strains										Virulence genes. No of strains (%)			
				08	09	10	11	12	13	14	15	16	17	*hly*	*prfA*	*inlA*	*inlB*
1/2a	114 (31)	8	106	26	42	17	3	2	7	9	0	6	2	114 (100)	83 (73)	101 (89)	95 (83)
1/2b	63 (18)	11	52	15	19	8	0	5	8	1	3	4	0	63 (100)	60 (95)	61 (97)	54 (86)
1/2c	30 (8)	0	30	5	2	14	3	1	3	1	1	0	0	30 (100)	15 (50)	26 (87)	18 (60)
4b	146 (40)	21	125	88	24	8	0	4	10	3	2	7	0	146 (100)	141 (97)	134 (92)	145 (99)
nt	12 (3)	0	12	6	6	0	0	0	0	0	0	0	0	11 (92)	11 (92)	8 (67)	6 (50)
Total	365 (100)	40	325	140	93	47	6	12	28	14	6	17	2	364 (99)	310 (85)	330 (90)	318 (87)

* Cheese (n = 49), cooked meat (n = 8), cooked sausage (n = 9), fresh fish (n = 11), fresh vegetables (n = 2), frozen seafood (n = 39), frozen vegetables (n = 27), ham (n = 5), ice cream (n = 4), mixed food (n = 39), pâté (n = 32), raw meat (n = 52), raw poultry (n = 20), raw sausages (n = 8) and smoked fish (n = 20).

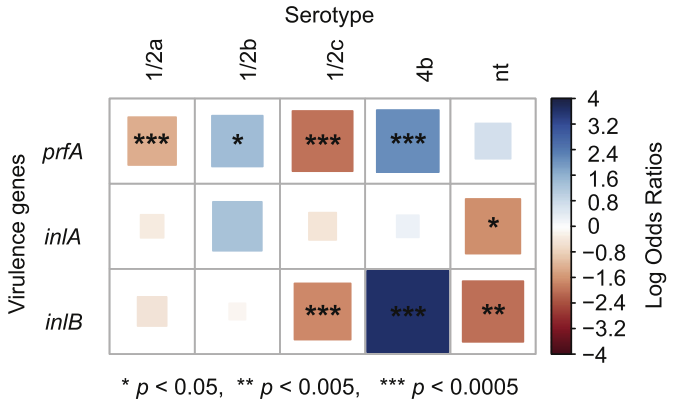


Fig. 1. Pairwise association plot for *Listeria monocytogenes* serotypes and virulence genes. Red and blue squares represent negative and positive associations, respectively. The color scale represents the magnitude of the association determined by Odds Ratios. Statistical significance of the associations was determined by Pearson's chi-square test or Fisher's exact test (when frequencies were less than 5). The figure was prepared using the package corrplot (Wei et al., 2017) in R (R Core Team, 2014). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

non-typeable (Table 1).

In order to examine the virulence potential of the strains, we searched by PCR a set of virulence associated genes, including the genes coding for listeriolysin (*hly*), the transcriptional regulator PrfA (*prfA*), internalin A (*inlA*) and internalin B (*inlB*). Of these genes, *hly* (364/365; 99,7%) was the most frequently detected followed by *inlA* (330/365; 90%), *inlB* (318/365; 87%) and *prfA* (310/365; 85%). In general, the frequency of detection of these genes was higher in serotype 4b when compared to other serotypes. In fact, serotype 4b, as opposed to serotype 1/2c, was positively associated to the presence of these genes, being these associations with *prfA* and *inlB* statistically significant (Fig. 1).

Initially a multiplex PCR assay which incorporated only one of the specific primers for *L. monocytogenes hlyA* gene (Hly-F/Hly-R, 1100 bp product) (Montero et al., 2015) was used as an internal control to verify the species identification of these isolates by PCR (Burall et al., 2011). However, in eight isolates only the primers pair Hly2-F/Hly2-R were positive for the PCR amplification of the *hlyA* gene, generating a PCR product of 852 bp (Montero et al., 2015). The serotypes associated to this pair of primers, i.e. positive for the 852 bp of the *hlyA* gene, were 1/2a (n = 4) or non-typeable (n = 4, one of them with a weak band of PCR product) strains. Only one non-typeable isolate was PCR negative for both *hlyA* pairs of primers, perhaps because it corresponds to a non-*Listeria monocytogenes* species. The lack of PCR product for *hlyA* (1100 bp) may be due to the miss-match of our primers or a sequence deletion containing our primer at the 3′ end.

3.2. Molecular subtyping and temporal distribution of strains

We investigated, by PFGE, the clonal relationships among the strains. As a result, 41 pulsogroups (A1 to O2) and 189 pulsotypes were identified (Fig. 2). In general, strains of a specific pulsogroup belonged to one or two serotypes. Additionally, some pulsogroups grouped strains isolated from a specific food type. For instance, most strains from pulsogroups D1, G1, N1, Z1, A2, F2, N2 were isolated from cheese, frozen seafood, mixed food, raw poultry, pâté, frozen vegetables and raw meat, respectively (Table S1). However, all other pulsogroups grouped strains isolated from several food types. This indicates a substantial genetic diversity of *L. monocytogenes* strains among the food samples.

On the other hand, a number of pulsotypes (4, 10, 14, 17, 22, 26, 27, 25 and 95) contained both clinical and food strains. For example, pulsotype 22 contained 12 clinical strains from the 2008 listeriosis outbreak, 32 strains isolated from cheese and one strain isolated from raw poultry (Fig. 3). Previously, we reported that these cheese samples were the origin of the 2008 listeriosis outbreak (Montero et al., 2015). In the other aforementioned pulsotypes, the clinical strains were isolated from sporadic cases of listeriosis and it was not possible to associate the food strains with disease due to lack of epidemiological data. However, it is interesting to note that all these pulsotypes contained strains isolated in 2008 and 2009 but not in later years. However, it is necessary to mention that our access to clinical strains is also very limited.

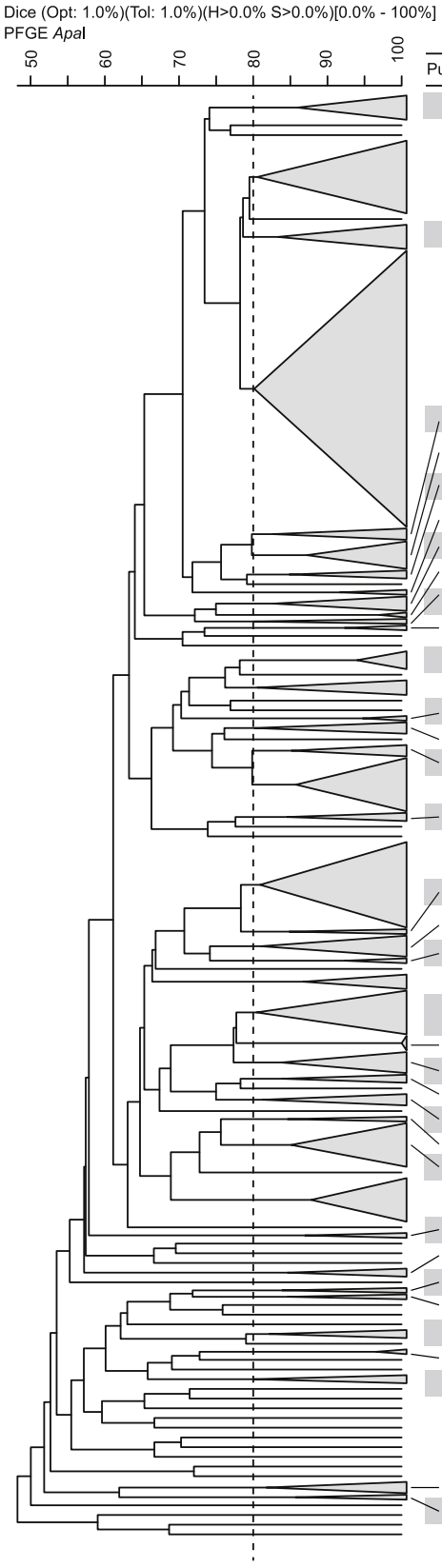
A large database of PFGE patterns corresponding to strains isolated during several years provides a historical baseline that may allow to investigate the dynamics and temporal distribution of specific pulsotypes (Barrett et al., 2006). In this sense, we were able to identify a number of pulsotypes (9, 15, 25, 46, 58, 84, 99, 116, 120, 122, 131 and 139) which were repeatedly found (persistent) in different years and food products (Fig. 4). In addition, we also found pulsotypes (65, 87 and 88) including strains isolated in the same year but from different food products. These results indicate the widespread distribution of some *L. monocytogenes* clonal populations, which may persist over the years.

In addition, several subtyping methods, including PFGE, have demonstrated that *L. monocytogenes* strains can be grouped into at least three genetic lineages called genetic lineages I, II and III (Fox et al., 2012; Galvão et al., 2012; Okwumabua et al., 2005). Although most subtyping studies of *L. monocytogenes* have defined these lineages with characteristic serotype groupings, the nomenclature of these three lineages is not always consistent (Fugett et al., 2007). In our work, mainly serotypes 1/2b (3b-7) and 4b were consistently grouped into a lineage I while serotypes 1/2a and 1/2c were mostly grouped into lineage II. However, according to our PFGE results (> 80% similarity), some pulsogroups of strains associated to lineage I also included one 1/2c serotype and ten 1/2a serotype strains. Furthermore, three 4b strains and four 1/2b strains were grouped together with some pulsogroups of lineage II (Fig. 2). We also characterized twelve non-typeable strains isolated from food, including pâté (5), raw meat (5) or smoked fish (2) (Table S1). One of the non-typeable strains was *hlyA* negative and, therefore, it might belong to another species of the *Listeria* genus.

4. Discussion

L. monocytogenes has emerged as an important foodborne pathogen in Chile. In 2008, 2009, two massive listeriosis outbreaks occurred in the country and, since then, there has been a slight increase in the incidence of sporadic cases. According to official reports, in 2017 there were 86 sporadic cases of listeriosis, causing a mortality of 26% (MINSAL. Gobierno de Chile, 2017). Most of these clinical cases (53/86; 62%) were caused by strains belonging to serotype 4b. Previously, we reported that 4b is the most prevalent *L. monocytogenes* serotype in ready to eat food (Montero et al., 2015). In other South American countries, 4b is also among the most frequently serotypes identified in food products and clinical cases (Braga et al., 2017; Macedo et al., 2017; Vallim et al., 2015). In the present study, we characterized a group of *L. monocytogenes* strains isolated during ten years in several food products distributed in Chile. The serotyping of these strains by PCR indicated that serotypes 1/2a and 4b were the most frequently detected.

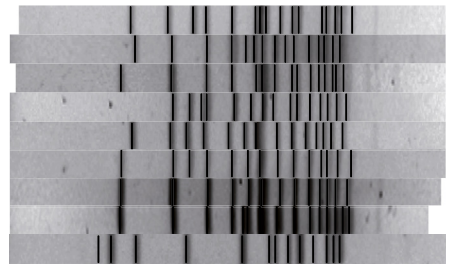
An important finding was that virulence associated genes *hly*, *prfA*, *inlA* and *inlB* were detected with a high frequency (> 90%) in serotype 4b strains (Table 1), which could perhaps be related to the higher incidence of this serotype in human listeriosis cases. Among these genes, *hly* and *prfA* are located in the *Listeria* pathogenicity island 1 (LIPI-1) and therefore, the molecular detection of these genes can be extrapolated to the presence of this genetic element (Montero et al., 2015).



Pulsogroup	Similarity	Serotypes (No. of isolates)	Pulsotypes (No. of isolates)
A	86	1/2b(3), 4b(5)	1(2), 2(1), 3(2), 4(3)
B	81	4b(23)	7(2), 8(1), 9(5), 10 (3), 11(2), 12(5), 13(1), 14(2), 15(2)
C	83	1/2b(6), 1/2c(1), 4b(1)	17(4), 18(1), 19(1), 20(1), 21(1)
D	80	1/2b (9), 4b (77)	22(45), 23(1), 24(9), 25(3), 26(8), 27(3), 28 (1), 29(1), 30(1), 31(1), 32(1), 33(1), 34(1), 35(2), 36 (1), 37 (1), 38 (1), 39 (1), 40(1), 41(3)
E	83	1/2a(1), 1/2b(2), nt(1)	42(2), 43(1), 44(1)
F	87	1/2b(8), 4b (1)	45(2), 46(5), 47(1), 48(1)
G	85	4b(3)	49(2), 50(1)
H	92	1/2b(2)	52(1), 53(1)
I	83	1/2b(4), 4b(1)	54(2), 55(1), 56(1), 57(1)
J	97	1/2b(2)	58(2)
K	80	1/2a(1), 1/2b(1)	59(1), 60(1)
L	92	4b(2)	61(1), 62(1)
M	94	1/2a(6)	65(5), 66(1)
N	81	1/2b(4), nt(1)	68(4), 69(1)
O	94	1/2a(2)	72(1), 73(1)
P	80	1/2b(4)	74(2), 75(1), 76(1)
Q	85	1/2b(2), 4b(2)	78(1), 79(1), 80(1), 81(1)
R	86	4b(17)	82(4), 83(2), 84(2), 85(1), 86(1), 87(4), 88(3)
S	85	4b(3)	89(2), 90(1)
T	81	1/2a(17), 1/2c(6), 4b(1) nt(3)	93(1), 94(1), 95(4), 96(3), 97(2), 98(1), 99(6), 100(2), 101(1), 102(1), 103(1), 104(3), 105(1)
U	85	1/2a(1), 1/2c(1)	106(1), 107(1)
V	81	1/2a(4), 1/2b(1), 1/2c(2)	108(1), 109(1), 110(2), 111(2), 112(1)
W	92	1/2a(2)	113(1), 114(1)
X	87	1/2a(4),1/2c(1)	116(2), 117(2), 118(1)
Y	80	1/2a(13), nt(1)	119(3), 120(3), 121(1), 122(2), 123(1), 124(1), 125(2), 126(1)
Z	100	1/2a(5)	127(5)
A2	84	1/2a(7)	128(5), 129(1), 130(1)
B2	84	1/2a(3)	131(2), 132(1)
C2	81	1/2a(3), nt(1)	134(3), 135(1)
D2	85	1/2a(1), 4b(1)	137(1), 138(1)
E2	85	1/2a(12), nt(2)	139(11), 140(1), 141(1), 142(1)
F2	88	1/2a(12), 1/2c(2)	144(11), 145(3)
G2	87	1/2a(1), nt(1)	147(1), 148(1)
H2		1/2a(2), 1/2b(1)	152(2), 153 (1)
I2	84	1/2c(1), 4b(1)	155(1), 156 (1)
J2	85	1/2c(1), 1/2c(1)	157(1), 158(1)
K2	82	1/2a(2), 1/2c(1)	162(1), 163(1), 164(1)
L2	97	1/2b(1), 1/2c(1)	166(2)
M2	80	1/2b(1), 1/2c(2)	169(2), 170(1)
N2	82	1/2c(4)	181(2), 182(1), 183(1)
O2	86	1/2a(1), 1/2c(1)	184(1), 185(1)

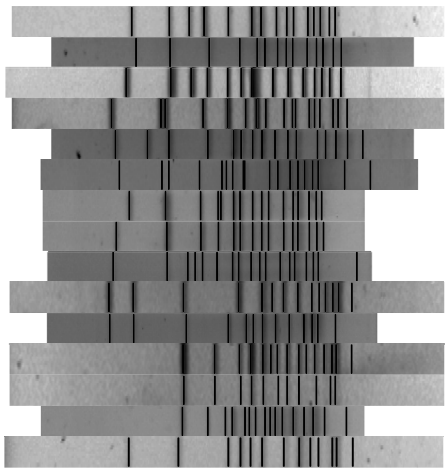
Fig. 2. Dendrogram showing the PFGE analysis of 365 *Listeria monocytogenes* strains isolated from clinical cases or foods during 2008–2017. A total of 41 pulsogroups (A to O2) and 189 pulsotypes were identified. Serotypes and pulsotypes belonging to each pulsogroup are shown.

In contrast to serotype 4b and non-typeable strains, the simultaneous detection of *hly* and *prfA* genes in serotype 1/2c strains was infrequent (Table 1). The above could be explained by deletions and recombination events or by genomic variability in the LIPI-1 carried by these strains. For instance, a recent study showed that there is a considerable nucleotide variability among the LIPI-1 harbored by strains of



*Apa*I pattern	Pulsotype (n)	Serotype (n)	Clinical isolates	Year (n)	Food type (n)	Year (n)
	4(3)	1/2b(3)	1	2008(1)	Cooked sausage(1), Pâté(1)	2009(2)
	10(3)	4b(3)	1	2008(1)	Frozen seafood(2)	2008(2)
	14(2)	4b(2)	1	2008(1)	Frozen seafood(1)	2009(1)
	17(4)	1/2b(3), 1/2c(1)	2	2008(2)	Frozen seafood(1), Pâté(1)	2009(2)
	22(45)	1/2b(3), 4b(42)	12 *	2008(12)	Cheese(32), Raw poultry(1)	2008(33)
	26(8)	4b(8)	1	2008(1)	Frozen vegetables(6), Frozen seafood(1)	2008(6), 2009(1)
	27(3)	4b(3)	2	2008(1), 2009(1)	Frozen seafood(1)	2009(1)
	35(2)	1/2b(1), 4b(1)	1	2009(1)	Frozen vegetables(1)	2009(1)
	95(4)	1/2a(2), 4b(1), nt(1)	1	2008(1)	Raw meat(2), Pâté(1)	2008(2), 2009(1)

Fig. 3. Representative *ApaI* pattern for selected pulsotypes isolated from foods or clinical cases. Serotypes, sources and dates of isolation are shown. Note that there are pulsotypes including strains isolated in different years or foods. * Clinical strains from the 2008 outbreak.



*Apa*I pattern	Pulsotype (n)	Serotype (n)	Food type (n)	Year (n)
	9(5)	4b(5)	Raw meat(2), Frozen seafood(2), Smoked fish(1)	2008(3), 2009(2)
	15(2)	4b(2)	Cooked sausage(1), Mixed food(1)	2010(1), 2013(1)
	25(3)	4b(3)	Cheese(2), Frozen vegetables(1)	2008(2), 2009(1)
	46(5)	1/2b(4), 4b(1)	Cooked sausage(1), Raw poultry(1), Smoked fish(2), Pâté(1)	2008(3), 2009(2)
	58(2)	1/2b(2)	Cream Ice(1), Mixed food(1)	2010(1), 2013(1)
	65(5)	1/2a(5)	Cooked sausages(3), Ham(1), Frozen seafood(1)	2014(5)
	84(2)	4b(2)	Mixed food(1), Fresh fish(1)	2012(1), 2013(1)
	87(4)	4b(4)	Mixed food(3), Fresh fish(1)	2016(4)
	88(3)	4b(4)	Raw meat(1), Raw poultry(2)	2010(3)
	99(6)	1/2a(4),1/2c(1), nt(1)	Raw meat(3), Frozen vegetable(1), Pâté(2)	2008(3), 2009(3)
	116(2)	1/2a(2)	Raw meat(1), Cooked meat(1)	2010(1), 2016(1)
	120(3)	1/2a(3)	Raw poultry(1), Pâté(2)	2008(1), 2009(2)
	122(2)	1/2a(2)	Smoked fish(1), Pâté(1)	2008(1), 2009(1)
	131(2)	1/2a(2)	Mixed food(1), Cooked meat(1)	2012(1), 2016(1)
	139(11)	1/2a(9), nt(2)	Smoked fish(3), Frozen seafood(6), Pâté(2)	2008(1), 2009(10)

Fig. 4. Representative *ApaI* pattern for selected pulsotypes that were repeatedly found (persistent) in food samples between 2008 and 2016, or which were found in more than one food sample in the same year. Serotypes, sources and dates of isolation are shown.

serotypes 4b and 1/2a, which was consistent with the serotype assignment of the isolates (Hadjilouka et al., 2018). Thus, genome sequencing of the serotype 1/2c may provide more information regarding the LIPI-1 harbored by them. Therefore, non-typeable strains could possess changes in the target sequences of the markers selected for PCR serotyping, which means that the protocol cannot associate them with a particular serotype. On the other hand, considering that these strains were positive for the *prs* and *hlyA* genes, some strains could belong to 4c or 4d serotypes (Table S1). In this sense, perhaps an alternative could be to work in parallel with serology (more expensive and technically more complex), without ruling out that this may also give negative results if the strain fails to express the antigens or these may have changed structurally and become undetectable by sera. The existence of *L. monocytogenes* strains belonging to serotypes other than those presently recognized within the species could be associated to new strains having a pathogenic potential, which cannot be disregarded.

In this study we used a cut-off value of similarity of ≥95% to establish pulsotypes rather than indistinguishable PFGE patterns. The reason for this is that we analyzed strains isolated from several years, and therefore, it could be expected that some clonal populations were subjected to evolutionary pressures and diversification events, which could lead to difference in one or two bands in PFGE patterns. In spite of this, we found that, in general, strains from a specific pulsotype in most cases belonged to the same serotype (Figs. 3 and 4). Besides, only few pulsotypes were persistent over the years, reflecting a rapid diversification or a high degree of genetic variability of these bacteria. Other studies have also identified by PFGE a number of persistent and widespread *L. monocytogenes* pulsotypes in food products and in food-associated environments (Autio et al., 2002; Vongkamjan et al., 2013). However, recent studies have shown that whole-genome sequencing (WGS) and single-nucleotide polymorphism (SNP) based phylogenetic analyzes improve the subtyping and determination of the genomic diversity of *L. monocytogenes* (Stasiewicz et al., 2015; Toledo et al., 2018). Consequently, WGS and SNPs analyses would complement and improve the molecular epidemiology and surveillance of this pathogen in Chile.

5. Conclusions

This study summarizes a permanent 10-year surveillance (2008–2017) of *Listeria monocytogenes* in the city of Santiago, Chile. *L. monocytogenes* is an emerging foodborne pathogen in the country and the serotypes 1/2a, 1/2b and 4b are the most frequently detected in food products and from clinical cases of listeriosis. Although some clonal populations are persistent and widespread, the genomic diversity of this pathogen in Chilean food products is considerable and change over time.

The availability of a large, diverse and time extended PFGE patterns database is a valuable tool for the molecular epidemiology of *L. monocytogenes* as well as of other pathogens. In this sense, novel approaches such as WGS and SPNs analyses will complement and increase our understanding of the population and epidemiology of this pathogen. This study contributes to the knowledge of the population dynamics of this pathogen in Chile and highlights the need to continue its rigorous surveillance.

Author contributions

Conceptualization, Roberto Vidal and Maricel Vidal; Data curation, Carolina Paduro and David Montero; Formal analysis, Carolina Paduro, David Montero, Nayaret Chamorro, Leandro Carreño, Roberto Vidal and Maricel Vidal; Funding acquisition, Roberto Vidal; Investigation, Maricel Vidal; Methodology, David Montero and Roberto Vidal; Resources, Roberto Vidal and Maricel Vidal; Supervision, Roberto Vidal; Validation, David Montero; Writing – original draft, David Montero; Writing – review & editing, Roberto Vidal and Maricel Vidal.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.103280.

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