

SUPPLEMENTAL DATA

Figure S1. Robustness of reference-based shared genome hqSNV analysis pipeline. Maximum likelihood phylogenetic analyses for 39 *L. monocytogenes* isolates using data collected in (A) 2010 using GAIIX/454/Sanger and (B) 2014 using high quality MiSeq data (136,302 and 142,935 shared hqSNVs, respectively) (refer to **Methods**). Technical sequencing replicates generated by different platforms, laboratories/users, sample preparation methods, and with various data qualities and read lengths exhibit similar phylogenetic topologies and genetic distances. Genetic distance bars indicating the shared hqSNV frequency are shown. Reference genome: 08-5578 (3,032,624 bp); shared genome sizes: 2,554,801 bp (2010 data set) and 2,596,205 bp (2014 data set).

Figure S2. Species-wide *L. monocytogenes* shared genome hqSNV phylogeny. Species-wide shared hqSNV phylogeny for *L. monocytogenes* isolates ($n=52$, 9 distinct outbreaks). A total of 143,045 hqSNV positions were identified across a shared genome of 2,595,673 bp. Reference genome: 08-5578. Additional details in **Methods**. (A) Minimum spanning tree, Isolates are colour-coded by epidemiology (inset). MLST clonal complex (CC) groups and number of hqSNVs between isolates are indicated. Food isolate, f; food processing equipment isolate, e; ready-to-eat, RTE. (B) Maximum likelihood tree.

Figure S3. *L. monocytogenes* Lineage I shared hqSNV phylogeny. Shared hqSNV phylogeny for lineage I *L. monocytogenes* isolates ($n=11$, 4 distinct outbreaks). A total of 15,435 hqSNV positions were identified across a shared genome of 2,720,701 bp. Reference genome: 81-0861. Additional details in **Methods**. (A) Minimum spanning tree; Isolates are colour-coded by epidemiology (inset); MLST clonal complex (CC) groups and number of hqSNVs between isolates are indicated; Food isolate, f; food processing equipment isolate, e; ready-to-eat, RTE. (B) Maximum likelihood tree.

Figure S4. *L. monocytogenes* CC8 shared hqSNV phylogeny. Shared hqSNV phylogeny for Clonal Complex 8 (CC8) lineage II *L. monocytogenes* isolates ($n=32$, 2 distinct outbreaks and 15 sporadic cases) (**Table S1**). A total of 361 hqSNV positions were identified across a shared genome of 2,790,598 bp. Reference genome: 08-5578. (A) Minimum spanning tree (B) Maximum likelihood tree. Isolates are colour-coded by epidemiology (inset). MLST clonal complex (CC) groups and number of hqSNVs between isolates are indicated. Food isolate, f; food processing equipment isolate, e; ready-to-eat, RTE.

Figure S5. Population structure of clinical *Listeria monocytogenes* in Canada. The population structure of 536 disease-causing *L. monocytogenes* from Canada (1981-2013) represented by a 7-gene MLST-based minimum-spanning tree generated in BioNumerics v6.5 [1]. Nodes correspond to sequence type (ST); node diameters are proportional the frequency of each ST, and the serotype is indicated (colour legend). Branch numbers show the number of allelic differences between connected STs. Grey zones identify STs in the same clonal complex (CC).

Figure S6. Whole-genome Comparison of Canadian Isolates With Publicly Available *L. monocytogenes* Genomes. Neighbour-joining phylogeny of 39 finished *L. monocytogenes*

Canadian genomes (**Table S1**) and 23 publically available genomes (**Table S7**) based on global pairwise alignments generated using ClustalW [2] and MAUVE v20150226 [3]. Epidemiologically related isolates listed in are highlighted, and serotypes and lineages are shown. The blue zone indicated CC8 isolates. A genetic distance bar (bottom left) is indicated.

Figure S7. PFGE analysis of *L. monocytogenes* isolates in this study. PFGE dendrograms for 42 *L. monocytogenes* isolates were generated using standard PulseNet protocols and BioNumerics v6.5 and *AscI* (**Panel A**) or *ApaI* (**Panel B**) restriction enzymes. The PFGE pattern identification and *p*-values for dendrogram nodes are shown. NLEP#, isolate name. Additional details in **Methods**.

Table S1. Canadian *Listeria monocytogenes* isolates analyzed in this study.

Table S2A. Shared hqSNV matrix for 39 *L. monocytogenes* isolates sequenced in 2010. A total of 136,302 hqSNV positions were identified across a shared genome of 2,554,801 bp. Reference genome: 08-5578. Additional details in **Methods**.

Table S2B. Shared hqSNV matrix for 39 *L. monocytogenes* isolates sequenced in 2013. A total of 142,935 hqSNV positions were identified across a shared genome of 2,596,205 bp. Reference genome: 08-5578. Additional details in **Methods**.

Table S3. Species-wide shared hqSNV matrix for *L. monocytogenes* isolates ($n=52$, 9 distinct outbreaks). A total of 143,045 hqSNV positions were identified across a shared genome of 2,595,673 bp. Reference genome: 08-5578. Additional details in **Methods**.

Table S4. Shared hqSNV matrix for lineage I *L. monocytogenes* isolates ($n=11$, 4 distinct outbreaks). A total of 15,435 hqSNV positions were identified across a shared genome of 2,720,701 bp. Reference genome: 81-0861. Additional details in **Methods**.

Table S5. Shared hqSNV matrix for lineage II *L. monocytogenes* isolates ($n=41$, 5 distinct outbreaks). A total of 49,359 hqSNV positions were identified across a shared genome of 2,709,893 bp. Reference genome: 08-5578. Additional details in **Methods**.

Table S6. Shared hqSNV matrix for Clonal Complex 8 (CC8) lineage II *L. monocytogenes* isolates ($n=32$, 2 distinct outbreaks and 15 sporadic cases) (**Table S1**). A total of 361 hqSNV positions were identified across a shared genome of 2,790,598 bp. Reference genome: 08-5578. Additional details in **Methods**.

Table S7. Publically available *Listeria monocytogenes* genomes used in this study.

Table S8. Sequences of substituted primers used for MLST.

A

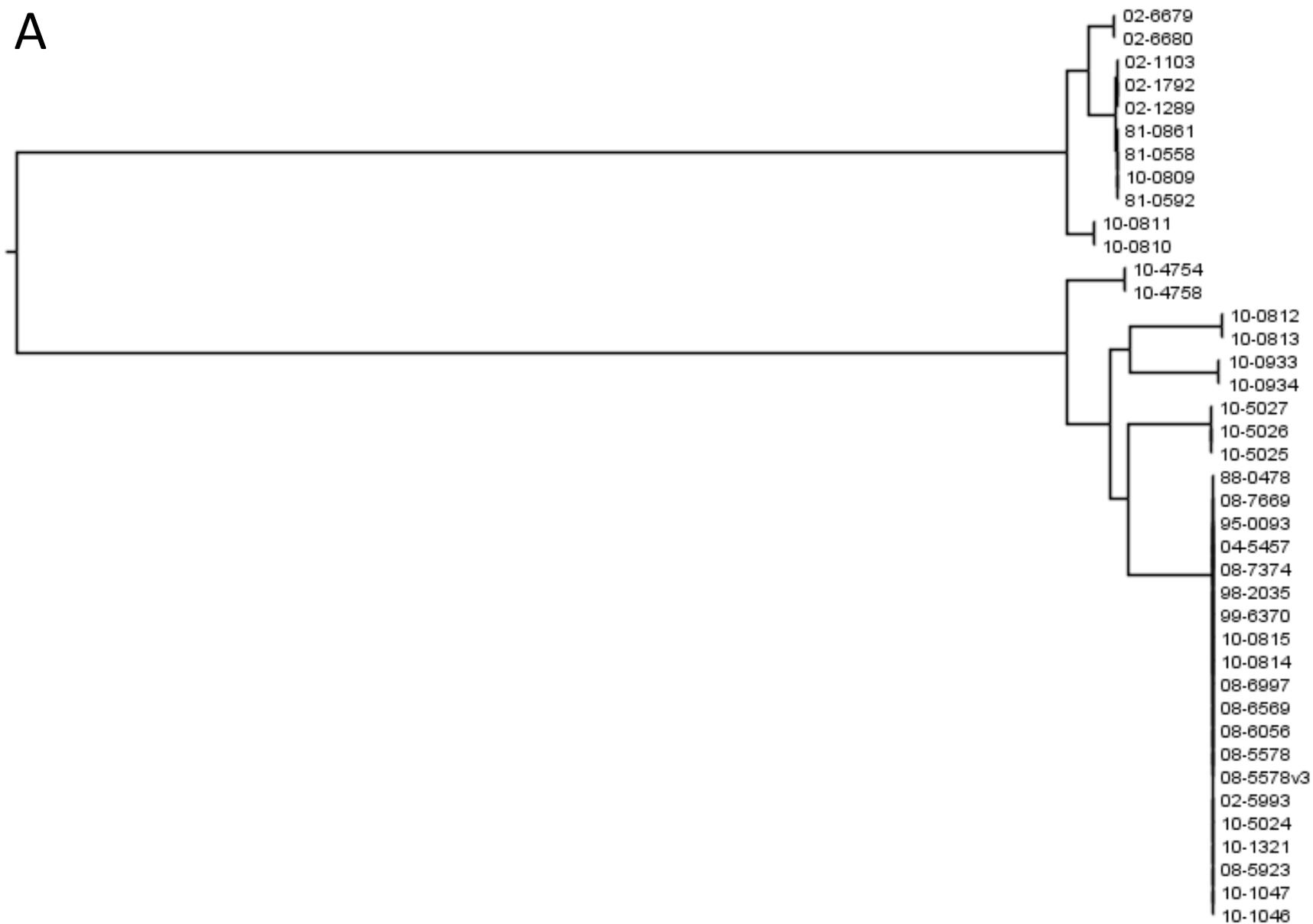


FIGURE S1A

0.2

B

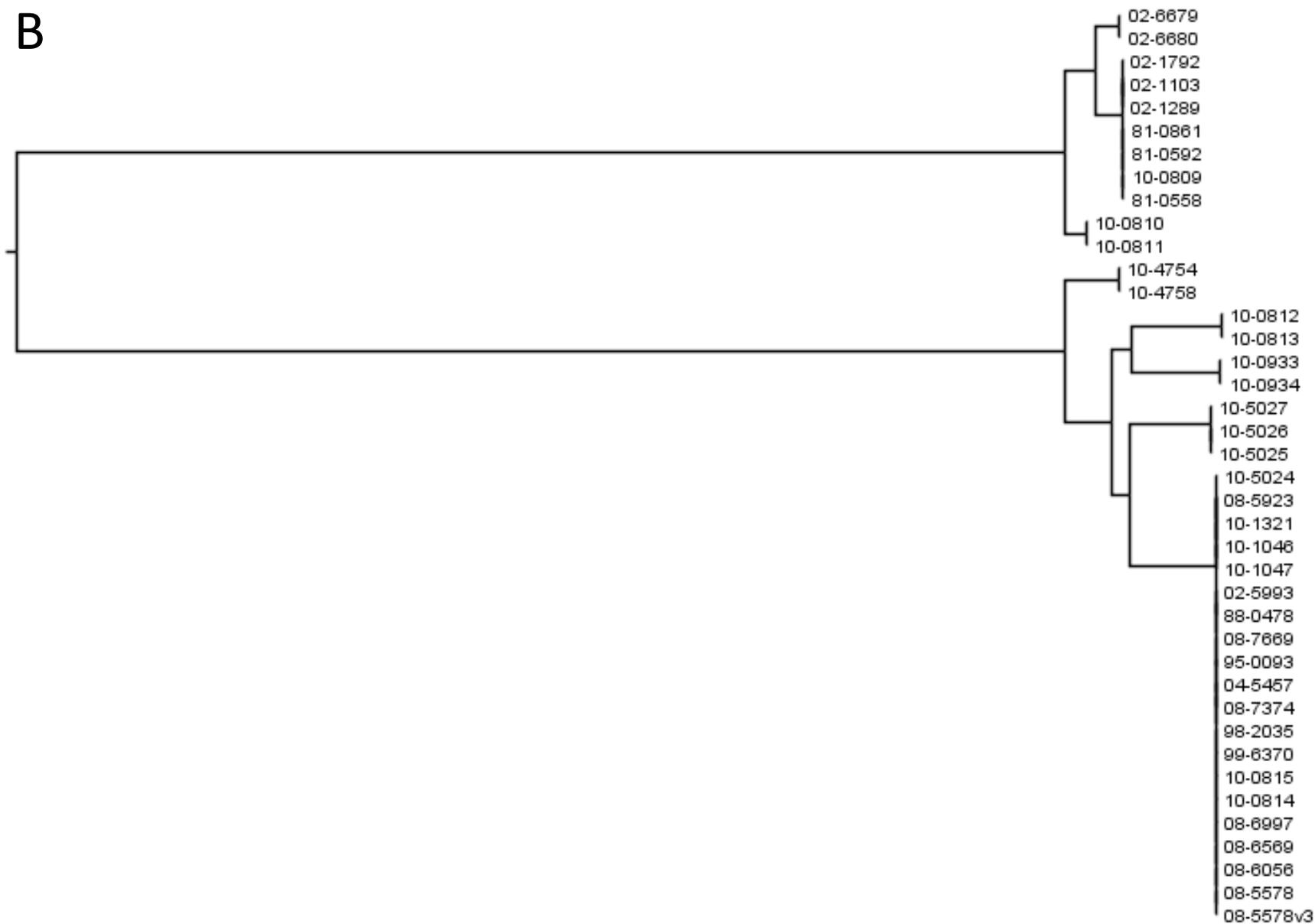


FIGURE S1B

0.2

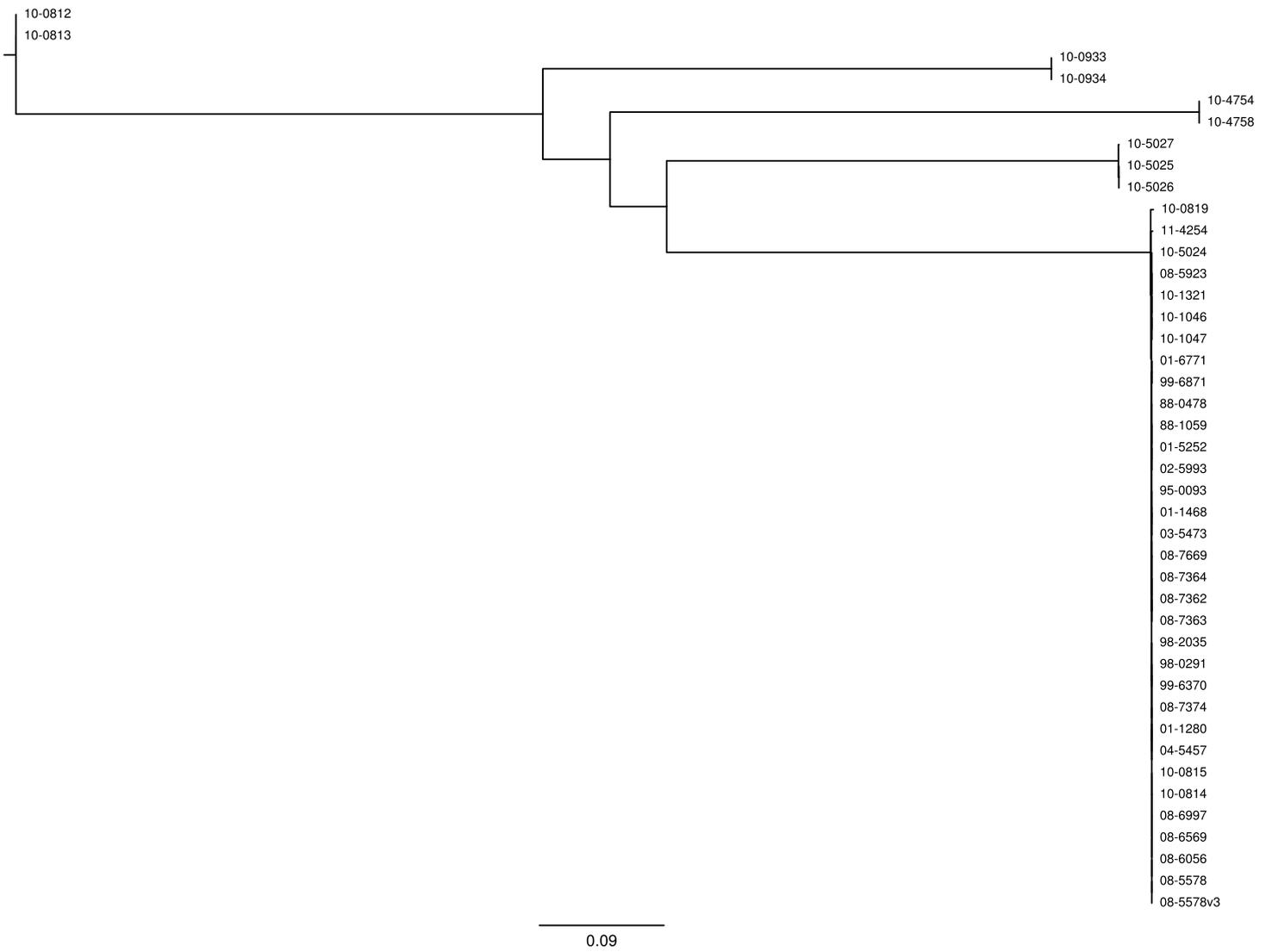
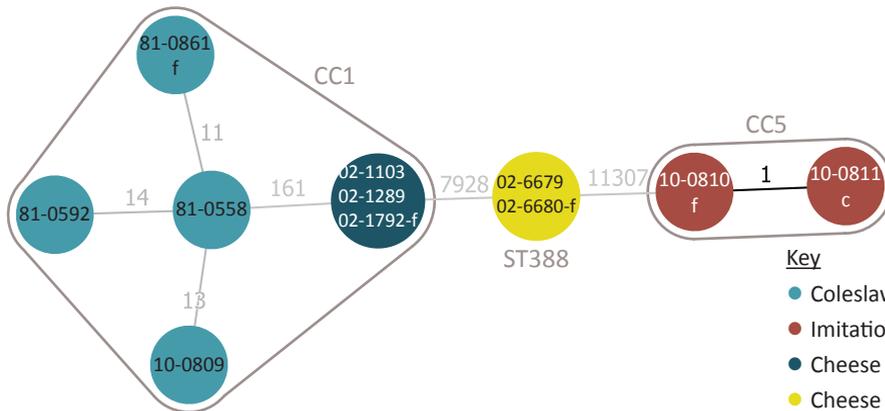


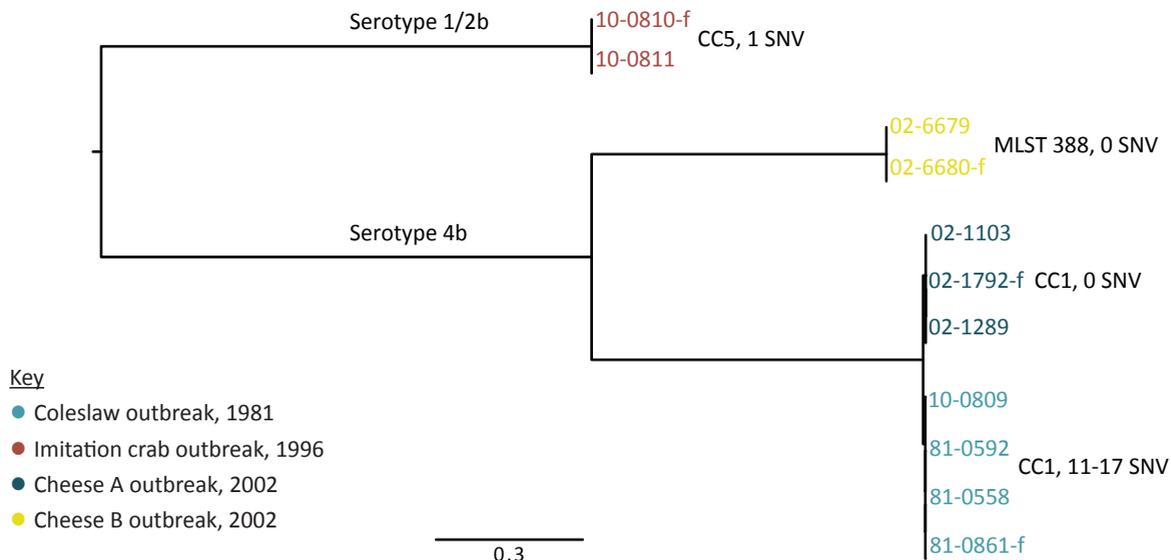
FIGURE S2B

FIGURE S3

A



B



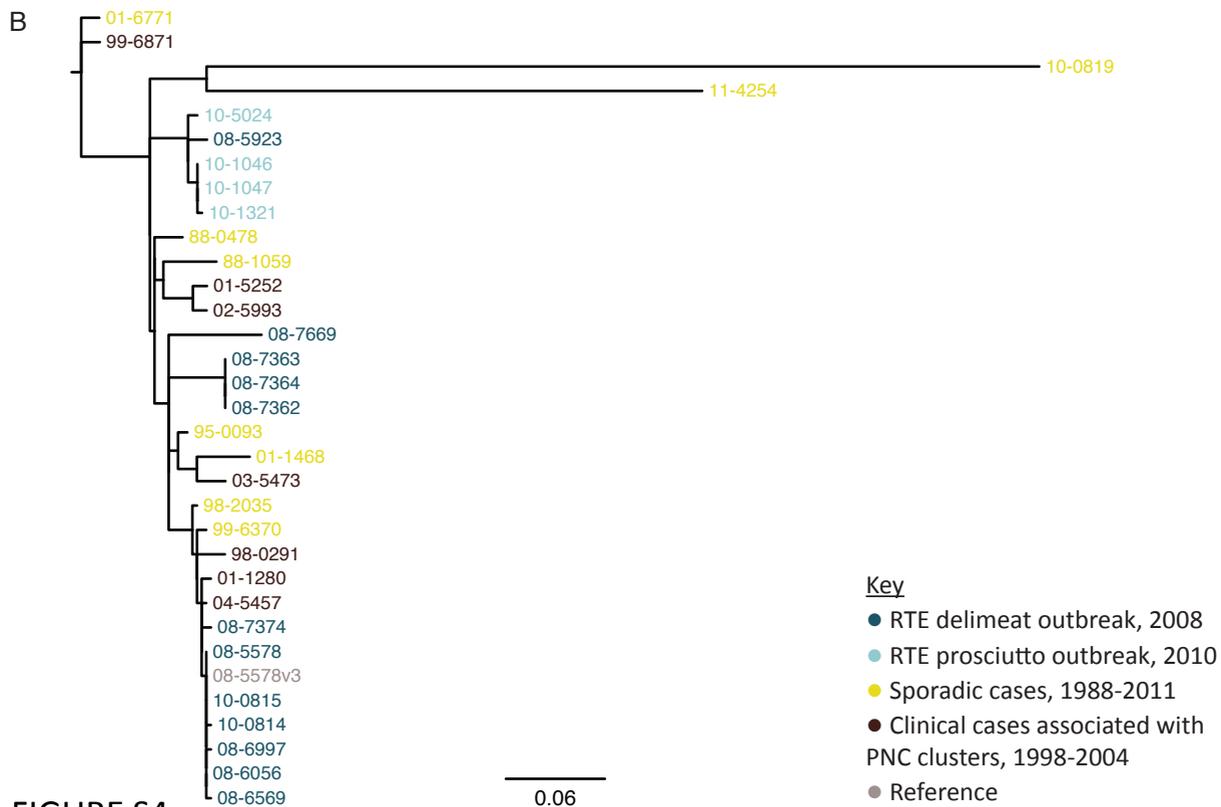
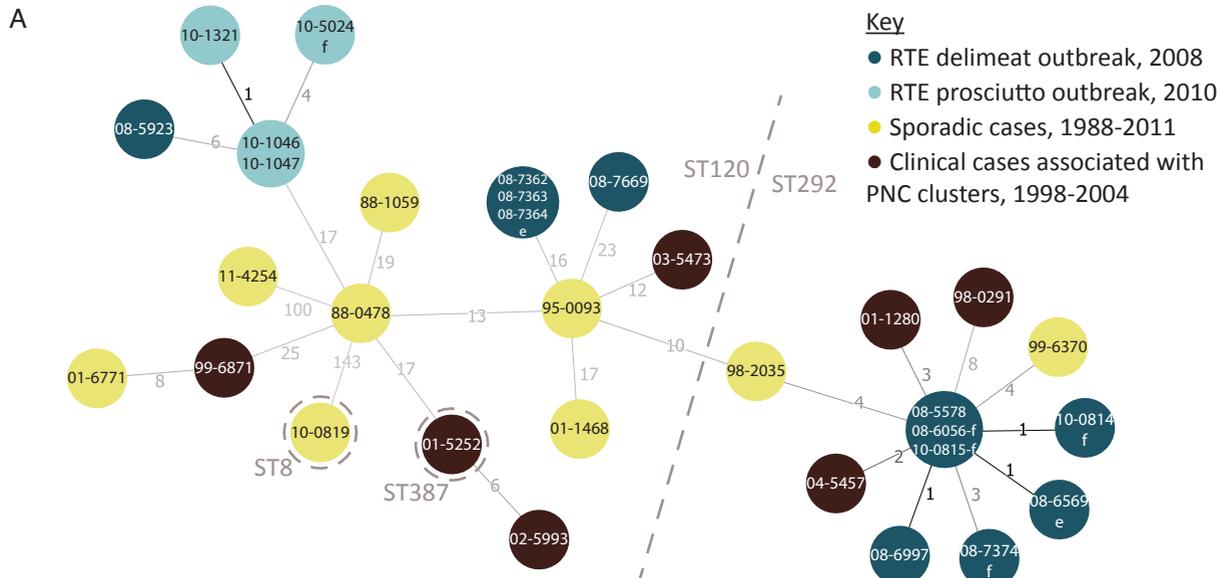


FIGURE S4

Multi-Locus Sequence Typing (MLST) Minimum Spanning Tree (MST) Canadian *L. monocytogenes* Isolates, all isolates in database as of 2013-06

MLST method by Ragon et al. 2008 with alignment and MST created via Bionumerics v6.5; numbers on branches indicate allele differences between connected sequence types (ST); node diameter is proportional to number of isolates; node colour shows isolate serotype.

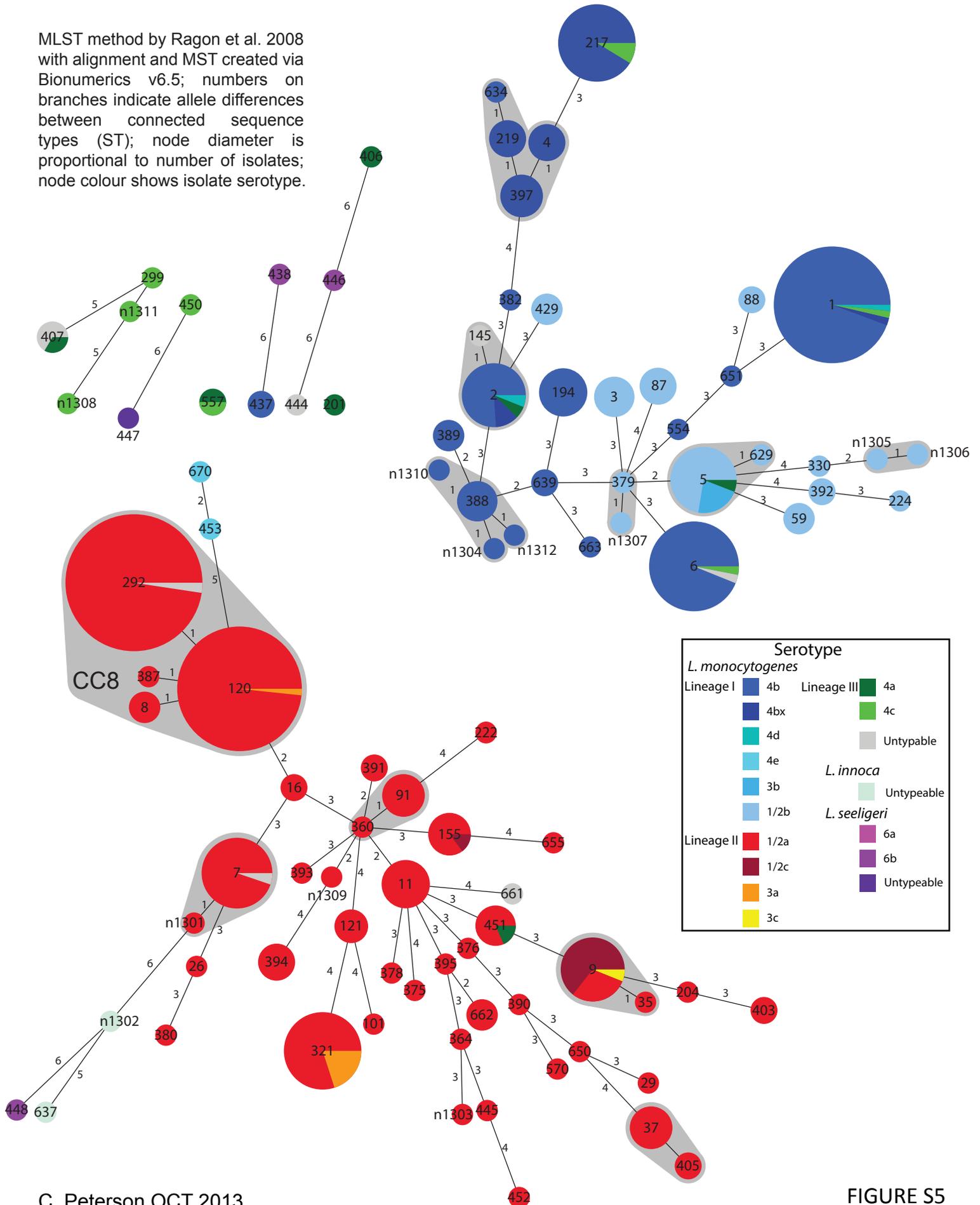
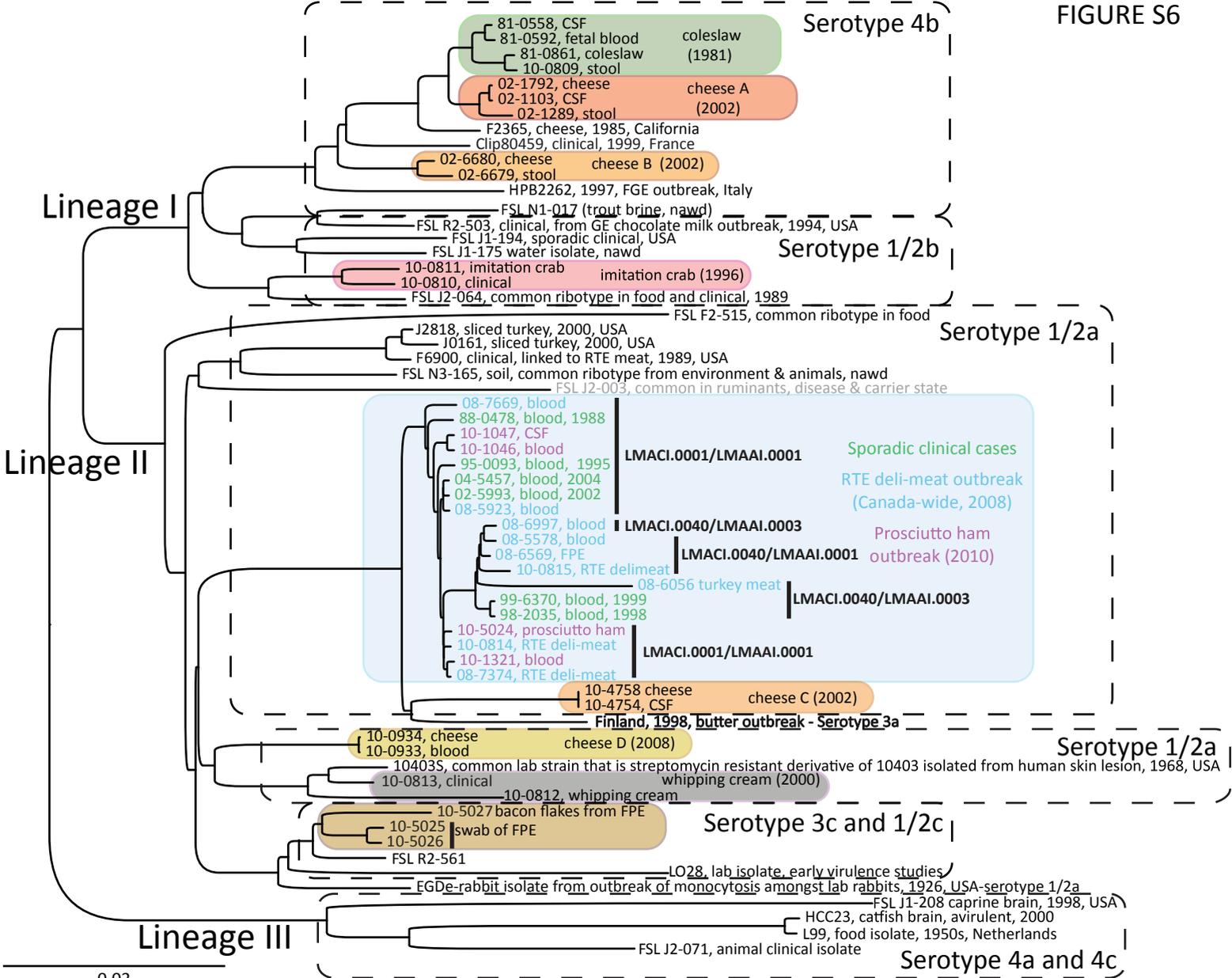


FIGURE S6



0.03

PFGE-Ascl

PFGE-Ascl

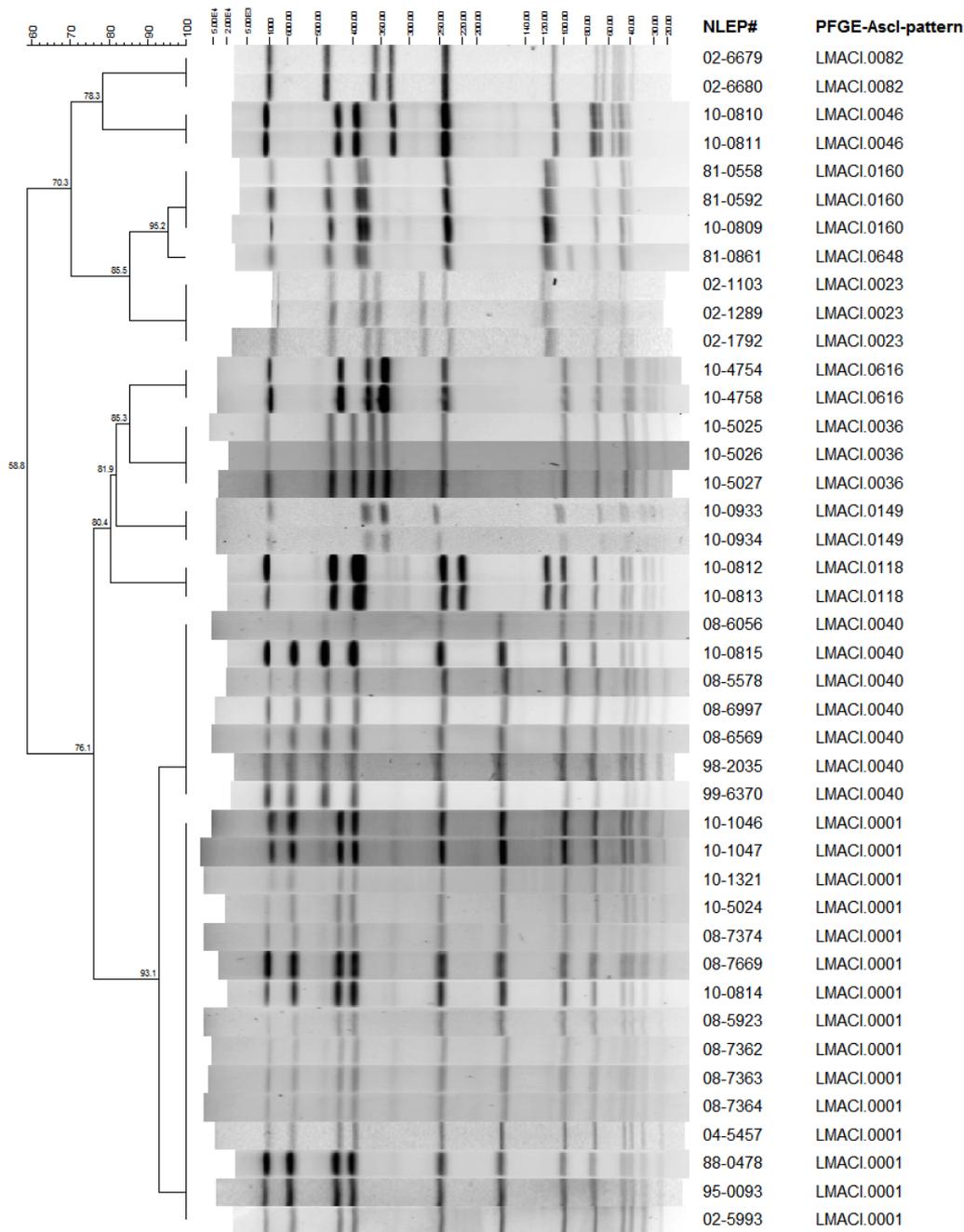


FIGURE S7A

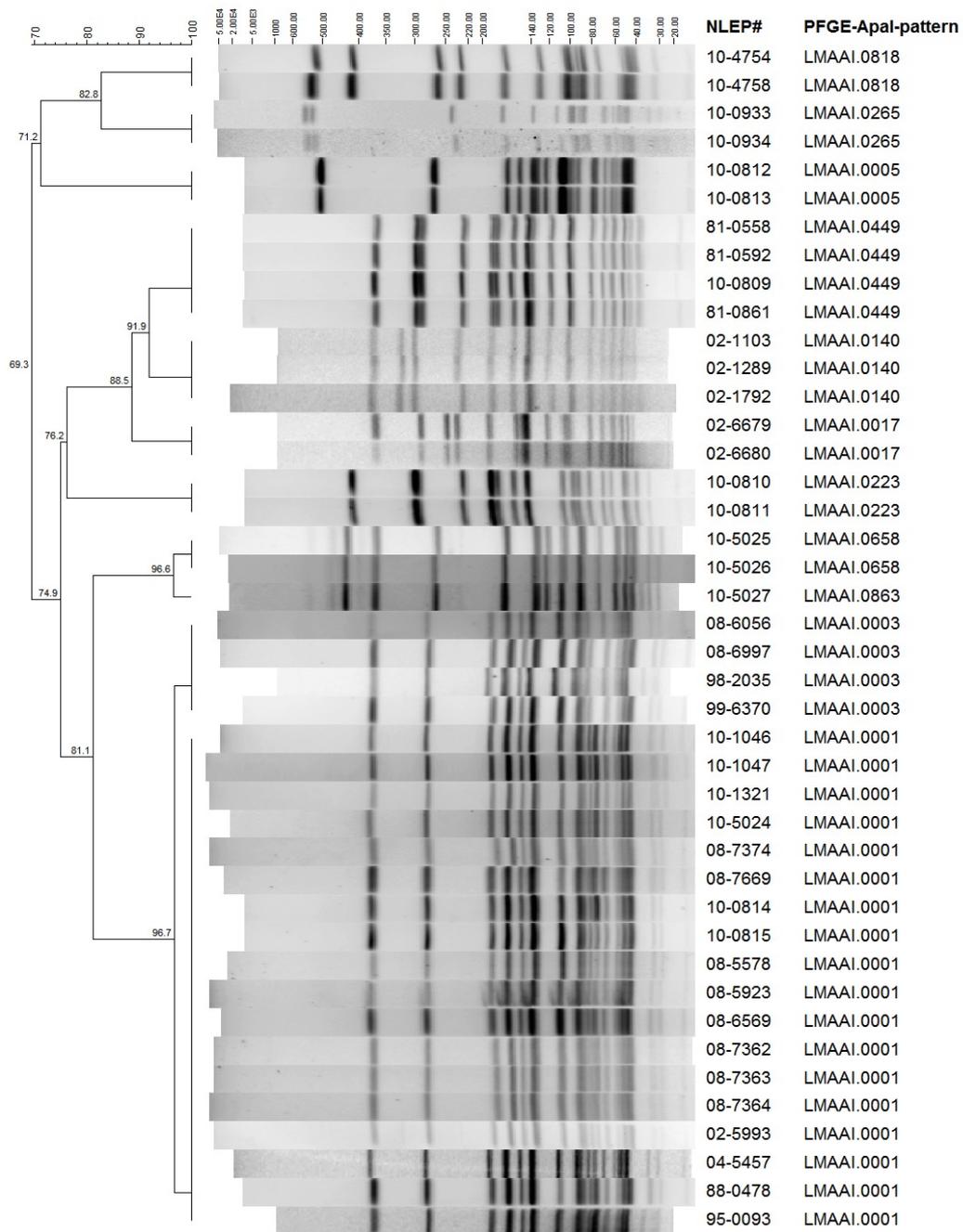


FIGURE S7B

Table S1. Canadian *Listeria monocytogenes* isolates analyzed in this study

Outbreak Investigation	Isolate	Figure 3 designation	Year Isolated	Submitting Province/Region	Specimen Type	Source	Serotype	MLST	Clonal complex	PFGE		Epidemiology	Reference	Assembly status	Genome Accession	Bioproject	BioSample	
										<i>AscI</i>	<i>Apal</i>							
Coleslaw, 1981	81-0861	NA	1981	Nova Scotia	Food	Coleslaw	4b	1	1	LMACI.0648	LMAAI.0449			Complete	CP006874	PRJNA167874	SAMN05771116	
	10-0809	NA	1981	Nova Scotia	Clinical	Stool	4b	1	1	LMACI.0160	LMAAI.0449	41 cases; 17 deaths including 2 adults with meningitis, 4 stillbirths, 5 [19] perinatal		Complete	CP007167	PRJNA167875	SAMN07124730	
	81-0592	NA	1981	Nova Scotia	Clinical	Fetal blood	4b	1	1	LMACI.0160	LMAAI.0449			Complete	CP007526	PRJNA167873	SAMN02713778	
	81-0558	NA	1981	Nova Scotia	Clinical	CSF	4b	1	1	LMACI.0160	LMAAI.0449			Complete	CP007525	PRJNA167872	SAMN02713777	
Imitation crab, 1996	10-0811	NA	1996	Ontario	Food	Imitation crab	1/2b	5	5	LMACI.0046	LMAAI.0223		2 cases; 0 deaths, severe gastroenteritis, 1 septic and hospitalized	Farber JM, Daley EM, MacKie MT, Limerick B: A small outbreak of listeriosis potentially linked to the consumption of imitation crab meat. <i>Lett Appl Microbiol</i> 2000, 31(2):100-104.	Complete	CP007169	PRJNA167877	SAMN07124732
	10-0810	NA	1996	Ontario	Clinical	Stool	1/2b	5	5	LMACI.0046	LMAAI.0223			Complete	CP007168	PRJNA167876	SAMN07124731	
Whipping cream, 2000	10-0812	NA	2000	Manitoba	Food	Whipping Cream	1/2a	7	7	LMACI.0118	LMAAI.0122	7 cases: 6 mild, 1 hospitalized and CSF positive	Pagotto F, Ng LK, Clark C, Farber J, Canadian Public Health Laboratory Network: Canadian listeriosis reference service. <i>Foodborne Pathog Dis</i> 2006, 3(1):132-137.	Complete	CP007170	PRJNA167878	SAMN07124733	
	10-0813	NA	2000	Manitoba	Clinical	Blood	1/2a	7	7	LMACI.0118	LMAAI.0122			Complete	CP007171	PRJNA167879	SAMN07124734	
Cheese C, 2002	10-4758	NA	2002	Quebec	Food	Cheese	1/2a	37	37	LMACI.0616	LMAAI.0818	17 cases: 11 hospitalized, 2 perinatal with premature births	Gaulin C, Currie A, Gravel G, Hamel M, Leblanc MA, Ramsay D, Bekal S: Summary of 11 years of enteric outbreak investigations and criteria to initiate an investigation, Province of Quebec, 2002 through 2012. <i>J Food Prot</i> 2014, 77(9):1563-1570.	Complete	CP007198	PRJNA167881	SAMN07124736	
	10-4754	NA	2002	Quebec	Clinical	CSF	1/2a	37	37	LMACI.0616	LMAAI.0818			Complete	CP007197	PRJNA167880	SAMN07124735	
Cheese A, 2002	02-1792	NA	2002	British Columbia	Food	Cheese	4b	1	1	LMACI.0023	LMAAI.0140	47 cases including: 2 meningitis and 2 perinatal bacteremia	[21]	Complete	CP007461	PRJNA167886	SAMN07124741	
	02-1289	NA	2002	British Columbia	Clinical	Stool	4b	1	1	LMACI.0023	LMAAI.0140				Complete	CP007460	PRJNA167885	SAMN07124740
	02-1103	NA	2002	British Columbia	Clinical	CSF	4b	1	1	LMACI.0023	LMAAI.0140				Complete	CP007459	PRJNA167884	SAMN07124739
Cheese B, 2002	02-6680	NA	2002	British Columbia	Food	Cheese	4b	388	n/a	LMACI.0082	LMAAI.0017	86 febrile gastrointestinal cases	[21]	Complete	CP007462	PRJNA167888	SAMN07124742	
	02-6679	NA	2002	British Columbia	Clinical	Stool	4b	388	n/a	LMACI.0082	LMAAI.0017				Complete	CP008821	PRJNA167887	SAMN02944848
Cheese D, 2008	10-0934	NA	2008	Quebec	Food	Cheese	1/2a	394	415	LMACI.0149	LMAAI.0265	39 cases including: 7 perinatal, 1 adult death, 1 stillborn	This study	Complete	CP007200	PRJNA167883	SAMN07124738	
	10-0933	NA	2008	Quebec	Clinical	Blood	1/2a	394	415	LMACI.0149	LMAAI.0265				Complete	CP007199	PRJNA167882	SAMN07124737
Ready to eat (RTE) delimeat, 2008	08-6569	08-E01	2008	Ontario	Processing Environment	Food processing	1/2a	292	8	LMACI.0040	LMAAI.0001			Complete	CP006858	PRJNA167896	SAMN05771122	
	08-7374	08-F02	2008	Ontario	Food	RTE Coorsh smoked meat	1/2a	292	8	LMACI.0001	LMAAI.0001			Complete	CP007010	PRJNA167898	SAMN07124749	
	08-6056	08-F01	2008	Ontario	Food	RTE turkey meat	1/2a	292	8	LMACI.0040	LMAAI.0003			Complete	CP007009	PRJNA167895	SAMN07124748	
	10-0814	08-F04	2008	Ontario	Food	RTE meat	1/2a	292	8	LMACI.0001	LMAAI.0001			Complete	CP008836	PRJNA167900	SAMN02873948	
	10-0815	08-F05	2008	Saskatchewan	Food	RTE meat	1/2a	292	8	LMACI.0040	LMAAI.0001			Complete	CP006860	PRJNA167901	SAMN05771120	
	08-5923 ¹	08-C16	2008	Ontario	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAI.0001	57 cases including 24 listeriosis-confirmed deaths and 0 perinatal deaths	[8, 10] and this Study	Complete	CP001604	PRJNA36363	SAMN02603721	
	08-7669 ¹	08-C18	2008	Saskatchewan	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAI.0001				Complete	CP007011	PRJNA167899	SAMN07124750
	08-5578	08-C15	2008	Ontario	Clinical	Blood	1/2a	292	8	LMACI.0040	LMAAI.0001			Complete	CP001602/ CP001603	PRJNA36361	SAMN02603776	
	08-6997	08-C17	2008	Ontario	Clinical	Blood	1/2a	292	8	LMACI.0040	LMAAI.0003			Complete	CP006859	PRJNA167897	SAMN05771121	
	08-7362	08-E02	2008	Ontario	Processing Environment	Unknown	1/2a	120	8	LMACI.0001	LMAAI.0001			Draft	CP008765	PRJNA215186	SAMN02725201	
	08-7363	08-E03	2008	Ontario	Processing Environment	Unknown	1/2a	120	8	LMACI.0001	LMAAI.0001			Draft	CP008766	PRJNA215187	SAMN02725202	
08-7364	08-E04	2008	Ontario	Processing Environment	Unknown	1/2a	120	8	LMACI.0001	LMAAI.0001			Draft	CP008767	PRJNA215188	SAMN02725203		
Ready to eat (RTE) prosciutto ham, 2010	10-5024	10-F03	2010	Ontario	Clinical	RTE prosciutto	1/2a	120	8	LMACI.0001	LMAAI.0001			Complete	CP008837	PRJNA167905	SAMN02725209	
	10-1321	10-C24	2010	Ontario	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAI.0001	2 cases, 0 deaths	This study	Complete	CP007018	PRJNA167904	SAMN07124752	
	10-1046 ²	10-C22	2010	Ontario	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAI.0001				Complete	CP007017	PRJNA167902	SAMN07124751

	10-1047 ²	10-C23	2010	Ontario	Clinical	CSF	1/2a	120	8	LMACI.0001	LMAAL.0001		Complete	CP006861	PRJNA167903	SAMN05771119	
	88-0478	88-C01	1988	Ontario	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAL.0001		Complete	CP006862	PRJNA167889	SAMN05771118	
	88-1059	88-C02	1988	Newfoundland	Clinical	Blood	3a	120	8	LMACI.0001	LMAAL.0001		Draft	CP008770	PRJNA215191	SAMN02725206	
	95-0093	95-C03	1995	Alberta	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAL.0001		Complete	CP007019	PRJNA167890	SAMN07124743	
	98-0291	98-C04	1998	Ontario	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAL.0001		Draft	CP008771	PRJNA215192	SAMN02725207	
	98-2035	98-C05	1998	Ontario	Clinical	Blood	1/2a	292	8	LMACI.0040	LMAAL.0003		Complete	CP007020	PRJNA167891	SAMN07124744	
	99-6370	99-C06	1999	Newfoundland	Clinical	Blood	1/2a	292	8	LMACI.0040	LMAAL.0003		Complete	CP007021	PRJNA167892	SAMN07124745	
	99-6871	99-C07	1999	Ontario	Clinical	Tissue	1/2a	120	8	LMACI.0001	LMAAL.0001		Draft	CP008772	PRJNA215193	SAMN02725208	
Sporadic	01-1280	01-C08	2001	Ontario	Clinical	Blood	1/2a	292	8	LMACI.0001	LMAAL.0001	Unknown epidemiology		CP006940	PRJNA215181	SAMN05771117	
	01-1468	01-C09	2001	Newfoundland	Clinical	Brain tissue	1/2a	120	8	LMACI.0098	LMAAL.0081		Draft	CP007527	PRJNA215182	SAMN02693102	
	01-5252	01-C10	2001	Manitoba	Clinical	Blood	1/2a	387	8	LMACI.0001	LMAAL.0003		Draft	CP007538	PRJNA215183	SAMN02725064	
	01-6771	01-C11	2001	Ontario	Clinical	Vitreous	1/2a	120	8	LMACI.0001	LMAAL.0142		Draft	CP008703	PRJNA215184	SAMN02725199	
	02-5993	02-C12	2002	Ontario	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAL.0001		Complete	CP007007	PRJNA167893	SAMN07124746	
	03-5473	03-C13	2003	Ontario	Clinical	Blood	1/2a	120	8	LMACI.0001	LMAAL.0001		Draft	CP008773	PRJNA215185	SAMN02725200	
	04-5457	04-C14	2004	Ontario	Clinical	Blood	1/2a	292	8	LMACI.0001	LMAAL.0001		Complete	CP007008	PRJNA167894	SAMN07124747	
	10-0819	10-C21	2010	Ontario	Clinical	Blood	1/2a	8	8	LMACI.0618	LMAAL.0003		Draft	CP008768	PRJNA215189	SAMN02725204	
	11-4254	11-C25	2011	British Columbia	Clinical	Blood	1/2a	120	8	LMACI.0573	LMAAL.0214		Draft	CP008769	PRJNA215190	SAMN02725205	
	10-5025	NA	2009	Ontario	Food	Fully cooked bacon flakes	1/2c	9	9	LMACI.0036	LMAAL.0658	Same establishment as 10-5025	Complete	CP007194		PRJNA167906	SAMN07124753
Non-Outbreak Associated FPE ³	10-5026	NA	2009	Ontario	Processing environment	Composite swab: dicer exit and blade	1/2c	9	9	LMACI.0036	LMAAL.0658	Same establishment as 10-5026	Complete	CP007195		PRJNA167907	SAMN07124754
	10-5027	NA	2009	Ontario	Processing environment	Composite swab: chute, 2 trays, scoop, polybag liner, inside hopper, hopper leveling bar, belt top & bottom	3c	9	9	LMACI.0036	LMAAL.0863	Distinct establishment	Complete	CP007196		PRJNA167908	SAMN07124755

RTE, ready to eat; CSF, cerebrospinal fluid; FPE, Food Processing Equipment; PFGE, pulsed-field gele electrophoresis

Average depth of coverage from 115-1227x completed genomes (n=37) using Roche 454 and Illumina data, and from 46-171x (or >50x) for draft genomes (n=13) using GAIIX data. Genomes for 08-5578 and 08-5923 were published previously [10]

¹Isolates 08-5923 and 08-7669 originally included in 2008 deli meat outbreak investigation deemed unlikely associated due to conflicting epidemiology and typing analyses [8]

²Same patient

³Common serotype in FPE but uncommon pathogen

Table S2B. Core hqSNV distance matrix for 39 *Listeria monocytogenes* isolates (2014 dataset)

Isolate	10-0933	10-0934	81-0592	10-0809	81-0558	81-0861	02-1289	02-1103	02-1792	10-5025	10-5026	10-5027	02-6679	02-6680	10-0812	10-0813	08-7669	08-5923	02-5993	08-6569	08-6997	08-7374	10-0814	10-1046	10-1047	10-1221	04-5457	08-5578	08-5378v3	08-6036	10-0815	10-5024	08-0478	98-2035	99-6370	95-0093	10-0810	10-0811	10-0738	10-4754				
10-0933	0	0	115441	115440	115440	115438	115431	115430	115430	21096	21096	21095	115397	115396	21294	21293	21594	21588	21586	21585	21585	21585	21585	21585	21585	21585	21584	21584	21584	21584	21584	21584	21584	21584	21584	21584	21582	114343	114342	23243	23242			
10-0934	0	0	115441	115440	115440	115438	115431	115430	115430	21096	21096	21095	115397	115396	21294	21293	21594	21588	21586	21585	21585	21585	21585	21585	21585	21585	21584	21584	21584	21584	21584	21584	21584	21584	21584	21584	21584	21584	21582	114343	114342	23243	23242	
81-0592	115441	115441	0	15	13	17	147	148	148	115400	115400	115399	6862	6863	114888	114887	114466	114461	114459	114459	114459	114459	114459	114459	114459	114458	114458	114458	114458	114458	114458	114458	114458	114458	114458	114458	114458	114458	114458	114456	10103	10104	114343	114342
10-0809	115440	115440	15	0	12	18	148	149	149	115399	115399	115398	6861	6860	114885	114884	114465	114460	114458	114458	114458	114458	114458	114458	114458	114458	114457	114457	114457	114457	114457	114457	114457	114457	114457	114457	114457	114457	114455	10100	10103	114342	114341	
81-0558	115440	115440	13	12	0	14	146	147	147	115399	115399	115398	6859	6860	114885	114884	114465	114460	114458	114458	114458	114458	114458	114458	114458	114457	114457	114457	114457	114457	114457	114457	114457	114457	114457	114457	114457	114455	10100	10101	114342	114341		
81-0861	115438	115438	17	18	14	0	150	149	149	115397	115397	115396	6863	6864	114883	114882	114463	114458	114456	114456	114456	114456	114456	114456	114456	114456	114455	114455	114455	114455	114455	114455	114455	114455	114455	114455	114455	114455	114455	114453	10104	10101	114340	114339
02-1289	115431	115431	147	148	146	150	0	1	3	115394	115394	115393	6858	6859	114879	114878	114462	114457	114455	114455	114455	114455	114455	114455	114455	114454	114454	114454	114454	114454	114454	114454	114454	114454	114454	114454	114452	10100	10101	114339	114338			
02-1103	115430	115430	148	149	147	149	1	0	2	115393	115393	115392	6859	6860	114878	114877	114461	114456	114454	114454	114454	114454	114454	114454	114454	114454	114453	114453	114453	114453	114453	114453	114453	114453	114453	114453	114451	10101	10102	114338	114337			
02-1792	115430	115430	148	149	147	149	3	2	0	115393	115393	115392	6859	6860	114878	114877	114461	114456	114454	114454	114454	114454	114454	114454	114454	114453	114453	114453	114453	114453	114453	114453	114453	114453	114453	114451	10101	10102	114338	114337				
10-5025	21096	21096	115400	115399	115399	115397	115394	115393	115393	0	0	23	115386	115385	21845	21844	20046	20040	20038	20038	20038	20038	20038	20037	20037	20037	20037	20037	20037	20037	20037	20037	20037	20037	20037	20037	20036	20037	20037	20034	114414	114413	21490	21489
10-5026	21096	21096	115400	115399	115399	115397	115394	115393	115393	0	0	23	115386	115385	21845	21844	20046	20040	20038	20038	20038	20038	20038	20037	20037	20037	20037	20037	20037	20037	20037	20037	20037	20037	20037	20036	20037	20037	20034	114414	114413	21490	21489	
10-5027	21095	21095	115399	115398	115398	115396	115393	115392	115392	23	0	115385	115384	21846	21845	20047	20041	20039	20039	20039	20039	20039	20039	20038	20038	20038	20038	20038	20038	20038	20038	20038	20038	20038	20038	20038	20035	114413	114412	21491	21490			
02-6679	115397	115397	6862	6861	6859	6863	6858	6859	6859	115386	115386	115385	0	1	114845	114844	114395	114390	114388	114388	114388	114388	114388	114388	114388	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114385	9769	9770	114372	114371		
02-6680	115396	115396	6863	6860	6860	6864	6859	6860	6860	115385	115385	115384	1	0	114844	114843	114394	114389	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114387	114385	9768	9771	114371	114370	
10-0812	21294	21294	114888	114885	114885	114883	114879	114878	114878	21845	21845	21846	114845	114844	0	1	22450	22444	22442	22441	22441	22441	22441	22441	22441	22441	22441	22440	22440	22440	22440	22440	22440	22440	22440	22440	22436	113760	113759	22959	22958			
10-0813	21293	21293	114887	114884	114884	114882	114878	114877	114877	21844	21844	21845	114844	114843	1	0	22449	22443	22441	22440	22440	22440	22440	22440	22440	22440	22440	22439	22439	22439	22439	22439	22439	22439	22439	22439	22435	113759	113758	22958	22957			
08-7669	21594	21594	114466	114465	114465	114463	114462	114461	114461	20046	20046	20047	114395	114394	22450	22449	0	28	24	22	22	22	22	22	25	25	25	21	21	21	21	21	21	21	21	21	18	113416	113415	22107	22106			
08-5923	21588	21588	114461	114460	114460	114458	114457	114456	114456	20040	20040	20041	114390	114389	22444	22443	28	0	20	20	20	20	20	5	5	5	19	19	19	19	19	19	19	19	19	19	16	113411	113410	22101	22100			
02-5993	21586	21586	114459	114458	114458	114456	114455	114454	114454	20038	20038	20039	114388	114387	22442	22441	24	0	16	16	16	16	16	17	17	15	15	15	15	15	15	15	15	15	15	15	15	12	113409	113408	22099	22098		
08-6569	21585	21585	114459	114458	114458	114456	114455	114454	114454	20038	20038	20039	114388	114387	22441	22440	22	0	16	2	4	2	17	17	17	3	1	1	1	1	1	1	1	1	1	1	10	113409	113408	22099	22098			
08-6997	21585	21585	114459	114458	114458	114456	114455	114454	114454	20038	20038	20039	114388	114387	22441	22440	22	0	16	2	0	4	2	17	17	3	1	1	1	1	1	1	1	1	1	1	10	113409	113408	22099	22098			
08-7374	21585	21585	114459	114458	114458	114456	114455	114454	114454	20038	20038	20039	114388	114387	22441	22440	22	0	16	4	0	4	2	17	17	3	3	3	3	3	3	3	3	3	3	3	10	113409	113408	22099	22098			
10-0814	21585	21585	114459	114458	114458	114456	114455	114454	114454	20038	20038	20039	114388	114387	22441	22440	22	0	16	2	0	4	2	17	17	3	1	1	1	1	1	1	1	1	1	1	1	10	113409	113408	22099	22098		
10-1046	21585	21585	114459	114458	114458	114456	114455	114454	114454	20037	20037	20038	114388	114387	22441	22440	25	5	17	17	17	17	17	0	0	0	16	16	16	16	16	16	16	16	16	16	16	13	113409	113408	22098	22097		
10-1047	21585	21585	114459	114458	114458	114456	114455	114454	114454	20037	20037	20038	114388	114387	22441	22440	25	5	17	17	17	17	17	0	0	0	16	16	16	16	16	16	16	16	16	16	16	13	113409	113408	22098	22097		
10-1321	21585	21585	114459	114458	114458	114456	114455	114454	114454	20037	20037	20038	114388	114387	22441	22440	25	5	17	17	17	17	17	0	0	0	16	16	16	16	16	16	16	16	16	16	16	13	113409	113408	22098	22097		
04-5457	21584	21584	114458	114457	114457	114455	114454	114453	114453	20037	20037	20038	114387	114386	22440	22439	21	19	15	3	3	3	3	16	16	16	0	2	2	2	2	2	2	2	2	2	9	113408	113407	22098	22097			
08-5578	21584	21584	114458	114457	114457	114455	114454	114453	114453	20037	20037	20038	114387	114386	22440	22439	21	19	15	1	1	1	16	16	16	0	2	2	2	2	2	2	2	2	2	2	9	113408	113407	22098	22097			
08-5578v3	21584	21584	114458	114457	114457	114455	114454	114453	114453	20037	20037	20038	114387	114386	22440	22439	21</																											

Table S4. Lineage I core hqSNV distance matrix

Clonal Complex	Outbreak Investigation	Isolate	10-0810	10-0811	02-6679	02-6680	02-1103	02-1289	02-1792	81-0592	10-0809	81-0861	81-0558	# Isolate Type
			C	F	F	C	C	C	F	C	C	F	C	
5		10-0810	0	1	11307	11307	11443	11443	11443	11450	11449	11449	11446	
5		10-0811	1	0	11308	11308	11444	11444	11444	11451	11450	11450	11447	
n/a	2002 Cheese B	02-6679	11307	11308	0	0	7928	7928	7928	7934	7933	7933	7930	
n/a		02-6680	11307	11308	0	0	7928	7928	7928	7934	7933	7933	7930	
	2002 Cheese A	02-1103	11443	11444	7928	7928	0	0	0	165	164	164	161	
		02-1289	11443	11444	7928	7928	0	0	0	165	164	164	161	
		02-1792	11443	11444	7928	7928	0	0	0	165	164	164	161	
1	1981 Coleslaw	81-0592	11450	11451	7934	7934	165	165	165	0	17	17	14	
		10-0809	11449	11450	7933	7933	164	164	164	17	0	16	13	
		81-0861	11449	11450	7933	7933	164	164	164	17	16	0	11	
		81-0558	11446	11447	7930	7930	161	161	161	14	13	11	0	

Isolate types: C, Clinical; F, Food; FPE, Food processing environment.

Note: Outbreak isolates without epidemiological links to outbreak were omitted from pairwise analysis (Figure 2). Epidemiologically-related pairs (green); pairs with known no epidemiological link belonging to the same CC (blue); pairs with known no epidemiological link belonging to a different CC (red);

Table S6. CC8 core hqSNV distance matrix

Outbreak Investigation	Strain (2008 deli meat investigation)	Isolate	10-1321	10-1046	10-1047	10-5024	08-6569	08-6997	08-5578	08-6056	10-0815	10-0814	08-7374	08-7362	08-7363	08-7364	08-7669	08-5923	98-0291	01-1280	04-5457	99-6370	98-2035	95-0093	88-0478	01-1468	01-6771	99-6871	03-5473	88-1059	01-5252	02-5993	11-4254	10-0819	# Isolate Type
			C	C	C	F	FPE	C	C	C	C	C	C	FPE	FPE	FPE	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
2010 Prosciutto		10-1321	0	1	1	5	24	24	23	23	23	24	24	27	27	27	34	7	27	24	23	23	21	19	18	32	29	29	27	25	23	23	104	147	
		10-1046	1	0	0	4	23	23	22	22	22	23	23	26	26	26	33	6	26	23	22	22	20	18	17	31	28	28	26	24	22	22	103	146	
		10-1047	1	0	0	4	23	23	22	22	22	23	23	26	26	26	33	6	26	23	22	22	20	18	17	31	28	28	26	24	22	22	103	146	
		10-5024	5	4	4	0	23	23	22	22	22	23	23	26	26	26	33	6	26	23	22	22	20	18	17	31	28	28	26	24	22	22	103	146	
	1	08-6569	24	23	23	23	0	2	1	1	1	2	4	21	21	21	28	25	9	4	3	5	5	13	18	26	31	31	21	25	23	23	106	149	
	1	08-6997	24	23	23	23	2	0	1	1	1	2	4	21	21	21	28	25	9	4	3	5	5	13	18	26	31	31	21	25	23	23	106	149	
2008 deli meat	1	08-5578	23	22	22	22	1	1	0	0	0	1	3	20	20	20	27	24	8	3	2	4	4	12	17	25	30	30	20	24	22	22	105	148	
	1	08-6056	23	22	22	22	1	1	0	0	0	1	3	20	20	20	27	24	8	3	2	4	4	12	17	25	30	30	20	24	22	22	105	148	
	1	10-0815	23	22	22	22	1	1	0	0	0	1	3	20	20	20	27	24	8	3	2	4	4	12	17	25	30	30	20	24	22	22	105	148	
	2	10-0814	24	23	23	23	2	2	1	1	1	0	4	21	21	21	28	25	9	4	3	5	5	13	18	26	31	31	21	25	23	23	106	149	
	3	08-7374	24	23	23	23	4	4	3	3	3	4	0	21	21	21	28	25	9	4	3	5	5	13	18	26	31	31	21	25	23	23	106	149	
		08-7362	27	26	26	26	21	21	20	20	20	21	21	0	0	0	31	28	24	21	20	20	18	16	21	29	34	34	24	28	26	26	109	152	
	4	08-7363	27	26	26	26	21	21	20	20	20	21	21	0	0	0	31	28	24	21	20	20	18	16	21	29	34	34	24	28	26	26	109	152	
		08-7364	27	26	26	26	21	21	20	20	20	21	21	0	0	0	31	28	24	21	20	20	18	16	21	29	34	34	24	28	26	26	109	152	
	5	08-7669	34	33	33	33	28	28	27	27	27	28	28	31	31	31	0	35	31	28	27	27	25	23	28	36	41	41	31	35	33	33	116	159	
	6	08-5923	7	6	6	6	25	25	24	24	24	25	25	28	28	28	35	0	28	25	24	24	22	20	19	33	30	30	28	26	24	24	105	148	
		98-0291	27	26	26	26	9	9	8	8	8	9	9	24	24	24	31	28	0	9	8	8	8	16	21	29	34	34	24	28	26	26	109	152	
		01-1280	24	23	23	23	4	4	3	3	3	4	4	21	21	21	28	25	9	0	3	5	5	13	18	26	31	31	21	25	23	23	106	149	
Sporadic		04-5457	23	22	22	22	3	3	2	2	2	3	3	20	20	20	27	24	8	3	0	4	4	12	17	25	30	30	20	24	22	22	105	148	
		99-6370	23	22	22	22	5	5	4	4	4	5	5	20	20	20	27	24	8	5	4	0	4	12	17	25	30	30	20	24	22	22	105	148	
		98-2035	21	20	20	20	5	5	4	4	4	5	5	18	18	18	25	22	8	5	4	4	0	10	15	23	28	28	18	22	20	20	103	146	
		95-0093	19	18	18	18	13	13	12	12	12	13	13	16	16	16	23	20	16	13	12	12	10	0	13	17	26	26	12	20	18	18	101	144	
		88-0478	18	17	17	17	18	18	17	17	17	18	18	21	21	21	28	19	21	18	17	17	15	13	0	26	25	25	21	19	17	17	100	143	
		01-1468	32	31	31	31	26	26	25	25	25	26	26	29	29	29	36	33	29	26	25	25	23	17	26	0	39	39	17	33	31	31	114	157	
		01-6771	29	28	28	28	31	31	30	30	30	31	31	34	34	34	41	30	34	31	30	30	28	26	25	39	0	8	34	32	30	30	111	154	
		99-6871	29	28	28	28	31	31	30	30	30	31	31	34	34	34	41	30	34	31	30	30	28	26	25	39	8	0	34	32	30	30	111	154	
		03-5473	27	26	26	26	21	21	20	20	20	21	21	24	24	24	31	28	24	21	20	20	18	12	21	17	34	34	0	28	26	26	109	152	
		88-1059	25	24	24	24	25	25	24	24	24	25	25	28	28	28	35	26	28	25	24	24	22	20	19	33	32	32	28	0	20	20	107	150	
		01-5252	23	22	22	22	23	23	22	22	22	23	23	26	26	26	33	24	26	23	22	22	20	18	17	31	30	30	26	20	0	6	105	148	
		02-5993	23	22	22	22	23	23	22	22	22	23	23	26	26	26	33	24	26	23	22	22	20	18	17	31	30	30	26	20	6	0	105	148	
	11-4254	104	103	103	103	106	106	105	105	105	106	106	109	109	109	116	105	109	106	105	105	103	101	100	114	111	111	109	107	105	105	0	183		
	10-0819	147	146	146	146	149	149	148	148	148	149	149	152	152	152	159	148	152	149	148	148	144	143	157	154	154	152	150	148	148	183	0			

Isolate types: C, Clinical; F, Food; FPE, Food processing environment.

*Isolates originally included in outbreak investigation due to PFGE patterns that were later excluded due to contrary epidemiological evidence.

Note: Outbreak isolates without epidemiological links to outbreak were omitted from pairwise analysis (Figure 2). Epidemiologically-related pairs (green); pairs with known no epidemiological link belonging to the same CC (blue); pairs with known no epidemiological link belonging to a different CC (red);

Table S7. Publically *Listeria monocytogenes* genomes

Strain	Year Isolated	Strain Background	Location	Specimen type	Source	Serotype	MLST	Clonal Complex	Epidemiology	Accession	Reference
F2365	1985	Mexican style soft cheese outbreak, 1985	California, USA	Food	Cheese	4b	1	1	142 cases including 48 deaths (20 in utero, 10 neonates, 18 adults)	AE017262	[4,5]
FSL N1-017				Food	Trout in brine	4b	3	3	Not associated with human disease	AARP00000000	[4,6]
Clip80459	1999	Jellied pork tongue outbreak, 1999	France	Human		4b	4	4	32 cases: CNS listeriosis, bacteremia, 6 deaths (5 adult, 1 in utero), 7 premature births	FM242711.1	[4,7]
HPB2262 (Aurelii1997)	1997	Corn salad outbreak, 1997	Northern Italy	Human	Corn salad (from 2 School cafeterias)	4b	2	2	1700 cases including 292 hospitalized (febrile gastroenteritis, mostly children)	AATL00000000	[4,8]
ScottA	1983	Pasteurized milk outbreak, 1983	Massachusetts, USA	Human		4b	2	2	49 cases: 7 fetus/infant, 42 adult, 14 deaths	CM001159	[4,9,10]
FSL R2-503 (G6054)	1994	Sporadic febrile gastroenteritis clinical case associated with chocolate milk, 1994	Illinois, USA	Human	Stool	1/2b	3	3	45 cases including 4 hospitalizations, 0 deaths	AARR00000000	[4,11,12]
FSL J1-194		Sporadic clinical case	USA	Human	Unknown	1/2b	88		Sporadic	AARJ00000000	[4,11]
FSL J1-175				Environment	Water	1/2b	87		Environmental isolate, not associated with disease	AARK00000000	[4,11]
FSL J2-064			USA	Human		1/2b	5	5		CP006592	[4,6]
10403S	1987	Lab derived strain	USA	Mouse		1/2a	85	7	Common lab strain, streptomycin resistant derivative of strain 10403 isolated from human skin lesion in 1968 at Montana State University	CP002002	[4,13]
EGD	1924		UK	Lab strain		1/2a	12	7	Derived from original strain isolated from rabbits by E.G.D. Murray	HG421741	[4,14]
EGD-e	1960		France	Lab strain	Unknown	1/2a	35	9	EGD obtained from Trudeau institute	AL591824	[4,5]
J2818	2000	RTE turkey deli meat outbreak, 2000	USA	Food	RTE turkey	1/2a	86		Processed turkey outbreak in 11 states sourced to single processing plant	AARX00000000	[4,15]
J0161 (FSL R2-499)	2000			Human		1/2a	11			CP002001	
F6900	1989	Sporadic clinical case, 1989	USA	Human		1/2a	86		Linked to processed meat from same facility as J0161 & J2818 isolates 11 yrs later	AARU00000000	[11]
FSL N3-165			USA	Environment	Soil	1/2a	90	90	Not associated with disease	AARQ00000000	[4,16]
Finland 1998	1999	Dairy butter outbreak, 1999	Finland			3a			25 cases including 20 sepsis, 4 meningitis, 1 abscess; 6 deaths	CP002004	[11,17]
FSL R2-561						1/2c				CP002003	[11]

LO28	<1985	<1985		Human	Stool	1/2c	210	9	Feces of healthy, pregnant clinical case	AARY00000000	[4,11,18]
FSL J1-208	1998	Farm animal outbreak, 1998	Georgia, USA	Animal	Caprine brain	4a				CM001469/CM001470	[11,19]
HCC23	2000	2000		Fish	Catfish brain	4a			Naturally avirulent	CP001175	[20]
L99	1950s	1950's	Netherlands	Food		4a			Weakly pathogenic to humans	FM211688	[21,22]
FSL J2-071		Clinical animal case		Animal		4c	131	71		AARN00000000	[11]

Table S8. Sequences of substituted primers used for MLST

Primer	Sequence
<i>NMLcat-R*</i>	CAGGAAACAGCTATGACCGCTTGCTCCGTCGAAAGGTTC
<i>NMLdapE-R*</i>	CAGGAAACAGCTATGACCATCGAACTATGGGSATTTTTACC
<i>NMLdat-R*</i>	CAGGAAACAGCTATGACCTGCGTCCATAATACACCRCTTTT
<i>NMLldh-F*</i>	GTAAAACGACGGCCAGTGGATTTRAGCCATGCCGTTCC
<i>NMLldh-R*</i>	CAGGAAACAGCTATGACCACRCCGTAGAATGTAGCGCC
<i>NMLlhkA-F*</i>	GTAAAACGACGGCCAGTCAAAGCCAAGTAGATGACTTAAC
<i>lhk-R2#</i>	CAGGAAACAGCTATGACCTACGCATTTTCATGAGAAACATCAG

* Primers for MLST were modified from Ragon et al. [1], based on an alignment of the following *L. monocytogenes* genomes ($n=16$): (08-5578, 08-5923, 08-6997, 08-7669, 10-0933, 10-1046, 10-4754, 10-4758, 10-5026, 81-0861, 98-2035, 99-6370, Clip81459, EGD-e, F2365, HCC23) at the seven MLST loci. Refer to **Tables S1** and **S2** for accession numbers. Specifically, the *cat* reverse primer, *ldh* forward and reverse primers, and the *lhkA* forward primer were redesigned, the *dapE* reverse and *dat* reverse primers had degenerate base substitutions.

lhkA-R2 (www.pasteur.fr/mlst) was used to replace the *lhkA* reverse primer

Supplemental References

- [1] **Ragon M, Wirth T, Hollandt F, Lavenir R, Lecuit M, Le Monnier A, et al.** A new perspective on *Listeria monocytogenes* evolution. *PLoS Pathog* 2008 Sep 5;4(9):e1000146.
- [2] **Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al.** Clustal W and Clustal X version 2.0. *Bioinformatics* 2007 Nov 1;23(21):2947-2948.
- [3] **Darling AE, Mau B, Perna NT.** progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 2010 Jun 25;5(6):e11147.
- [4] **Cantinelli T, Chenal-Francisque V, Diancourt L, Frezal L, Leclercq A, Wirth T, et al.** "Epidemic clones" of *Listeria monocytogenes* are widespread and ancient clonal groups. *J Clin Microbiol* 2013 Nov;51(11):3770-3779.
- [5] **Glaser P, Frangeul L, Buchrieser C, Rusniok C, Amend A, Baquero F, et al.** Comparative genomics of *Listeria* species. *Science* 2001 Oct 26;294(5543):849-852.
- [6] **Gray MJ, Zadoks RN, Fortes ED, Dogan B, Cai S, Chen Y, et al.** *Listeria monocytogenes* isolates from foods and humans form distinct but overlapping populations. *Appl Environ Microbiol* 2004 Oct;70(10):5833-5841.
- [7] **de Valk H, Vaillant V, Jacquet C, Rocourt J, Le Querrec F, Stainer F, et al.** Two consecutive nationwide outbreaks of Listeriosis in France, October 1999-February 2000. *Am J Epidemiol* 2001 Nov 15;154(10):944-950.
- [8] **Aureli P, Fiorucci GC, Caroli D, Marchiaro G, Novara O, Leone L, et al.** An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. *N Engl J Med* 2000 Apr 27;342(17):1236-1241.
- [9] **Briers Y, Klumpp J, Schuppler M, Loessner MJ.** Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak. *J Bacteriol* 2011 Aug;193(16):4284-4285.
- [10] **Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, et al.** Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N Engl J Med* 1985 Feb 14;312(7):404-407.
- [11] **Wiedmann M, Swaminathan B, Lauer P, Portnoy D, Cossart P, Buchrieser C, et al.** *Listeria monocytogenes* Database. Available at: http://www.broadinstitute.org/annotation/genome/listeria_group/GenomeDescriptions.html. Accessed 09/25, 2015.
- [12] **Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, et al.** An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *N Engl J Med* 1997 Jan 9;336(2):100-105.
- [13] **Bishop DK, Hinrichs DJ.** Adoptive transfer of immunity to *Listeria monocytogenes*. The influence of in vitro stimulation on lymphocyte subset requirements. *J Immunol* 1987 Sep 15;139(6):2005-2009.
- [14] **Becavin C, Bouchier C, Lechat P, Archambaud C, Creno S, Gouin E, et al.** Comparison of widely used *Listeria monocytogenes* strains EGD, 10403S, and EGD-e highlights genomic variations underlying differences in pathogenicity. *MBio* 2014 Mar 25;5(2):e00969-14.
- [15] **Olsen SJ, Patrick M, Hunter SB, Reddy V, Kornstein L, MacKenzie WR, et al.** Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin Infect Dis* 2005 Apr 1;40(7):962-967.
- [16] **Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, et al.** Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Appl Environ Microbiol* 2004 Aug;70(8):4458-4467.
- [17] **Lyytikainen O, Autio T, Maijala R, Ruutu P, Honkanen-Buzalski T, Miettinen M, et al.** An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. *J Infect Dis* 2000 May;181(5):1838-1841.

[18] **Vicente MF, Baquero F, Pérez-Díaz JC.** Cloning and expression of the *Listeria monocytogenes* haemolysin in *Escherichia coli*. *FEMS Microbiology Letters* 1985;30(1-2):77–79.

[19] **den Bakker HC, Bowen BM, Rodríguez-Rivera LD, Wiedmann M.** FSL J1-208, a virulent uncommon phylogenetic lineage IV *Listeria monocytogenes* strain with a small chromosome size and a putative virulence plasmid carrying internalin-like genes. *Appl Environ Microbiol* 2012 Mar;78(6):1876-1889.

[20] **Steele CL, Donaldson JR, Paul D, Banes MM, Arick T, Bridges SM, et al.** Genome sequence of lineage III *Listeria monocytogenes* strain HCC23. *J Bacteriol* 2011 Jul;193(14):3679-3680.

[21] **Hain T, Ghai R, Billion A, Kuenne CT, Steinweg C, Izar B, et al.** Comparative genomics and transcriptomics of lineages I, II, and III strains of *Listeria monocytogenes*. *BMC Genomics* 2012 Apr 24;13:144-2164-13-144.

[22] **Kuenne C, Billion A, Mraheil MA, Strittmatter A, Daniel R, Goesmann A, et al.** Reassessment of the *Listeria monocytogenes* pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome. *BMC Genomics* 2013 Jan 22;14:47-2164-14-47.