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## Genomic surveillance of *Listeria monocytogenes* in Taiwan, 2014-2019

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Running Head: *L. monocytogenes* in Taiwan

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## 1 **Abstract**

2 *Listeria monocytogenes* is a life-threatening foodborne pathogen. Here we report the genomic  
3 characterization of a nationwide dataset of 411 clinical and 82 food isolates collected in Taiwan  
4 between 2014 and 2019. The observed incidence of listeriosis increased from 0.83 to 7 cases  
5 per million population upon implementation of mandatory notification in 2018. Pregnancy-  
6 associated cases accounted for 2.8% of human listeriosis and all-cause 7-day mortality was of  
7 11.9% in non-maternal-neonatal listeriosis. *L. monocytogenes* was isolated from 90% of raw  
8 pork and 34% of chicken products collected in the supermarkets. Sublineages SL87, SL5 and  
9 SL378 accounted for the majority (65%) of clinical cases. SL87 and SL378 were also  
10 predominant (57%) in food products. Five cgMLST clusters accounted for 57% clinical cases,  
11 suggesting unnoticed outbreaks spanning up to 6 years. Mandatory notification allowed  
12 identifying the magnitude of listeriosis in Taiwan. Continuous real-time genomic surveillance  
13 will allow reducing contaminating sources and disease burden.

14

## 15 **Importance**

16 Understanding the phylogenetic relationship between clinical and food isolates is important to  
17 identify the transmission routes of foodborne diseases. Here we performed a nationwide study  
18 between 2014 and 2019 of both clinical and food *Listeria monocytogenes* isolates and  
19 sequenced their genomes. We show a 9-fold increase in listeriosis reporting upon  
20 implementation of mandatory notification. We found that sublineages SL87 and SL378  
21 predominated among both clinical (50%) and food (57%) isolates, and identified 5 cgMLST  
22 clusters accounting for 57% of clinical cases, suggestive of potential protracted sources of  
23 contamination over up to 6 years in Taiwan. These findings highlight that mandatory  
24 declaration is critical in identifying the burden of listeriosis, and the importance of genome  
25 sequencing for a detailed characterization of the pathogenic genotypes circulating in Asia.

## 26 **Introduction**

27        Listeriosis is a foodborne infection caused by the Gram-positive bacterial pathogen  
28 *Listeria monocytogenes*. Human listeriosis is a severe infection characterized by bacteremia,  
29 meningoencephalitis and maternal-neonatal infection (1, 2). While most human foodborne  
30 infections are associated with a high incidence but by a relative low morbidity and mortality,  
31 listeriosis has a low incidence varying between 0.1 and 12 per million people, but a high  
32 mortality rate (15-21%), even when active antimicrobial therapy is prescribed (3-5).

33        The population structure of *L. monocytogenes* is highly clonal, composed of four  
34 phylogenetic lineages, 15 serotypes that can be grouped into six PCR serogroups (6, 7), and  
35 hundreds of clonal complexes (CCs) and sublineages (SLs) as defined by multilocus sequence  
36 typing (MLST) and core genome MLST (cgMLST), respectively (8). Multiple listeriosis  
37 outbreaks associated with contaminated food products have been reported worldwide (9-13),  
38 mainly associated to serogroups IVb (lineage I) and IIa (lineage II). The virulence of *L.*  
39 *monocytogenes* is heterogeneous: some CCs are hypervirulent and more efficient in gut  
40 colonization (e.g., CC1, CC2, CC4 and CC6 from serogroup IVb), resulting in high clinical  
41 frequency; others (e.g., CC121 and CC9, from serogroups IIa and IIc) are hypovirulent, express  
42 a truncated form of internalin (InlA) (14, 15), a major virulence factor of *L. monocytogenes*  
43 (16, 17). The hypovirulent clones exhibit higher tolerance to surface disinfectants and are more  
44 prevalent in food isolates, infecting mainly highly immunocompromised individuals (15, 18).

45        In Asia, the incidence of listeriosis was thought to be low before the early 2000s. However,  
46 an increase of listeriosis incidence comparable to that of Western countries has been observed  
47 in the last decade (19-23), likely as a consequence of enhanced surveillance. In Taiwan, patient  
48 information and isolates of notifiable diseases are collected by the Taiwan Centers for Disease  
49 Control. No listeriosis outbreak had been reported prior to mandatory notification and only few  
50 cases have been sporadically identified in medical centers, mostly associated with CC87, CC19

51 and CC155 (20, 24). While listeriosis has been a notifiable disease in many Western countries  
52 since the 1990s, it was only implemented in Taiwan in January 2018, which led to a significant  
53 increase in reported listeriosis cases. Here we provide a comprehensive nationwide overview  
54 of listeriosis incidence prior and after mandatory declaration and a detailed genome-based  
55 characterization of *L. monocytogenes* genotypes circulating in Taiwan.

## 56 **Methods**

### 57 **Sampling**

58 We collected 411 clinical *L. monocytogenes* isolates from 2014 to 2019, of which 333 were  
59 obtained in the context of the nationwide mandatory notification between Jan. 1, 2018 and  
60 December 31, 2019 by the Taiwan Centers for Disease Control (CDC). The use of mandatory  
61 notification data was approved by the ethics committee of Institutional Review Board of  
62 Centers for Disease Control, Ministry of Health and Welfare, Taiwan. To assist epidemiological  
63 investigations, 280 food samples (113 raw chicken, 117 raw ground pork, and 50 ready-to-eat  
64 vegetable mixed salad products; Table S1) were also collected in 2016 and 2019 from  
65 supermarkets and open-air markets in the cities where the incidence of human listeriosis was  
66 the highest (Taipei, New Taipei and Taichung) and in Hsinchu (Table S1). Each food item of  
67 individual markets was sampled once for vegetable products from supermarkets and meat  
68 products from open-air markets. Raw meat commodities from supermarkets were sampled  
69 twice 2 weeks apart. The raw meat products were sampled from 46 markets of 5 districts in  
70 Taichung, 16 markets of 4 districts in Taipei, and 16 markets of 3 districts in New Taipei cities.  
71 *L. monocytogenes* isolation from food samples was performed according to the guidelines of  
72 the Taiwan food and drug administration (<http://www.fda.gov.tw>). Briefly, 25 g of each food  
73 sample was pre-enriched in 100 ml of *Listeria* enrichment broth UVM (CMP, Taiwan) and  
74 incubated at 37 °C, 200 rpm for 18 to 24 h. Secondary enrichment was performed by  
75 transferring 100 µL of the overnight culture to 10 ml of *Listeria* enrichment Fraser broth. After  
76 overnight incubation at 37 °C with 200 rpm agitation, 10 µL of the secondary enrichment broth  
77 was seeded onto Chromogenic Listeria Agar (CMP, Taiwan) and incubated at 37 °C for up to  
78 48 h. The suspected colonies were purified in Brain Heart Infusion Agar (CMP, Taiwan)  
79 cultured at 37 °C for 18 to 24 h. Species identification of the colonies were confirmed by  
80 MALDI-TOF mass-spectrometry as previously described (25). To reduce redundancy, isolates

81 obtained from the same sample and belonging to the same CT were considered as duplicates,  
82 and only one representative was considered in epidemiological analyses.

83

#### 84 **Whole genome sequencing and sequence analysis**

85 DNA libraries were prepared with Illumina DNA Prep and TruSeq® DNA PCR-Free Low  
86 Throughput Library Preparation Kits (Illumina Inc. USA). Whole genome sequencing was  
87 performed using Illumina MiSeq sequencing platform (Illumina Inc. USA) with MiSeq  
88 Reagent Kit v3 (2X 300 bp). Reads were trimmed with AlienTrimer (26) and assembled with  
89 SPAdes 3.11. Reads were trimmed from adapter sequences and non-confident bases using  
90 AlienTrimmer v.0.455 (minimum read length of 30 bases and minimum quality Phred score 20,  
91 i.e. 99% base call accuracy) and corrected with Musket v.1.156, implemented in fqCleaner  
92 v.3.0 (Alexis Criscuolo, Institut Pasteur). High-accuracy assemblies were obtained from using  
93 SPAdes 3.11 with the automatic k-mer, --only-assembler and --careful options, to reduce the  
94 number of mismatches and short indels and used in downstream analyses (27). Molecular  
95 typing based on genoserogrouping, MLST and cgMLST was performed with BIGSdb v.1.30  
96 (<https://bigsdbs.pasteur.fr/listeria/>) (8, 28). The presence of plasmids, intact prophages and  
97 *Listeria* genomic regions was inferred from the assemblies using MOB-suite v.2.0.1 (29),  
98 PHASTER (30) and BIGSdb-*Lm* (<http://bigsdbs.pasteur.fr/listeria/>), respectively. Antibiotic  
99 resistance genes were detected using BIGSdb-*Lm* and ABRicate v.1.0.1  
100 (<https://github.com/tseemann/abricate>). Minimum spanning trees and single linkage  
101 dendrograms were built from cgMLST profiles using BioNumerics v.7.6 and visualized in iTOL.

102

#### 103 **Statistical analysis**

104 The significance of associations of clones with each disease type or region was analyzed with  
105  $\chi^2$  tests as described by creating a contingency table for each clone (15).



106

107 **Data availability**

108 All sequence data are available on NCBI Sequence Read Archive under the BioProject

109 accession number PRJNA493675.

## 110 **Results**

### 111 **Epidemiology of listeriosis in Taiwan**

112 We included 411 cases of human listeriosis in this study. Before nationwide mandatory  
113 notification, only 78 listeriosis cases were reported from 2014 to 2017 in 10 cities of Taiwan,  
114 and an incidence of 75 cases per million people was estimated based on total patient admissions  
115 to a medical center in Taipei (20). Following mandatory notification, 333 human listeriosis  
116 cases were reported in Taiwan in 2018-2019 and disease information was available from 327  
117 of them. These comprised 278 (85%) bacteremia cases, 32 (9.8%) CNS infections, nine (2.8%)  
118 maternal-neonatal infections, and eight of other infections (peritonitis,  $n=5$ , cholecystitis,  $n=2$ ,  
119 pleurisy,  $n=1$ ) (Fig. 1A and Table 1). The annual incidence was of 7.16 and 6.95 listeriosis cases  
120 per million people in 2018 and 2019, respectively (Fig. 1A). Cases were mainly in the main  
121 island of Taiwan, with very few cases in the offshore islands (2 cases in Penghu and 1 case in  
122 Kinmen) (Fig. 1B).

123 The median age of the patients was 68.5 years (range, 0-96 years) for the bacteremia cases,  
124 65.5 years (range, 20-93 years) for the CNS infections, 60 years (range 41-87 years) for the  
125 peritonitis and cholecystitis, and 30 years (range, 26-38 years) for the maternal infections  
126 (Table 1). Diarrhea was noted in 5.71% of the patients and only in bacteremia patients (6.47%)  
127 (Table 1). Among the 318 non-maternal-neonatal listeriosis cases, 166 (52.2%) were female  
128 (Table 1). All-cause 7-day mortality was 11.9% in non-perinatal listeriosis (Table 1).

129

### 130 **Prevalence of *L. monocytogenes* in food samples**

131 *L. monocytogenes* was isolated from raw chicken products (25 of 113, 22.12%) and raw ground  
132 pork products (69 of 117, 58.97%) (Table S1). Notably, 90.41% (66/73) of raw ground pork  
133 products in the supermarkets were positive for *L. monocytogenes*, whereas only 6.8% (3/44) of  
134 those sampled in the open-air markets were positive for *L. monocytogenes*. In the case of

135 chicken products, 33.8% (25/74) of those sold in supermarkets were positive for *L.*  
136 *monocytogenes*, and all the chicken meat sampled from open-air markets were negative for *L.*  
137 *monocytogenes*. By contrast, all the vegetable products sampled in 2019 ( $n=50$ ) were negative  
138 for *L. monocytogenes*.

139

#### 140 **Molecular diversity of *L. monocytogenes* clinical and food isolates**

141 A total of 493 isolates were sequenced (Table S2). Clinical isolates ( $n=411$ ) were distributed  
142 across lineages I ( $n=267$ , 65%) and II ( $n=144$ , 35%) and 22 different cgMLST sublineages (of  
143 20 MLST CCs). SL87 ( $n=147$ , 36%), SL5 ( $n=62$ , 15%) and SL378 ( $n=58$ , 14%) were the most  
144 prevalent sublineages, accounting for 65% of clinical cases (Fig. S1B). SL5 was associated to  
145 CNS infection (odds ratio 2.495,  $p=0.0373$ , Fig. S1A).

146 Food isolates ( $n=82$ ) were distributed across lineages I ( $n=31$ , 38%) and II ( $n=51$ , 62%) and  
147 eight different cgMLST sublineages (of 7 CCs). The three most prevalent sublineages were  
148 SL87 ( $n=26$ , 32%), SL378 ( $n=21$ , 26%) and SL9 ( $n=15$ , 18%), which accounted for 76% of  
149 the food isolates. SL87, SL378, SL155 and SL9 predominated the raw meat products ( $n=72$ ,  
150 87.8%, Fig. S1B and S1C). SL155 was only isolated from raw ground pork products ( $n=10$ ,  
151 20.4% of the pork products) and SL8 was only isolated from raw chicken products ( $n=4$ , 12.1%  
152 of the chicken products) (Fig. S1C). Although frequently isolated from listeriosis patients,  
153 neither SL5 nor SL1 was isolated from the food products (Fig. S1B).

154 A total of 117 cgMLST types (CT) were identified: 96 ( $n=291$  isolates) found exclusively in  
155 clinical cases, 14 exclusively in food ( $n=18$ ) and seven found in both human and food isolates  
156 ( $n=184$ ). All isolates collected from the same food sample belonged to the same CT, except in  
157 two raw ground pork products sampled in Taichung, for which two different strains were  
158 observed in each (cgMLST types L1-SL87-ST87-CT58 and L2-SL378-ST378-CT4349, and  
159 L1-SL3-ST3-CT5507 and L1-SL288-ST330-CT5502).

160 There were 26 CTs (26/103, 25%) comprising more than one clinical case and 22 of which  
161 spanning multiple years (Table 2), suggesting high prevalence of common CTs for several years,  
162 which may imply unnoticed long-term outbreaks. There were five cgMLST types L1-SL87-  
163 ST87-CT58 ( $n=54$ , 14%), L1-SL87-ST87-CT4373 ( $n=53$ , 10%), L1-SL5-ST5-CT4358 ( $n=42$ ,  
164 13%), L1-SL87-ST87-CT4374 ( $n=28$ , 7%) and L2-SL378-ST378-CT4349 ( $n=57$ , 13%)  
165 accounting for 57% of human listeriosis, and spanned up to 6 years (Table 2 and Fig. 2B).  
166 These CTs also predominated in CNS and maternal-neonatal infections in 2018-2019 for which  
167 disease information was available (62.5%, Fig. S1D). While L1-SL87-ST87-CT58, L1-SL87-  
168 ST87-CT4373 and L2-SL378-ST378-CT4349 were widely spread across regions and did not  
169 exhibit a city-specific distribution, L1-SL5-ST5-CT4358 (odds ratio 8.274,  $p < 0.0001$ ) and L1-  
170 SL87-ST87-CT4374 (odds ratio 4.084,  $p = 0.0024$ ) were highly associated to Taichung city (Fig.  
171 S1E). In addition, 10 CTs (10/117, 8.5%) detected in this study have been previously reported  
172 in the context of listeriosis surveillance in North America, Europe, Oceania and Asia (Table  
173 S3).

174

### 175 **Virulence and stress resistance genetic traits**

176 Relevant traits of virulence and resistance were analyzed in all clinical and food isolates.  
177 The presence of virulence traits was consistent with isolate phylogeny. All the isolates carry  
178 intact *Listeria* Pathogenicity Island 1 (LIPI-1), which contains the virulence transcription  
179 regulator *prfA*, the listeriolysin gene *hly* and multiple genes involved in intracellular vacuole  
180 disruption (31) (Fig. 3). LIPI-3, which includes the listeriolysin S gene targeting intestinal  
181 bacteria (32, 33), was found in 11% (55/493) of the isolates in SL1, SL3, SL219, SL224, SL288  
182 and SL455 of lineage I (Fig. 3, Table S2). LIPI-4, which contributes to *L. monocytogenes*  
183 placental and blood-CNS barrier crossing in CC4 (15), is present in 35% (175/493) of the  
184 isolates in SL87 (CC87, serogroup IIb), SL219 (CC4, serogroup IVb) and SL455 (CC455,

185 serogroup IVb) (Fig. 3). Almost all the isolates (473/493, 96%) carry an *inlA* gene encoding  
186 for full-length internalin, a *L. monocytogenes* invasion protein involved in the crossing of the  
187 intestinal and placental barriers (14, 16, 17) (Fig. 3). In the remaining 4% isolates, mutations  
188 in *inlA* leading to premature stop codons (PMSC) were detected, resulting in the expression of  
189 a truncated InlA. InlA mutations identified concerned all SL9 isolates [(previously reported  
190 PMSC\_4 ( $n=15$ ), PMSC\_8 ( $n=1$ ) and PMSC\_11 ( $n=2$ )] (34, 35), and two SL3 isolates that  
191 carry a new PMSC. The new *inlA* PMSC [termed PMSC\_32, following the existing  
192 nomenclature (8, 36)] consists of a C→A substitution at nucleotide position 1041, within the  
193 leucine-rich repeat coding domain, leading to a shorter unanchored InlA protein of 346 amino  
194 acids.

195 Acquired resistance genes towards antibiotics were found in 52 isolates, coding for resistance  
196 towards tetracycline (*tetM*,  $n=50$ ; 10.1%) and aminoglycosides (*aadA2*,  $n=1$ ; *aacA4*,  $n=1$ ) (Fig.  
197 3). Genetic traits involved in resistance towards metals, antiseptics and/or biofilm formation  
198 were also found. These included the arsenic resistance listeria genomic islands LGI-2 ( $n=27$ ,  
199 in SL1 and SL2) and LGI-3 ( $n=21$ , in SL101) involved in metal resistance, the stress survival  
200 islet 1 (SSI-1,  $n=157$ , mainly in SL3, SL5, SL8, SL9, and SL155), and the resistance cassette  
201 *bcrABC* conferring resistance to benzalkonium chloride ( $n=4$ , in SL9 and SL155) (37-40) (Fig.  
202 3). Disrupted *comK*, proposed to be associated with biofilm formation on food-conditioned  
203 surfaces (41), was found in 29% (143/493) of the isolates (Fig. 3), and was significantly more  
204 prevalent in food isolates as compared to clinical isolates ( $p<0.05$ , Chi square test). All these  
205 traits have been shown to confer tolerance to environmental stress in *L. monocytogenes*,  
206 facilitating its long-term persistence in production environments (38, 39, 42, 43).

207

## 208 Discussion

209 Here we described the findings of a genomic nationwide study on clinical and food *L.*

210 *monocytogenes* isolates over a 6-year period, prior to and after mandatory notification of  
211 human listeriosis cases. The incidence of listeriosis has sharply increased from 0.83 to 7 cases  
212 per million population after the implementation of mandatory notification of listeriosis in 2018,  
213 illustrating the effectiveness of mandatory notification. As a result, since July 2021, the Taiwan  
214 Food and Drug Administration has extended food monitoring of *Listeria* from infant and dairy  
215 products to refrigerated and ready-to-eat food products (<https://consumer.fda.gov.tw>). As  
216 compared to the regulation before this date where food products were randomly sampled once,  
217 an extended sampling plan (5 to 10 sampling times per location according to the guidelines on  
218 sampling CAC GL 50-2004) is now in place for food microorganisms. This may help to identify  
219 more contamination sources of *L. monocytogenes* in different food products.

220 As compared to Europe and North America where pregnancy-associated listeriosis accounts  
221 for 9-14% of human listeriosis, only 2.8% of listeriosis cases in Taiwan were pregnancy-  
222 associated (2, 44, 45). This might be due to the difference in placental barrier crossing ability  
223 of circulating clones, and/or to the low total fertility rate in Taiwan, which is ranked the last in  
224 the world (<https://www.cia.gov/the-world-factbook/field/total-fertility-rate/>). Median patient  
225 age (67 years) in non-maternal-neonatal listeriosis was slightly lower than previously reported  
226 in Europe and North America (67-77 years) (2, 44, 45). Here, all-cause mortality (11.9%, within  
227 7 days) in non-maternal-neonatal listeriosis was similar to that in Germany (13%, within 7 days)  
228 but lower than previously reported in France (30-46%, 90 days) and the United States (21%)  
229 (2, 44, 45), although these differences might be due to varied follow-up periods. As observed  
230 in our previous study of listeriosis cases in a medical center in Taiwan, the all-cause mortality  
231 was 14.8% within 7 days and 25.2% within 30 days (20, 45). Thus, the mortality of listeriosis  
232 is likely underestimated when considering a short follow-up period, as listeriosis can have an  
233 impact on mortality at least up to three months (2).

234 While in Western countries serogroup IVb is the main cause of human listeriosis (9, 15, 18), in

235 Taiwan serogroups IIb (SL87/CC87, SL5/CC5, SL378/CC19) and IIa (SL155/CC155) are  
236 responsible for the majority of human listeriosis cases (74.5%, 306 of 411). Previously,  
237 SL87/CC87 (serogroup IIb) has been also reported to be responsible for 28-34% of clinical  
238 infections in China and highly prevalent in raw meat products (46-48). These geographical  
239 differences may result from different food habits in Asia, since serotype IVb (particularly  
240 SL1/CC1) appears to be strongly associated to dairy products (15, 18) and dairy consumption  
241 in Taiwan is relatively low across all age groups (49).

242 Whole genome sequencing of isolates collected prior to and after mandatory notification  
243 allowed the identification of multiple local and nationwide clusters of cases spanning up to six  
244 years. For instance, L1-SL87-ST87-CT58 (CC87, serogroup IIb) and L2-SL378-ST378-  
245 CT4349 (CC19, serogroup IIa) comprised both clinical and food (chicken and pork) isolates  
246 distributed across multiple geographic regions from 2014 to 2019. The presence of identical  
247 cgMLST types across locations and along the years suggests long-term contamination of either  
248 food-processing plants, meat refrigeration and/or large-scale distribution facilities, although  
249 trace-back investigations are needed to confirm the source of the outbreaks. In contrast, the  
250 region-associated distribution of L1-SL5-ST5-CT4358 to Taichung city suggests a local source  
251 of contamination. Food sampling extended to abattoir environments and meat distribution  
252 chains will help to identify the sources of contamination, if any (50). While up to 82% (96/117)  
253 of cgMLST types in this study were exclusively seen in clinical isolates, a more extensive food  
254 sampling will also benefit to identification of the sources of contamination, which remain  
255 unknown for most clusters observed (82%, 96/117). The introduction of new regulation on food  
256 microorganisms sampling in 2021 will help to identify the environmental niches of *Listeria* in  
257 Taiwan.

258 The majority (91%) of cgMLST types found were unique to this study, reinforcing the  
259 importance of comprehensive national surveillance programs to control *Listeria* transmission

260 (51). Nevertheless, 10 CTs have been previously reported within the scope of listeriosis  
261 surveillance in other countries (Table S3), suggestive of punctual cross-country contaminations  
262 or, in the case L1-SL87-ST87-CT58, of contamination sources with large scale distributions.  
263 This study constitutes the first nationwide genome-based characterization of *L. monocytogenes*  
264 genotypes circulating in Taiwan, and highlights the importance of mandatory declaration in  
265 understanding listeriosis incidence. Continuous real-time genome-based surveillance coupled  
266 with epidemiological investigations and regular surveillance of food products for *L.*  
267 *monocytogenes* will allow to reducing contaminating sources and lower the disease burden in  
268 Taiwan.

269

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276

#### 277 **Potential conflicts of interest**

278 The authors have no conflicts to disclose.



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460



461 Table 1. Demographic data and *L. monocytogenes* clone distribution of the 327 human  
 462 listeriosis cases between Jan. 1, 2018 and Dec. 31, 2019.

Disease	Bacteremia	CNS infection	Peritonitis & cholecystitis	Maternal-neonatal infection	All diseases
Total	278	32	7	9	327
Clinical information					
Female	145 (52%)	17 (53%)	3 (43%)	7 (77.8%)	173 (52.9%)
*Age (year)	68.5 (0, 96)	65.5 (20, 93)	60 (41, 87)	30 (26, 38) <sup>†</sup>	67 (0, 96) <sup>†</sup>
Diarrhea	18 (6.47%)	0 (0%)	0 (0%)	0 (0%)	19 (5.71%)
7-day mortality (%)	34 (12.2%)	4 (12.5%)	1 (14.3%)	0 <sup>‡</sup>	39 (11.9%) <sup>‡</sup>
Lineages					
L1	178 (64%)	27 (84.4%)	4 (57.1%)	6 (66.7%)	215 (66%)
L2	100 (36%)	5 (35.6%)	3 (42.9%)	3 (33.3%)	112 (34%)
Serogroups					
IVb	22 (7.9%)	3 (9.4%)	1 (14.2%)	0 (0%)	26 (7.95%)
IIb	156 (56.1%)	24 (75%)	3 (42.9%)	6 (66.7%)	189 (57.80%)
IIa	97 (34.9%)	5 (15.6)	3 (42.9%)	3 (33.3%)	109 (33.33%)
IIc	3 (1.1%)	0 (0%)	0 (0%)	0 (0%)	3 (0.92%)
Sublineages (Clonal Complexes)					
SL87 (CC87)	104 (37.4%)	12 (37.5%)	2 (28.6%)	4 (44.5%)	122 (37.31%)
SL5 (CC5)	37 (13.3%)	9 (28.13%)	1 (14.3%)	2 (22.2%)	49 (14.98%)
SL378 (CC19)	41 (14.7%)	2 (6.25%)	1 (14.3%)	1 (11.1%)	45 (13.76%)
SL155 (CC155)	22 (7.9%)	2 (6.25%)	0 (0%)	2 (22.2%)	27 (8.26%)
SL1 (CC1)	13 (4.7%)	1 (3.12%)	0 (0%)	0 (0%)	14 (4.28%)
Other SLs/CCs	61 (22.0%)	6 (18.75%)	3 (42.9%)	0 (0%)	70 (21.41%)

463 Data are represented as number (percentage) unless described.

464 \*Age is presented as median (min, max).

465 <sup>†</sup> The age of neonatal infection was excluded.

466 <sup>‡</sup> Perinatal infections were excluded.

467 Table 2. cgMLST types detected in this study comprising more than 2 isolates ( $n=33$  out of  
 468 117). cgMLST types with 10 or more clinical isolates are highlighted in bold.

cgMLST type (clonal complex, serogroup)	No. isolates (%, $N=493$ )	No. Clinical isolates (%, $n=411$ )	No. Food isolates (food type*)	Isolation Years	No. Regions
<i>Lineage I</i>					
<b>L1-SL87-ST87-CT58 (CC87, IIb)</b>	<b>77 (15.6%)</b>	<b>54 (13%)</b>	<b>23 (13 C, 10 P)</b>	<b>2014-19</b>	<b>17</b>
<b>L1-SL87-ST87-CT4373 (CC87, IIb)</b>	<b>53 (10.8%)</b>	<b>53 (13%)</b>		<b>2014-19</b>	<b>16</b>
<b>L1-SL5-ST5-CT4358 (CC5, IIb)</b>	<b>42 (8.5%)</b>	<b>42 (10%)</b>		<b>2014-19</b>	<b>9</b>
<b>L1-SL87-ST87-CT4374 (CC87, IIb)</b>	<b>28 (5.7%)</b>	<b>28 (7%)</b>		<b>2016-19</b>	<b>8</b>
<b>L1-SL1-ST1,ST1533-CT2384 (CC1, IVb)</b>	<b>11 (2.2%)</b>	<b>11 (3%)</b>		<b>2015, 2017-19</b>	<b>6</b>
L1-SL5-ST5-CT4360 (CC5, IIb)	5 (1.0%)	5 (1%)		2016, 2018-19	3
L1-SL3-ST3-CT4369 (CC3, IIb)	4 (0.8%)	4 (1%)		2016,2018-19	3
L1-SL288-ST330-CT5502 (CC288, IIb)	3 (0.6%)	1 (0.2%)	2 (P)	2019	2
L1-SL87-ST87-CT9095 (CC87, IIb)	3 (0.6%)	3 (1%)		2018	3
L1-SL1-ST1-CT4353 (CC1, IVb)	2 (0.4%)	2 (0.5%)		2014	1
L1-SL288-ST330-CT5035 (CC288, IIb)	2 (0.4%)	2 (0.5%)		2018-19	1
L1-SL5-ST5-CT4359 (CC5, IIb)	2 (0.4%)	2 (0.5%)		2015, 2019	1
L1-SL5-ST5-CT5033 (CC5, IIb)	2 (0.4%)	2 (0.5%)		2018	2
L1-SL5-ST5-CT5501 (CC5, IIb)	2 (0.4%)	2 (0.5%)		2019	1
L1-SL87-ST87-CT4372 (CC87, IIb)	2 (0.4%)		2 (C)	2016	1
L1-SL87-ST87-CT4376 (CC87, IIb)	2 (0.4%)	2 (0.5%)		2015-19	2
<i>Lineage II</i>					
<b>L2-SL378-ST378-CT4349 (CC19, IIa)</b>	<b>77 (15.6%)</b>	<b>57 (14%)</b>	<b>20 (7 C, 13 P)</b>	<b>2015-19</b>	<b>12</b>
<b>L2-SL101-ST101-CT4370 (CC101, IIa)</b>	<b>18 (3.7%)</b>	<b>18 (4%)</b>		<b>2016-19</b>	<b>7</b>
<b>L2-SL155-ST155-CT4362 (CC155, IIa)</b>	<b>10 (2.0%)</b>	<b>10 (2%)</b>		<b>2016-19</b>	<b>7</b>
L2-SL9-ST9-CT4379 (CC9, IIc)	11 (2.2%)	1 (0.2%)	10 (1 C, 9 P)	2016, 2019	4
L2-SL177-ST177-CT5041 (CC177, IIa)	8 (1.6%)	8 (2%)		2018-19	6
L2-SL155-ST155-CT4363 (CC155, IIa)	7 (1.4%)	7 (2%)		2016-19	4
L2-SL155-ST155-CT4364 (CC155, IIa)	6 (1.2%)	2 (0.5%)	4 (P)	2014-15, 2019	4
L2-SL1081-ST1081-CT4378 (ST1081, IIa)	5 (1.0%)	5 (1%)		2016, 2018-19	3
L2-SL155-ST155-CT5049 (CC155, IIa)	5 (1.0%)	5 (1%)		2018-19	4
L2-SL8-ST551-CT4347 (CC8, IIa)	5 (1.0%)	1 (0.2%)	4 (C)	2016, 2019	2
L2-SL155-ST155-CT5050 (CC155, IIa)	4 (0.8%)	3 (1%)	1 (P)	2018-19	4
L2-SL155-ST1524-CT9039 (CC155, IIa)	3 (0.6%)	3 (1%)		2018-19	3
L2-SL155-ST155-CT4365 (CC155, IIa)	2 (0.4%)	2 (0.5%)		2015-16	2
L2-SL155-ST155-CT5496 (CC155, IIa)	2 (0.4%)		2	2019	2
L2-SL155-ST155-CT5539 (CC155, IIa)	2 (0.4%)		2	2019	1
L2-SL26-ST26-CT5754 (CC26, IIa)	2 (0.4%)	2 (0.5%)		2019	2
L2-SL9-ST9-CT5533 (CC9, IIc)	2 (0.4%)		2	2019	1

469

470 \*Food type: C, chicken; P, pork.

## Figure legends

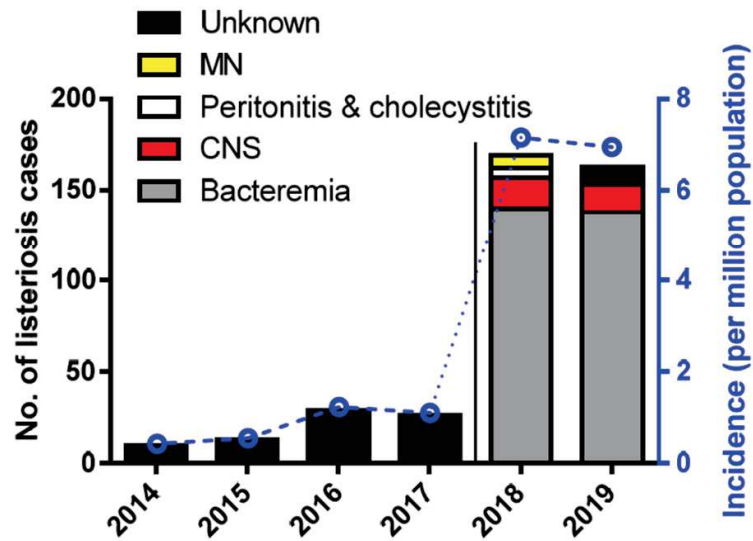
Figure. 1. Incidence of listeriosis in Taiwan. A total of non-redundant 411 clinical isolates between January 2014 and December 2019 were collected. (A) Total no. of listeriosis cases and incidence per million population before (2014-2017) and after (2018-2019) mandatory notification in Taiwan. (B) Total no. of listeriosis cases in the different administrative regions of Taiwan before (2014-2017) and after (2018-2019) mandatory. The regions in white color indicates no reported case in the period.

Figure. 2. cgMLST analyses of the 493 *L. monocytogenes* isolates collected between January 2014 and December 2019. (A) Minimum spanning tree of isolates' core genome MLST allelic profiles (based on 1748 loci). cgMLST profiles are represented by circles proportional to the number of isolates and the number of allelic distances between profiles are indicated in the connecting lines. The dashed bar delimits *L. monocytogenes* phylogenetic lineages, dashed circles delimit sublineages (SL; i.e. isolates sharing 150 or fewer allelic differences) and grey shadows denote clusters of isolates within the same cgMLST type (CT; i.e. isolates sharing 7 or fewer allelic differences). CT names are shown for clusters with 2 or more isolates. CTs comprising 1 or more human isolates are shown in colors. Non-clinical isolates are labeled as E (environmental), P (pork) and C (chicken). (B) Prevalence of cgMLST types with 2 or more isolates according to phylogenetic lineage and isolation source.

Figure. 3. Genetic diversity of the 493 *L. monocytogenes* isolates. A) Single linkage clustering based on the cgMLST profiles. Branches are colored by phylogenetic lineage (L1, red; L2, blue). Sublineages (SL) with more than 5 isolates are labelled. Information on the serogroup, year of isolation and source are provided, using the color code indicated in the legend. The presence of virulence, acquired resistance traits, prophages and plasmids are shown as blue color-filled boxes.

FIGURE 1

A



B

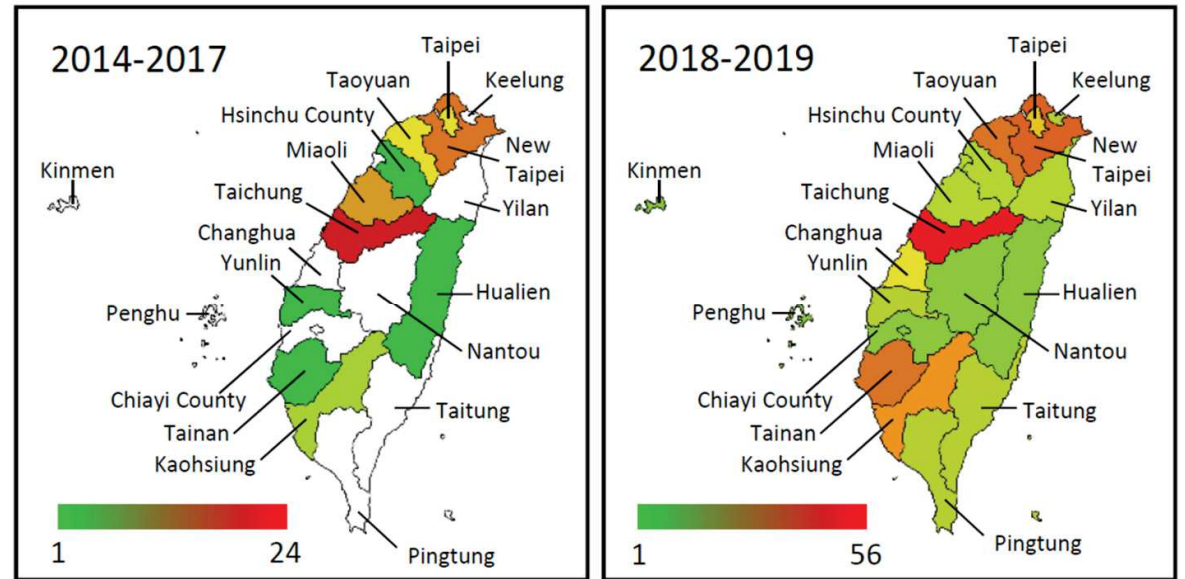


FIGURE 2

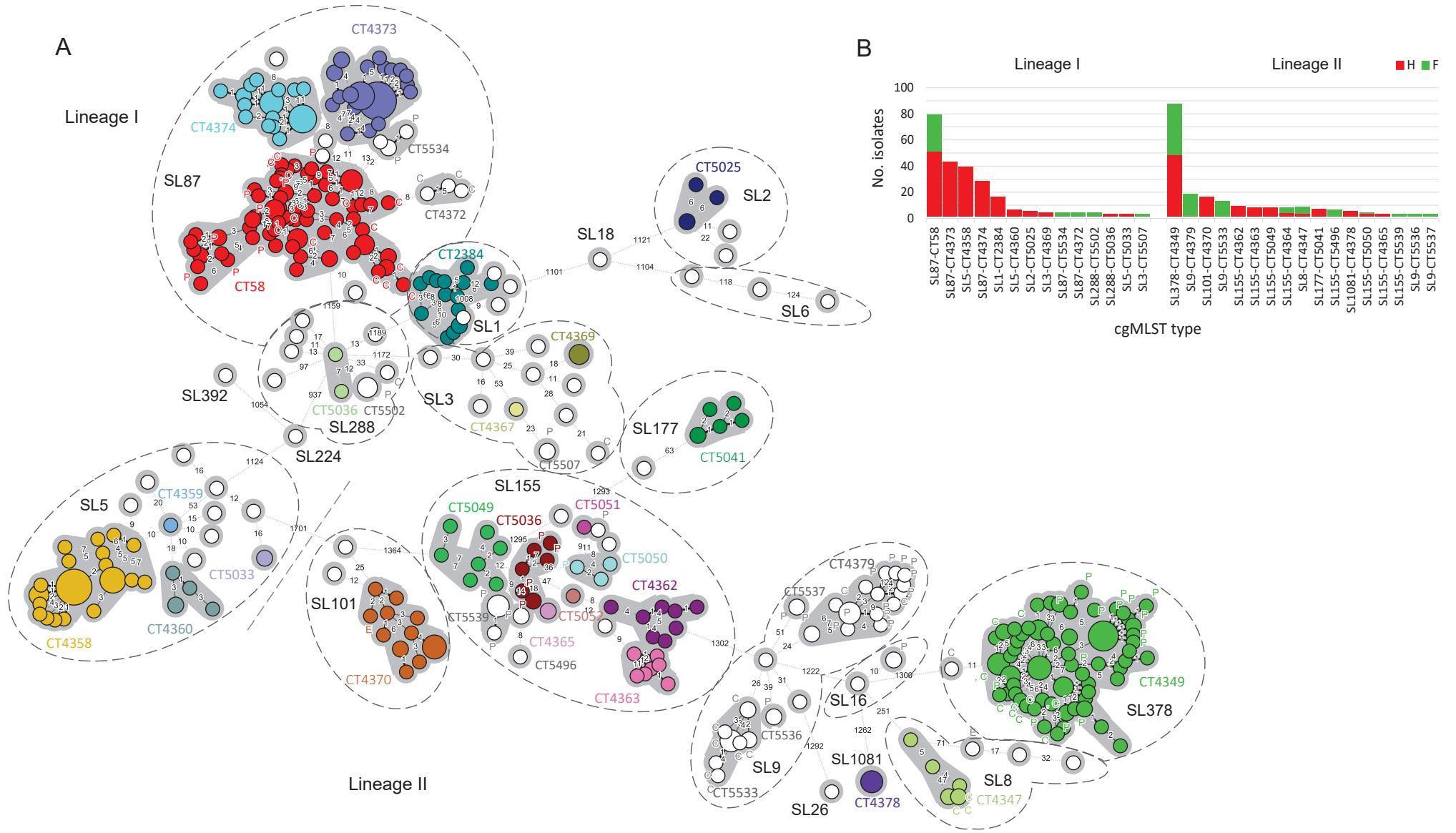
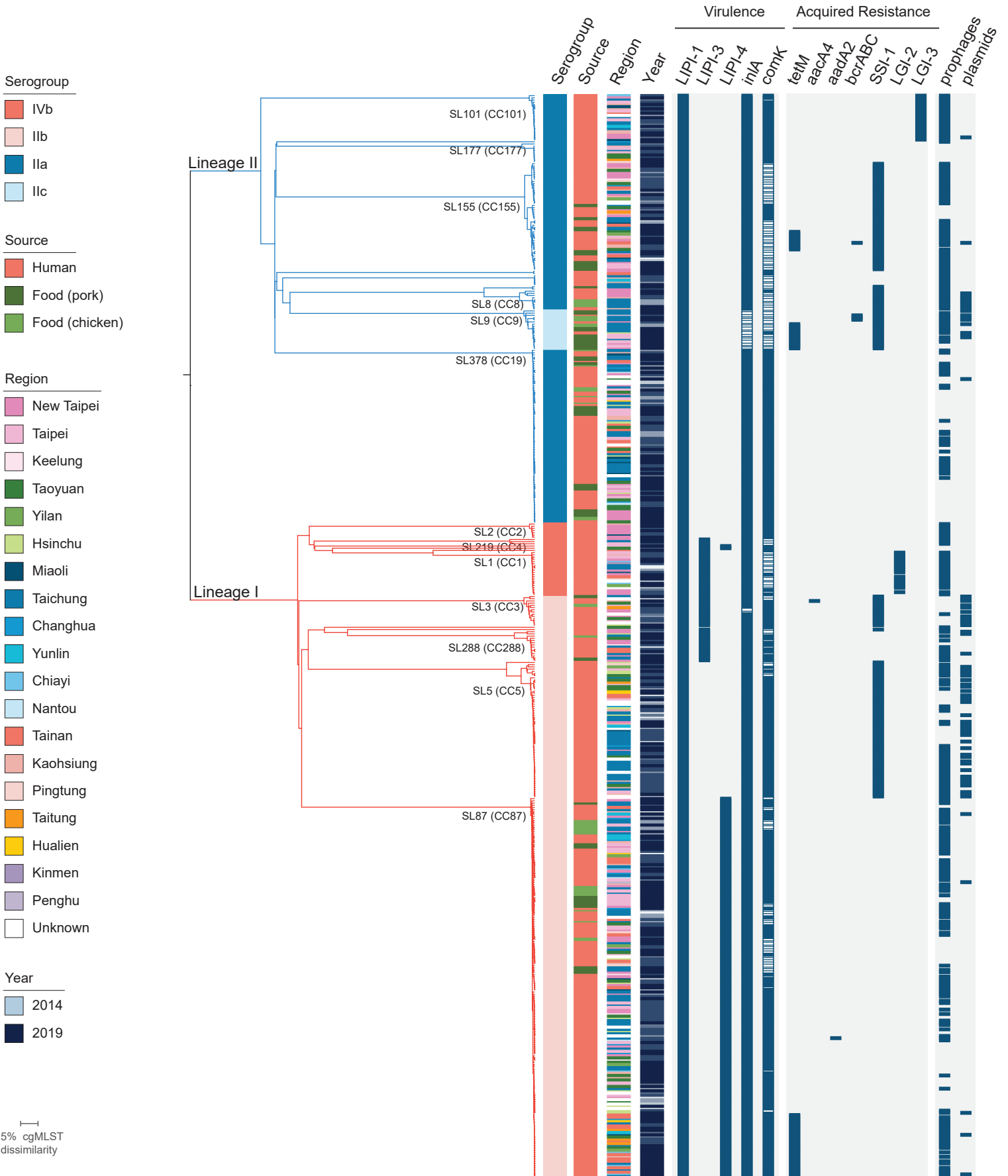


FIGURE 3



## SUPPLEMENTAL MATERIAL

### Genomic surveillance of *Listeria monocytogenes* in Taiwan, 2014-2019

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Table S1. Number of *Listeria monocytogenes*-positive food samples collected per region and food type in this study.

Product	Taipei	New Tapei	Taichung	Hsinchu	Total
Raw ground pork	18/27 (67%)	19/28 (68%)	32/62 (52%)	-	69/117 (59%)
Raw chicken	5/23 (22%)	8/27 (30%)	12/63 (19%)	-	25/113 (22%)
Mixed salads	-	-	-	0/50 (0%)	0/50 (0%)
Total	23/50 (46%)	27/55 (49%)	44/125 (35%)	0/50 (0%)	94/290 (32%)



Table S2. Characteristics of *Listeria monocytogenes* isolates retrieved in this study (N=493).

[extended excel file]

Table S3. Previously reported cgMLST types detected in this study ( $n=10$ ).

cgMLST type (clonal complex, serogroup)	source(s) (this study)	source(s) (other studies)	countries (other studies)	source lab	Min. cgMLST similarity (%)
<i>Lineage 1</i>					
L1-SL1-ST1-CT2384 (CC1, IVb)	H	H,F,PE	US	FDA,CDC	99.6%
L1-SL224-ST845-CT21 (CC224, IIb)	H		MX,US	FDA,SENEICA	99.9%
L1-SL87-ST87-CT58 (CC87, IIb)	H,F	H,F,PE	AU,CA,CN,CO,DE,FR,SK,US	CDC,CFIA,DI,FDA,IP,RKI	99.6%
<i>Lineage 2</i>					
L2-SL101-ST101-CT4370 (CC101, IIa)	H	n.a.	n.a.	n.a.	99.7%
L2-SL155-ST155-CT4364 (CC155, IIa)	H,F	n.a.	n.a.	n.a.	99.6%
L2-SL16-ST16-CT5516 (CC8, IIa)	F	F	CA	CFIA	99.7%
L2-SL177-ST177-CT5041 (CC177, IIa)	H	H	KH	CDC-TW	99.8%
L2-SL378-ST378-CT4349 (CC19, IIa)	H,F	H	KH	CDC-TW	99.6%
L2-SL8-ST8-CT5167 (CC8, IIa)	H	n.a.	n.a.	n.a.	99.9%
L2-SL9-ST9-CT5537 (CC9,IIa, IIc)	F	H,F	FR	IP	99.8%

Source abbreviations: H, human; F, food; PE, food production environment.

Country abbreviations: AU, Australia, CA, Canada; CN, China; CO, Colombia; FR, France; KH, Cambodia; MX, Mexico; SK, South Korea; US, United States of America; n.a., not available.

Source lab abbreviations: CDC, Centers for Disease Control, United States; CDC-TW, Centers for Disease Control Taiwan; CFIA, Canadian Food Inspection Agency, Canada; DI, Doherty Institute, Australia; FDA, Food and Drug Administration, United States; IP, Institut Pasteur, France; RKI, Robert Koch Institut, Germany; SENESICA, Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria, Mexico; n.a., not available.

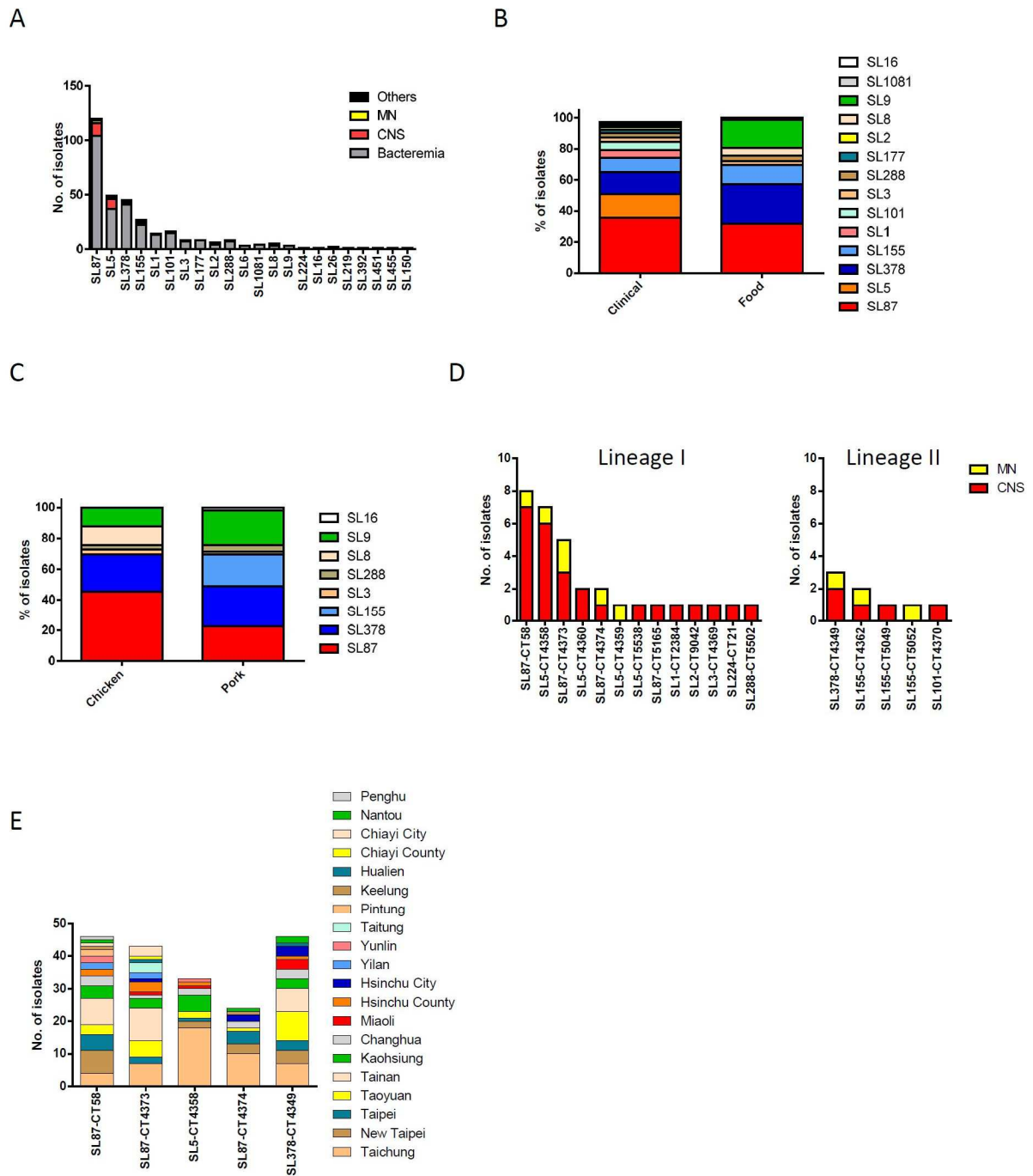


Figure S1. Distribution of *L. monocytogenes* major cgMLST sublineages (SLs) and types (CTs) in clinical and food sources in Taiwan. (A) No. isolates per SL and clinical form (2018-2019). (B) Percentage of larger SLs in clinical and food sources (2014-2019). (C) Percentage of larger SLs per food type (2014-2019). (D) Prevalence of cgMLST types in maternal-neonatal and CNS infections, for which the clinical information was available ( $n=327$  isolates). (E) Distribution of major CTs of clinical isolates in different administrative regions of Taiwan. Abbreviations: CNS, central nervous system infection; MN, maternal-neonatal infections.