International Journal of Food Microbiology 410 (2024) 110515

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Listeria monocytogenes in ready-to-eat (RTE) delicatessen foods: Prevalence, genomic characterization of isolates and growth potential

E. Tirloni [a,*], G. Centorotola [b], F. Pomilio [b], M. Torresi [b], C. Bernardi [a], S. Stella [a]

[a] Department of Veterinary Medicine and Animal Sciences, University of Milan, via dell'Università 6, 26900 Lodi, Italy
[b] IZSAM, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, via Campo Boario, Teramo 64100, Italy

ARTICLE INFO

Keywords:
Growth potential
Listeria monocytogenes
Delicatessen
Ready-to-eat
Whole genome sequencing

ABSTRACT

This study investigated *Listeria monocytogenes* prevalence and count in 132 ready-to-eat (RTE) delicatessen samples belonging to different categories (starters with/without mayonnaise pasta/rice-based courses, meat/fish-based main courses) produced by an Italian industry. Whole Genome Sequencing characterized the isolates to map the pathogen circulation. Moreover, the growth potential of L. *monocytogenes* in the most contaminated product was investigated by a challenge test.

L. monocytogenes was detected in 23 samples, giving an estimated prevalence of 17.4 %. Starters with mayonnaise showed a very high prevalence (56.7 %), showing the role of the sauce in the diffusion of the pathogen within the plant. A total of 49 isolates were obtained; they belonged to two different serogroups, IIb and IIa, and were related to two clonal complexes (CCs) and sequence types (STs) (CC288-ST330 and CC121-ST717), suggesting the possible persistence and circulation of the pathogen within the plant.

The results of the challenge test showed a limited ability to grow in the selected product thanks to the presence of lactic microflora.

1. Introduction

Listeria monocytogenes is a highly noteworthy pathogen in the food industry as it widely occurs in agricultural, aquaculture and food environments; thanks to its resistance to high salt concentrations, low temperatures (it can replicate from −2 °C to 45 °C in experimental conditions), low oxygen concentration and low pH, it may persist for decades in environmental niches inside the food processing plants (Buchanan et al., 2017; EURL Lm, 2021; Ferreira et al., 2014; Malley et al., 2015; Walker et al., 1990). Despite the efforts made in recent years to implement food safety control measures, *L. monocytogenes* is still a risk to consumers' health. Several reported illnesses due to L. *monocytogenes*, according to the European Food Safety Authority (EFSA), did not decrease in the last years, and, even if rare and generally characterized by mild symptoms, listeriosis may become a systemic invasive disease in susceptible subjects (e.g. pregnant women, newly born, elderly, immunocompromised) (EFSA, 2018; EFSA-ECDC, 2021a, 2021b, 2022; FAO/WHO, 2001; FAO/WHO, 2004). Moreover, listeriosis is characterized by the highest proportion of hospitalized cases among all the zoonoses in the European Union (EU) (almost 97.1 % of the cases) and by the highest fatality rate (13.7 %) (EFSA-ECDC, 2022). Previous risk assessments identified ready-to-eat foods (RTE) as the top source of human listeriosis in the USA (Pradhan et al., 2010), with a critical role of deli meats (US-FDA, 2003); 83 % of human listeriosis cases were associated with consumption of RTE deli products that were contaminated at the retail level. Even in Europe, RTE products are of great concern for consumers according to several risk assessment studies (EFSA, 2018; Pérez-Rodríguez et al., 2017; Tsaloumi et al., 2021). In recent years, outbreaks due to the consumption of contaminated RTE retail delicatessen were reported, with an essential role of meat-derived delicatessen: in Italy, an outbreak occurred between May 2015 and January 2016, involving 24 cases (4 fatal cases) due to the consumption of a contaminated meat product called "coppa di testa" (Duranti et al., 2018), while various outbreaks were reported in Austria (jellied pork), in France (jellied pork, rillettes), in Germany (sausage salad) and in Spain (stuffed pork) (Fernandez-Martinez et al., 2022; Pichler et al., 2009; de Valk et al., 2001; International Meeting on Emerging Diseases and Surveillance, 2009, Abstract 10.098, reported by Allerberger and Wagner, 2010). Moreover, RTE stored in vacuum or modified atmospheres and with extended refrigerated shelf lives offer the opportunity for the pathogen to replicate through the storage period.

In the present study, both prevalence and genomic characterization,

* Corresponding author.
E-mail address: erica.tirloni@unimi.it (E. Tirloni).

https://doi.org/10.1016/j.ijfoodmicro.2023.110515
Received 12 September 2023; Received in revised form 28 November 2023; Accepted 4 December 2023
Available online 5 December 2023

by whole genome sequencing (WGS) approach, of L. *monocytogenes* in different RTE delicatessen products produced by an Italian industry were evaluated; moreover, the growth potential of L. *monocytogenes* was investigated in a selected product.

2. Materials and methods

2.1. The products

Various types of RTE delicatessen, produced by a medium-scale industry located in Northern Italy, were considered in this study. A total of 132 samples, including 34 different products, were analysed; the products were selected based on a retrospective analysis of the whole plant production, including all the RTE product types. The selected products were classified into five categories, namely starters with mayonnaise sauce (SM), starters without mayonnaise (SW), pasta/rice-based courses (PR), meat-based main courses (MB) and fish-based main courses (FB) (Table 1); each product category is produced in a separate section. Some ingredients are used in more than one section, and some operators can circulate from one section to another.

For each product type, samples from 4 different batches (one sample per batch, produced in 4 different weeks) were obtained. Each product was packed under Modified Atmosphere Packaging (MAP) condition (25 % CO2, 75 % N2). The products were supposed to be stored at temperatures below 4 °C and consumed with an assigned expiry date of 15 days.

2.2. Detection and count of L. monocytogenes

The prevalence and count of L. *monocytogenes* were evaluated in all samples. For the detection of L. *monocytogenes*, 25 g of the product were sampled and diluted in 225 mL of Half Fraser Broth (Biogenetics, Ponte San Nicolò, Padova, Italy), homogenized for 60 s in a Stomacher 400 (Seward Medical, London, UK) and incubated at 30 °C for 48 h. Afterwards, 100 µl of the homogenate were plated on Rapid L'mono agar (Generon, Modena, Italy) and incubated at 37 °C for 24–48 h (AFNOR BRD 07–0405–09/98 method; AFNOR, 1998). In parallel, from the same sample, 1 mL of the homogenate was added to 9 mL of whole Fraser broth that was incubated at 37 °C for 24 h; after incubation, 100 µl of the broth were newly seeded onto Rapid L'mono agar and incubated in the same conditions.

To enumerate L. *monocytogenes*, 10–15 g of each sample were 5-fold diluted in pre-chilled sterile saline solution (0.85 % NaCl and 0.1 % peptone) and homogenized for 60 s in a Stomacher. *L. monocytogenes* was enumerated by spread plating on Rapid L'mono agar, incubated at 37 °C for 48 h (AFNOR BRD 07/05–09/01 method; AFNOR, 2001) with a detection limit of 0.7 Log CFU/g.

From plates (obtained by detection method), 1 to 2 presumptive L. *monocytogenes* colonies (blue colonies without any yellow halo) were picked for subsequent identification. From plates (obtained from the count method), 1 to 5 presumptive *L. monocytogenes* colonies were randomly picked for subsequent identification. The species identification was performed by Microbact™ Listeria 12 L Kit (Oxoid,

Table 1
results obtained from detection and enumeration of L. *monocytogenes* in RTE delicatessen samples obtained from four sampling sessions.

ID	Product	Session 1		Session 2		Session 3		Session 4	
SM	STARTERS WITH MAYONNAISE	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g
SM1	DICED VEGETABLES WITH VEGAN MAYONNAISE	+	<0.70	+	1.70	+	2.28	na	
SM2	DICED VEGETABLES WITH MAYONNAISE	+	<0.70	+	<0.70	+	<0.70	+	<0.70
SM3	JULIENNE VEGETABLES WITH VEGAN MAYONNAISE	+	2.30	+	2.08	+	2.08	na	–
SM4	JULIENNE VEGETABLES WITH MAYONNAISE	–	–	–	–	–	–	–	–
SM5	HAM FILLED WITH PRODUCT 2	+	<0.70	+	<0.70	–	–	+	<0.70
SM6	CANAPES WITH MAYONNAISE AND OTHER INGREDIENTS	–	–	–	–	–	–	+	<0.70
SM7	SHRIMPS COCKTAIL	–	–	–	–	–	–	–	–
SM8	SLICED BEEF IN TUNA SAUCE	+	1.85	+	3.22	+	1.30	–	–
SW	STARTERS WITHOUT MAYONNAISE	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g
SW1	CHICKEN, VEGETABLES, OLIVES	–	–	–	–	–	–	–	–
Sw2	TOMATOES, CHEESE, OLIVES	–	–	–	–	–	–	–	–
SW3	HAM, VEGETABLES, PICKLED VEGETABLES	–	–	–	–	–	–	–	–
SW4	SPECK WITH CREAM CHEESE	–	–	–	–	–	–	–	–
SW5	NERVETTI	–	–	–	–	–	–	–	–
SW6	NERVETTI WITH BEANS	–	–	–	–	–	–	–	–
PR	PASTA/RICE-BASED COURSES	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g
PR1	COLD PASTA WITH VEGETABLES AND CHEESE	–		+	<0.70	+	1.00	–	
PR2	RICE WITH SHRIMPS	+	<0.70	–		–		–	
PR3	EMMER WITH VEGETABLES, SPECK AND CHEESE	–		+	<0.70	–		–	
PR4	BARLEY WITH VEGETABLES	–		–		–		–	
PR5	COLD PASTA WITH PESTO AND VEGETABLES	–		–		–		–	
PR6	COLD PASTA WITH TUNA	–		–		–		+	<0.70
PR7	RICE WITH VEGETABLES, HAM AND TUNA	–		–		–		–	
PR8	GNOCCHI WITH VEGETABLES AND SHRIMPS	–		–		–		–	
PR9	COLD PASTA WITH VEGETABLES	–		–		–		–	
PR10	EMMER WITH VEGETABLES, OLIVES AND CHEESE	na		–		–		–	
Mb	MEAT-BASED MAIN COURSES	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g
mb1	VEAL WITH SPINACH AND EGGS	–		–		–		–	
mb2	VEAL WITH SPINACH, CHEESE, PEAS AND EGGS	–		–		–		–	
mb3	ROAST-BEEF	–		–		na	na	–	
fb	FISH-BASED MAIN COURSES	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g	Det	Log CFU/g
fb1	SALMON TROUT	–		–		–		–	
fb2	COD	–		–		–		–	
fb3	OCTOPUS WITH VEGETABLES	–		+	<0.70	–		–	
fb4	SHRIMPS	–		–		–		–	
fb5	SHRIMPS WITH CELERY	–		–		–		–	
fb6	SEAFOOD SALAD	–		–		–		–	
fb7	SEAFOOD SALAD WITH VEGETABLES	–		–		–		–	

Det: detection.
Na: not applicable, not present at the moment of sampling session.

Basingstoke, UK). A total of 54 colonies were isolated.

2.3. L. monocytogenes serogroup and whole genome sequencing (WGS)

From the isolates' population identified by biochemical methods as L. *monocytogenes*, 49 strains were stored in microbank tubes (Pro-Lab Diagnostics, Round Rock, TX, USA) at −20 °C and sent to the Italian National Reference Laboratory for L. *monocytogenes* for DNA extraction, molecular serotyping and WGS analysis to define in silico Clonal Complex (CC), Sequence Type (ST), core genome Multi Locus Sequence Type (cgMLST) and relevant virulence and resistance genes absence/presence.

Molecular serotyping was performed using a multiplex PCR assay according to the European Union Reference Laboratory for L. *monocytogenes* protocol (Doumith et al., 2004; Doumith et al., 2005; Kérouanton et al., 2010), with minor modifications. Briefly, each strain was streaked on an ALOA plate (Liofilchem, Roseto degli Abruzzi, Teramo, Italy) and incubated for 18 to 24 h at 37 °C ± 1 °C. One L. *monocytogenes* colony was then picked and streaked on Brain Heart Infusion (BHI) (Liofilchem). DNA extraction was performed by suspending one or two L. *monocytogenes* colonies in 100 µl of Instagene matrix (Bio-rad, Milan, Italy), incubating the suspension for 30 min at 56 °C ± 1 °C, then for 15 min at 95 °C ± 1 °C. After centrifugation for 5 min at 5000*g*, DNA amplification was carried out in a Gene Amp PCR system 9700 thermal cycler. For molecular serotyping, PCR products were run using QIAxcel (QIAGEN, Milan, Italy), an automated capillary electrophoresis system.

The DNA extraction for sequencing was performed according to Portmann et al. (2018), with minor modifications, using the QIAamp DNA Mini Kit (QIAGEN Hilden, Germany), following the manufacturer's protocol. The purity of DNA (A260/280 and A260/230) was evaluated by Biospectrometer fluorescence (Eppendorf, Milan, Italy). DNA was quantified using a Qubit dsDNA HS (High Sensitivity) Assay Kit (Invitrogen, Carlsbad, CA, USA). Starting from 1 ng of input DNA, the Nextera XT DNA chemistry (Illumina, San Diego, CA) was used for library preparation according to the manufacturer's protocols. WGS was performed on the NextSeq 500 platform (Illumina, San Diego, CA, US) with the NextSeq 500/550 mid-output reagent cartridge v2 (300 cycles, standard 150-bp paired-end reads). For the WGS data analysis, an in-house pipeline (Cito et al., 2018) was used, which included steps for trimming (Trimmomatic v0.36) (Bolger et al., 2014) and quality control check of the reads (FastQC 129 v0.11.5) (Wingett and Andrews, 2018). Genome de novo assembly of paired-end reads was performed using SPAdes v3.11.1 (Bankevich et al., 2012) with default parameters for the Illumina platform 2 × 150 chemistry (−only-assembler –careful –k21, 33, 55, 77). Subsequently, the genome assembly quality check was performed with QUAST v.4.3 (Gurevich et al., 2013). The genome quality was checked according to the parameters recommended by Timme et al. (2020). The multilocus sequence typing (MLST) and core genome multilocus sequence typing (cgMLST) analysis were performed according to Pasteur's reference schemes (https://bigsdb.pasteur.fr/). A threshold of ≤7 allelic distance (AD) was considered for the cgMLST cluster definition (Moura et al., 2016). The software GrapeTree (Zhou et al., 2018) was used to obtain the Minimum Spanning tree (MSTreeV2). The 49 *L. monocytogenes* genomes were also characterized in silico using BIGSdb-Lm database tools (accessed on May 30, 2022), querying for "Virulence", "Metal and disinfectant resistance", and "Stress Islands".

The genome assemblies were deposited at DDBJ/ENA/GenBank under the BioProject PRJNA982763.

2.4. Growth potential of L. monocytogenes in an RTE deli food

2.4.1. Challenge test

In order to evaluate the growth potential of L. *monocytogenes* in the selected deli product, challenge tests were carried out by inoculating the pathogen and storing the product for the declared shelf life.

The samples (sliced beef in tuna sauce, packaged in 220 g portions), produced on the same day, were inoculated with a mixture of three L. *monocytogenes* strains selected from the stock supplied by the National Reference Laboratory for *Listeria monocytogenes* EURL to perform challenge tests. Based on the ability of the strains to grow at low temperatures and low pH conditions, to the origin (meat substrate) and the genoserotype, the strains 12MOB045LM, 12MOB85LM and 12MOB112LM were selected (EURL LM, 2013). From the strain stocks, kept frozen at −80 °C in Microbank Cryogenic vials (Pro-Lab Diagnostics UK, Merseyside, UK), a loop was transferred to BHI broth (Oxoid) and incubated at 37 °C for 24 h. Cultures were then re-inoculated in BHI broth and incubated at 8 °C to pre-adapt the bacteria to the environmental conditions. The strains were harvested in a late exponential growth phase, defined as a relative change in absorbance of 0.05–0.2 at 540 nm (Jenway 6105, Staffordshire, UK). Cell concentrations were determined by microscopy at 1000× magnification (Motic B310, Wetzlar, Germany). Lastly, pre-cultures of individual strains were diluted in sterile saline water (0.85 % NaCl) to reach a concentration of 4.3 Log CFU/mL, aiming to obtain an initial count of ~2 log CFU/g in the product. To minimize changes in substrate characteristics, the inoculum volume did not exceed 1 % of the total weight (1 mL in each portion) (EURL Lm, 2021). The samples were assigned to three series that were contaminated with a sterile pipette at different points:

- ✓ LM on the upper beef slice, in contact with tuna sauce (A);
- ✓ LM in the sauce that overlaps the product (B);
- ✓ LM between two slices of beef (C).

All the series (A, B, C) were then repacked in MAP with the same gas concentrations used by the producer and stored at 8 °C (mild thermal abuse). At t_0, the day of inoculation, and after 4, 10 and 15 days (end of the assigned shelf-life), *L. monocytogenes* and LAB were enumerated as reported in Section 2.2.

2.5. Statistical analysis

Data on the prevalence of L. *monocytogenes* in the samples (Section 2.2) were submitted to Fisher exact test to evaluate the difference among the RTE product categories. Values of L. *monocytogenes* obtained from the sample series during the challenge tests performed on the selected product (Section 2.4.1) were compared by one-way ANOVA. For all the statistical analyses, a threshold of $P < 0.05$ was applied to define the significance of the differences.

3. Results and discussion

3.1. Prevalence of L. monocytogenes

In 2003, a quantitative risk assessment determined that the majority of human listeriosis cases should be attributed to RTE delicatessen and that deli meats sliced at retail were the cause of >4/5 of deli meat-associated listeriosis cases annually (US-FDA, 2003). In general, RTE delicatessen can be easily contaminated by L. *monocytogenes* due to the natural presence of the pathogen in the raw materials and the handling (especially when the product is manually assembled by operators and packaged). Moreover, the RTE delicatessen production plant is an ideal environment for the pathogen: the presence of complex machinery and tools that are difficult to clean as they need to be disassembled daily could allow the persistence of food residues. This was already stated in previous studies, where L. *monocytogenes* was found on equipment used to transport, store, or prepare food. *L. monocytogenes* was already isolated from tools directly in contact with food, like tables (especially when present cracks), slicers, cutting boards, and knives, but also from niches not directly in contact with food, like drains, wheels of food transport carts, cooling fans in chilling chambers (Ferreira et al., 2004; Lakicevic et al., 2015).

In the present study, 34 different RTE delicatessen were considered and sampled in four sampling sessions for a total of 132 samples: *L. monocytogenes* was detected in 23 samples, giving an estimated total prevalence of 17.4 % (Table 2). This value should be carefully considered, as it indicates frequent exposure of the consumers to the presence of the pathogen; it also indicates the need to adopt implemented hygiene procedures, and the potential circulation of L. *monocytogenes* strains within the plant.

The evaluation of the results obtained from the different product categories showed a clear difference in the role played by the different delicatessen and in the diffusion within the production lines. The category of starters with mayonnaise showed a very high prevalence of the pathogen (56.7 %); this value was by far higher than those obtained from the other categories; when comparing this value with that obtained from all the samples without mayonnaise sauce (6/102, 5.9 %), a significant difference was revealed ($P < 0.01$). The frequent detection of L. *monocytogenes* in mayonnaise-based RTE foods was already investigated by Uyttendaele et al. (1999), who reported a prevalence of 21.3 % of the pathogen in mayonnaise-based salads obtained from the Belgian retail market. *L. monocytogenes* was also detected at a relatively high rate in pasta/rice-based courses (12.8 %) and in fish-based main courses (3.6 %). In contrast, it was never detected in starters without mayonnaise and meat-based main courses. If we analyse the counts of the pathogen more in detail, the starters with mayonnaise confirmed their role of interest, with 26.7 % of samples showing values >0.70 Log CFU/g (limit of quantification of the method) and 13.3 % of the counts >2 Log CFU/g, the threshold value (100 CFU/g) indicated by EU Reg. 2073/2005 (EC, 2005). In particular, counts overcoming the threshold were detected in diced vegetables with vegan mayonnaise, julienne vegetables with vegan mayonnaise, and sliced beef in tuna sauce. These data indicate that the mayonnaise sauce could be the most important source of the pathogen. Among the other product categories, only one sample of pasta-based courses from the 39 samples analysed resulted in a > 0.70 (1 Log CFU/g) Log CFU/g count. In contrast, no detectable counts were obtained from the other samples.

This contamination scenario suggests frequent contamination of the mayonnaise sauce production line, with an occasional spilling of the pathogen in the other production areas, with operators and/or equipment being potential cross-contamination vehicles. As reported above, equipment can become a suitable niche for L. *monocytogenes* survival and biofilm formation. According to Huffman and Butts (2008), niches can be defined as "locations harbouring the organism after the routine sanitation"; regarding the products analysed in this study, various equipment can be involved, in particular when mayonnaise sauce was used as an ingredient: mixers and dispensers can be difficult to be adequately cleaned and sanitised owing to difficult accessibility of the whole internal surfaces. Also, although this aspect was not investigated in this study, the accumulation of sauce debris could have determined the persistence of the pathogen through the sampling sessions.

A case of concern is related to sliced beef in tuna sauce, which showed a mean L. *monocytogenes* count of 2.12 Log CFU/g and a maximum of 3.22 Log CFU/g. This result suggests a role of the production line specifically dedicated to this product; in this light, it has to be noted that the contamination of other similar products (e.g. julienne vegetables with vegan mayonnaise) was markedly lower: this finding could confirm that the contamination could be related to the inadequate hygiene management of the production line rather than to the use of contaminated raw matter.

Considering the homogeneity (mutability) of the data, applying the Gini mutability index to evaluate the relative frequency of the individual product values calculated in the respective samplings, a value of 0.1961 was obtained; thus, recalling that the normalized index has values between 0 and 1, the data showed a low mutability, meaning they are pretty homogenous.

Table 2
prevalence and frequency of enumerable counts of L. *monocytogenes* in the RTE delicatessen categories.

	N° of analysed samples	Prevalence (%)	N° of samples with counts > 0.70 Log CFU/g (%)	N° of samples with counts > 2.00 Log CFU/g (%)
Starters with mayonnaise (SM)	30	17 (56.7)^{A}	8 (26.7)^{A}	4 (13.3)
Starters without mayonnaise (SW)	24	0 (0.0)^{B}	0 (0.0)^{B}	0 (0.0)
Pasta/rice-based courses (PR)	39	5 (12.8)^{B}	1 (2.6)^{B}	0 (0.0)
Meat-based main courses (MB)	11	0 (0.0)^{B}	0 (0.0)	0 (0.0)
Fish-based main courses (FB)	28	1 (3.6)^{B}	0 (0.0)^{B}	0 (0.0)
Total	132	23 (17.4)	9 (6.8)	4 (3.0)

^{A,B} Statistically significant difference ($P < 0.01$) among the RTE delicatessen categories.

3.2. Genomic characterization of L. monocytogenes strains

A total of 49 isolates were obtained from the positive samples and were identified as L. *monocytogenes*. Isolates characterization revealed the presence of two different serogroups, IIb and IIa, known to be the main ones isolated in food matrices, including RTE products (Henriques et al., 2017; Maćkiw et al., 2020). Moreover, *L. monocytogenes* strains IIb and IIa detected in this study were related to two different CCs and STs, in particular CC288-ST330 and CC121-ST717, respectively. All the detailed results are reported in Table 3 and Fig. 1.

The majority of the L. *monocytogenes* strains tested were classified as IIb, CC288-ST330 (40 up to 49 strains). Isolates belonging to CC288 were previously reported across facilities and consistently found in meat and from the environment (Burnett et al., 2022). CC288 *L. monocytogenes* strains are known to be prevalent throughout North America and Europe; they were often associated with food contamination (Amato et al., 2015; Chenal-Francisque et al., 2011) and were also implicated in invasive disease in Taiwan (Huang et al., 2015). In particular, ST330 was reported by Chen et al. (2019) in meat and meat products from China and by Suo et al. (2022) in Shanghai (China) poultry farm products.

Within the CC288-ST330 strains, two clusters were identified, showing no AD or AD ≤7 each other (Fig. 2). Specifically, in detail, the first cluster (cluster I) was characterized by L. *monocytogenes* strains isolated from different SM products: SM1 (diced vegetables with vegan mayonnaise), SM2 (diced vegetables with mayonnaise), SM3 (julienne vegetables with vegan mayonnaise) and SM8 (sliced beef in tuna sauce). Moreover, in the second cluster (cluster II) *L. monocytogenes* strains isolated from three different RTE categories were present: SM (SM1, SM2, SM3 and SM8), FB3 (octopus with vegetables) and PR2 (rice with shrimps). These findings confirmed how the same L. *monocytogenes* strain circulated among the tested RTE categories, probably residing and persisting within the processing plant. Furthermore, all the 40 CC288-ST330 *L. monocytogenes* strains of this study showed the same virulence profiles (Fig. 1). In agreement with the literature information, all the CC288-ST330 strains showed the presence of *Listeria* Pathogenicity Island 1 (LIPI-1) and a full-length *inlA* (Burnett et al., 2022). According to our findings, the ST330 was also newly reported to carry the *llsX* gene related to LIPI-3. This gene is known to encode the Listeriolysin S (LLS), a cytotoxic and hemolytic virulence factor essential for gut microbiota destruction, and also to be critical for L. *monocytogenes* infection and survival in the neutrophils (Chen et al., 2019). The presence of both

Table 3
L. monocytogenes isolates details (strain identification, accession number, molecular and genomic results), related ready to eat (RTE) category positive for L. *monocytogenes* detection and sampling session number.

Strain id	RTE category	RTE detailed category	Sampling session number	Accession number	Serogroup	Clonal Complex	Sequence Type
Lm_1	SM	SM1	1	SAMN35713243	IIb	CC288	ST330
Lm_2	SM	SM1	1	SAMN35713244	IIb	CC288	ST330
Lm_3	SM	SM1	2	SAMN35713245	IIb	CC288	ST330
Lm_4	SM	SM1	2	SAMN35713246	IIb	CC288	ST330
Lm_5	SM	SM1	2	SAMN35713247	IIb	CC288	ST330
Lm_6	SM	SM1	3	SAMN35713248	IIb	CC288	ST330
Lm_7	SM	SM1	3	SAMN35713249	IIb	CC288	ST330
Lm_8	SM	SM1	3	SAMN35713250	IIb	CC288	ST330
Lm_9	SM	SM2	1	SAMN35713251	IIb	CC288	ST330
Lm_10	SM	SM2	2	SAMN35713252	IIb	CC288	ST330
Lm_11	SM	SM2	2	SAMN35713253	IIa	CC121	ST717
Lm_12	SM	SM2	3	SAMN35713254	IIb	CC288	ST330
Lm_13	SM	SM2	4	SAMN35713255	IIa	CC121	ST717
Lm_14	SM	SM3	1	SAMN35713256	IIb	CC288	ST330
Lm_15	SM	SM3	1	SAMN35713257	IIb	CC288	ST330
Lm_16	SM	SM3	1	SAMN35713258	IIb	CC288	ST330
Lm_17	SM	SM3	1	SAMN35713259	IIb	CC288	ST330
Lm_18	SM	SM3	1	SAMN35713260	IIb	CC288	ST330
Lm_19	SM	SM3	1	SAMN35713261	IIb	CC288	ST330
Lm_20	SM	SM3	2	SAMN35713262	IIb	CC288	ST330
Lm_21	SM	SM3	2	SAMN35713263	IIb	CC288	ST330
Lm_22	SM	SM3	2	SAMN35713264	IIb	CC288	ST330
Lm_23	SM	SM3	3	SAMN35713265	IIb	CC288	ST330
Lm_24	SM	SM3	3	SAMN35713266	IIb	CC288	ST330
Lm_25	SM	SM3	3	SAMN35713267	IIb	CC288	ST330
Lm_26	SM	SM5	1	SAMN35713268	IIa	CC121	ST717
Lm_27	SM	SM5	1	SAMN35713269	IIa	CC121	ST717
Lm_28	SM	SM5	2	SAMN35713270	IIa	CC121	ST717
Lm_29	SM	SM5	4	SAMN35713271	IIa	CC121	ST717
Lm_30	FB	FB3	2	SAMN35713272	IIb	CC288	ST330
Lm_31	FB	FB3	2	SAMN35713273	IIb	CC288	ST330
Lm_32	PR	PR2	1	SAMN35713274	IIb	CC288	ST330
Lm_33	PR	PR3	2	SAMN35713275	IIa	CC121	ST717
Lm_34	PR	PR3	2	SAMN35713276	IIa	CC121	ST717
Lm_35	PR	PR6	4	SAMN35713277	IIa	CC121	ST717
Lm_36	SM	SM8	1	SAMN35713278	IIb	CC288	ST330
Lm_37	SM	SM8	1	SAMN35713279	IIb	CC288	ST330
Lm_38	SM	SM8	1	SAMN35713280	IIb	CC288	ST330
Lm_39	SM	SM8	1	SAMN35713281	IIb	CC288	ST330
Lm_40	SM	SM8	1	SAMN35713282	IIb	CC288	ST330
Lm_41	SM	SM8	1	SAMN35713283	IIb	CC288	ST330
Lm_42	SM	SM8	2	SAMN35713284	IIb	CC288	ST330
Lm_43	SM	SM8	2	SAMN35713285	IIb	CC288	ST330
Lm_44	SM	SM8	2	SAMN35713286	IIb	CC288	ST330
Lm_45	SM	SM8	2	SAMN35713287	IIb	CC288	ST330
Lm_46	SM	SM8	2	SAMN35713288	IIb	CC288	ST330
Lm_47	SM	SM8	2	SAMN35713289	IIb	CC288	ST330
Lm_48	SM	SM8	3	SAMN35713290	IIb	CC288	ST330
Lm_49	SM	SM8	3	SAMN35713291	IIb	CC288	ST330

SM: starters with mayonnaise sauce.
FB: fish-based main course.
PR: pasta/rice-based courses.
SM1: diced vegetables with vegan mayonnaise.
SM2: diced vegetables with mayonnaise.
SM3: julianne vegetables with vegan mayonnaise.
SM5: ham filled.
SM8: sliced beef in tuna sauce.
FB3: octopus with vegetables.
PR2: rice with shrimps.
PR3: emmer with vegetables, speck and cheese.
PR6: cold pasta with tuna.

complete LIPI-1 and LIPI-3 is considered responsible for the virulence increase in some L. *monocytogenes* strains (Vilchis-Rangel et al., 2019) and this aspect could represent a significant concern for the consumers of RTE products, which by definition will not need any further treatment before consumption.

The other L. *monocytogenes* strains were classified as IIa, CC121-ST717 (9 up to 49 strains). Clonal complex CC121 is overrepresented in food, particularly in meat and meat products and in food production plants (Centorotola et al., 2021; Maury et al., 2019; Torresi et al., 2021), whereas it is less frequent in animals and the environment (Henri et al., 2016; Sévellec et al., 2020). Two clusters were detected within the CC121-ST717 strains tested, showing how this strain circulated in different RTE categories (Fig. 3). In particular, in the first cluster (cluster I) with AD $\leq$7, *L. monocytogenes* strains isolated from SM2 and SM5 (ham filled)) and PR3 (emmer with vegetables, speck and cheese) were highlighted. The second cluster (cluster II), instead was represented by

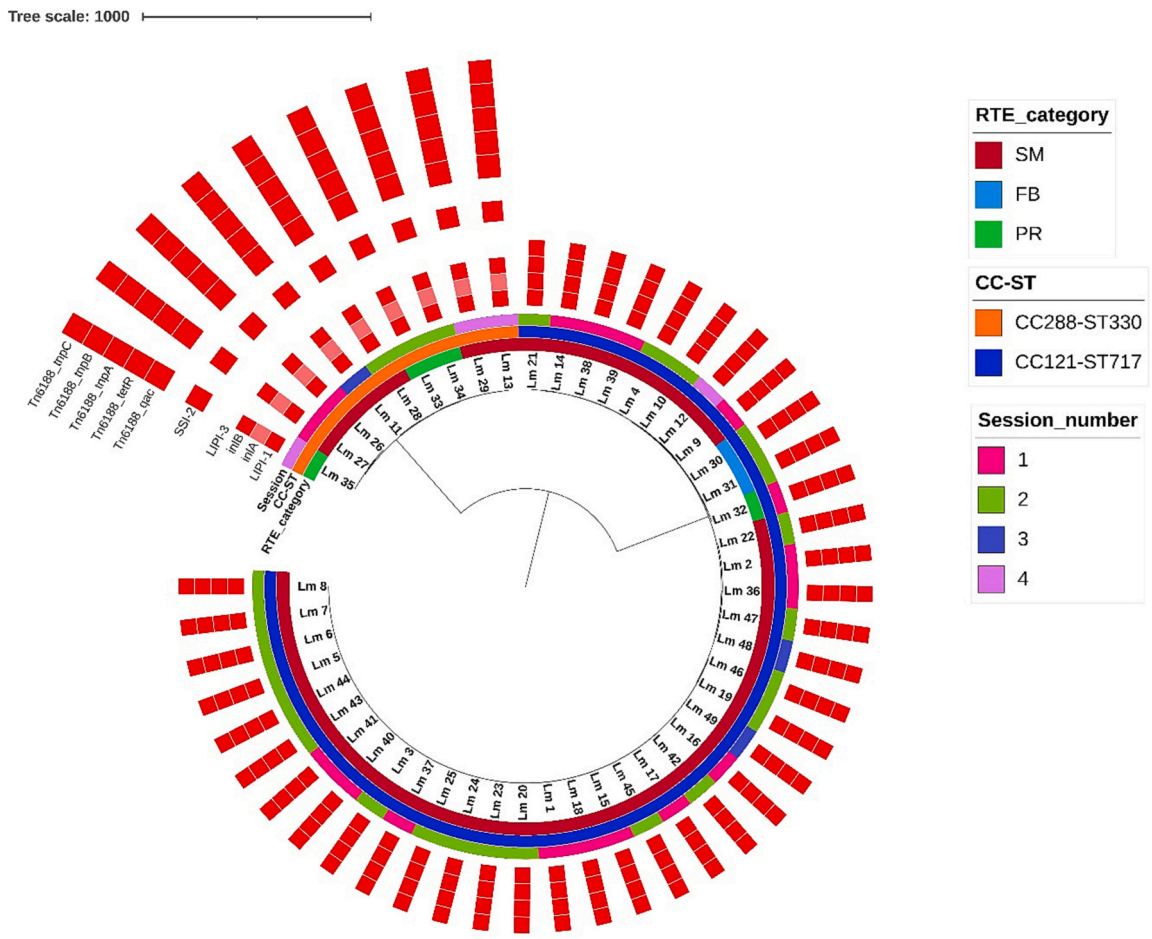

Fig. 1. Core genome multilocus sequence typing (cgMLST) phylogenetic tree grouping of virulence and resistance across the 49 *L. monocytogenes* isolates. Virulence and resistance profiles are shown in the heatmap. Red square: presence of the gene; light red: presence of premature stop codon and truncated *inlA*. Visualizing the genes' profiles and their presence/absence according to their cgMLST was possible using the Interactive Tree of Life (iTOL) (https://itol.embl.de/).
SM: Starters with mayonnaise
FB: Fish-based main courses
PR: Pasta/rice-based courses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

L. *monocytogenes* strains isolated from SM5 and PR6 (cold pasta with tuna), showing at least 5 AD.

CC121 is known to be able to resist and persist in the food processing plant (Centorotola et al., 2021; Guidi et al., 2021; Palma et al., 2020; Stoller et al., 2019); all the CC121 strains of this study carried several resistance genes, such as specific stress island and transposons (Fig. 1), that could increase the resistance of L. *monocytogenes* strains. In particular, the Stress Survival Islet 2 (SSI-2), found in all the L. *monocytogenes* CC121 of this study, is well known to be mainly harboured by strains of CC121-ST121, conferring resistance to alkaline and oxidative stresses (Harter et al., 2017; Maury et al., 2019). Moreover, all the CC121-ST717 strains also confirmed the presence of transposon Tn6188_qac, specific for tolerance to benzalkonium chloride, the primary disinfectant used during the cleaning and disinfection of food production plants. In this study, other transposons were identified only for this CC, such as Tn6188_tetR, Tn6188_tnpA, Tn6188_tnpB, Tn6188_tnpC. Despite several resistance factors, CC121 isolates are usually not linked to clinical cases. They are known to be hypovirulent clones due to the presence of an internalin A gene truncation produced by a premature stop codon (PMSC), leading to attenuated virulence and increased ability to adapt and survive in food and food processing plants (Guidi et al., 2021; Magagna et al., 2023; Maury et al., 2019). Regarding ST717, few data are nowadays available in the literature; according to findings obtained using the BIGSdb-Lm database (accessed on 10 June 2023), this new ST was first assigned to L. *monocytogenes* strains isolated in 2011 from a dairy facility environment in Italy. Moreover, according to our results, Magagna et al. (2023) reported this recently assigned ST, which was detected from food and environmental samples and characterized by PMSC mutation. No more data are available on ST717; therefore, these results could help to increase knowledge on new minor STs related to the CC121 group circulating in RTE products.

According to our genomic findings, the presence of two specific CCs-STs L. *monocytogenes* isolates in different RTE products tested in this study, taken during different sampling sessions, could be related to the potential persistence of these strains within the food processing plant during the years. It could be necessary to improve this aspect by sampling the food processing plant environments and considering both the food and non-food contact surfaces. Moreover, the persistence of L. *monocytogenes* in food environments could facilitate the cross-contamination of various RTE products (EFSA, 2018), probably in the production line or during particular production phases. Furthermore, an interesting result derives from the RTE category SM2, in which both CC288-ST330 and CC121-ST717 strains were observed. This finding could be useful to define the source attribution and the routes of L. *monocytogenes* contamination.

3.3. Selection of RTE delicatessen and growth potential of L. monocytogenes

Based on the results obtained from the analysis of the products, beef

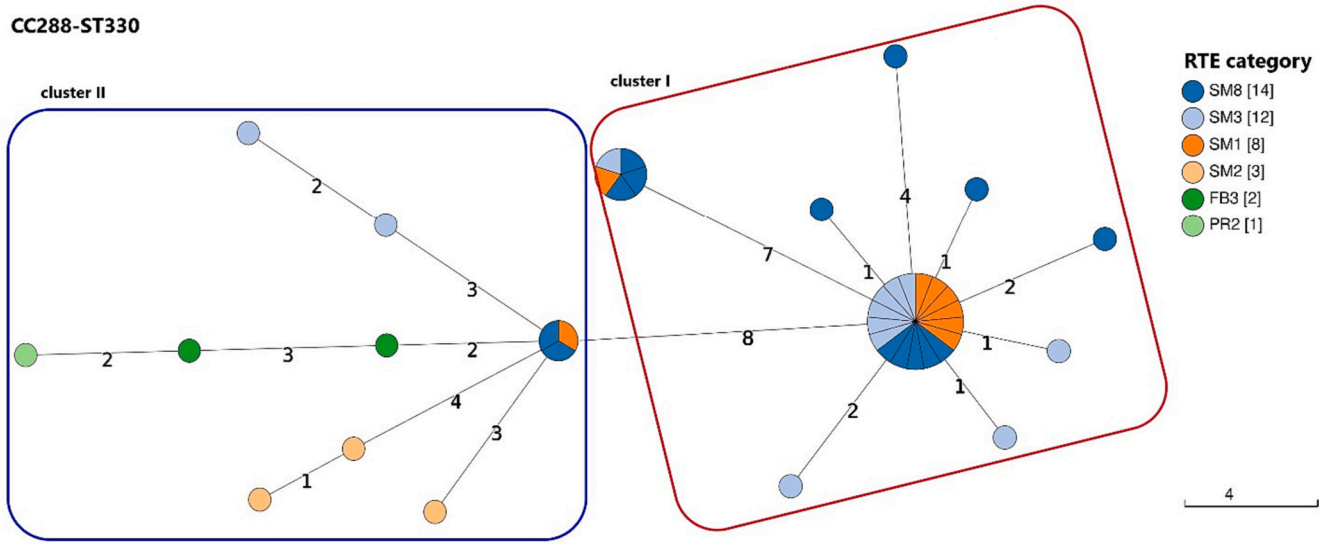

Fig. 2. Minimum spanning tree (MST) based on the cgMLST profiles of L. *monocytogenes* CC288-ST330, coloured according to RTE category. CC288-ST330 clusters are highlighted with boxes: the red box is for the first cluster (cluster I), and the blue red box is for the second cluster (cluster II).
SM: Starters with mayonnaise
FB: Fish-based main courses
PR: Pasta/rice-based courses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

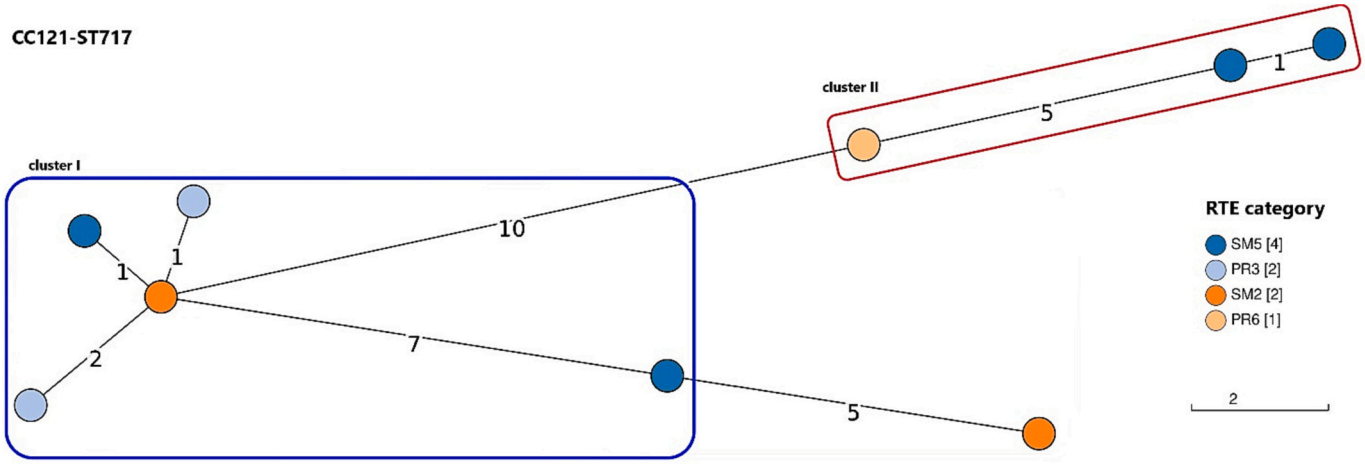

Fig. 3. Minimum spanning tree (MST) based on the cgMLST profiles of L. *monocytogenes* CC121-ST717, coloured according to RTE category. CC121-ST717 clusters are highlighted with boxes: the blue box is for the first cluster (cluster I), and the red box is for the second cluster (cluster II).
SM: Starters with mayonnaise
PR: Pasta/rice-based courses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in tuna sauce was selected as it combined a high prevalence of the pathogen with the highest bacterial count.

The challenge tests performed on beef in tuna sauce aimed to mimic different contamination scenarios, namely the contamination of meat slices after cooking with the effect of meat/sauce interface (A), previous contamination of the sauce added to the product (B) and the same contamination route of scenario (A), but without the effect of meat/sauce interface (C).

Data are reported in Table 4. The comparison among the three series inoculated with L. *monocytogenes* showed a difference between series B (inoculated in the sauce) and the other two. However, the difference was not statistically significant. Considering a value of 0.5 Log CFU/g as the threshold to define a food substrate as suitable for supporting L. *monocytogenes* growth (EURL LM, 2021), the sauce used for the production showed to allow only a minimal increase in the counts ($\Delta = 0.25$ Log CFU/g), and could be considered as the least critical substrate. Such a scenario represented by sauce contamination could result from the persistence of the pathogen within production equipment, such as a dispenser, due to insufficient cleaning/disinfection procedures. In the

Table 4
Mean count of L. *monocytogenes* during storage of the product (sliced beef in tuna sauce).

Series	t_0	t_4	t_{10}	t_{15}
A	2.93 ± 0.10	3.64 ± 0.14	3.27 ± 0.09	3.36 ± 0.29
Δ	–	0.71 (t_4-t_0)	0.34 (t_{10}-t_0)	0.43 (t_{15}-t_0)
B	2.96 ± 0.12	3.00 ± 0.04	3.21 ± 0.12	3.03 ± 0.11
Δ	–	0.04 (t_4-t_0)	0.25 (t_{10}-t_0)	0.07 (t_{15}-t_0)
C	2.62 ± 0.54	3.21 ± 0.15	3.05 ± 0.18	2.77 ± 0.42
Δ	–	0.60 (t_4-t_0)	0.43 (t_{10}-t_0)	0.16 (t_{15}-t_0)

Series considered: A: LM on the upper beef slice, in contact with tuna sauce; B: LM in the sauce; C: LM between beef slices.

other two scenarios, where the contamination of meat surface was hypothesised, an initial increase (until t_4 sampling) was observed, exceeding the 0.5 Log threshold (0.71 and 0.60 Log CFU/g in series A and C, respectively), followed by a decrease until the end of the experimental period (15 days). An increase of 0.65 Log CFU/g at 7 °C was reported in meat salad with mayonnaise inoculated on the surface with L. *monocytogenes* by Uyttendaele et al. (2009); these results in relation to our findings suggest the importance of the possible contamination of meat surface that acts as a potentially suitable environment for *Listeria* growth, at least during the first days of shelf life. In these scenarios, too, an insufficient sanitation of production equipment (e.g. the slicer) could be hypothesised as the likely source of contamination.

In the same study by Uyttendaele et al. (2009), many challenge tests were executed on RTE foods, but growth of L. *monocytogenes* occurred only in 9.9 % of cases. The authors emphasised the role of an appropriate product formulation; in our case, the sauce was a sufficient hurdle to prevent growth. This trend observed during the trial could also be presumably explained by the antagonistic activity of lactic acid bacteria that showed fast growth in all the experimental series, starting from values around 4 Log CFU/g and reaching values above 6 and 8 Log CFU/g after 4 and 10 days, respectively. LAB were already proven to exhibit promising competitive action towards L. *monocytogenes* in meat substrates (Fadda et al., 2008; Stella et al., 2013).

4. Conclusions

RTE deli foods are susceptible to contamination by L. *monocytogenes*, owing to the complexity of the production process, to the environmental diffusion of the pathogen and its ability to persist in several niches along the production chain. The high prevalence detected (17.4 % of the RTE delicatessen considered, >50 % for starters with mayonnaise) was associated with a close similarity among the clones isolated. These results indicate the possibility of persistence and circulation of the pathogen within the plant, thus highlighting the critical role of hygienic practices during production, especially cleaning and disinfection procedures. Because of the lack of environmental samples, related L. *monocytogenes* isolates, and different food product sampling sessions, it was impossible to confirm the presence of residing and persisting strains within the processing plant. Further studies are needed to evaluate the presence of L. *monocytogenes* and the possible persistence of these two CCs-STs in the food production plant environments, sampling both the food contact and non-food contact surfaces to detect L. *monocytogenes*.

In order to evaluate the relative risk posed by the consumption of beef with tuna sauce, three parameters should be considered (Uyttendaele et al., 2009), namely the prevalence of contaminated samples, the count of L. *monocytogenes* and the growth potential of the pathogen in the food substrate. Our data indicated a frequent exposure of the consumers to the pathogen; the substrate allowed a moderate growth (lower than 1 Log, observed only in the first part of the trial), but the detection, in some cases, of high counts should be carefully considered. The threshold demanded by EU Reg. 2073/2005 of 2 Log CFU/g, which was exceeded in some samples of different starters with mayonnaise sauce, is, correctly, very conservative. The risk posed by consuming these products should also be carefully evaluated when considering different population strata (e.g. older people or pregnant women). The Food Business Operators play a crucial role in preventing severe RTE food contamination by L. *monocytogenes* by limiting cross-contamination and reducing the presence of the pathogen in the production plan by applying correct sanitation procedures.

Funding

This work was supported by the University of Milan – Call: Research Support Plan 2020 [Piano di Sostegno alla Ricerca 2020], project: Listeria monocytogenes in RTE food: prevalence, genetic characterization of the isolates, growth potential and performance of bioprotective approaches.

CRediT authorship contribution statement

E. Tirloni: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. **G. Centorotola:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **F. Pomilio:** Data curation, Writing – review & editing. **M. Torresi:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **C. Bernardi:** Investigation, Methodology, Writing – review & editing. **S. Stella:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The genome assemblies of the strains were deposited at DDBJ/ENA/GenBank under the BioProjects PRJNA982763 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA982763).

References

AFNOR (Association Française de Normalisation), 1998. Detection of *Listeria monocytogenes* and *Listeria* spp. AFNOR BRD 07/04-09/98.

AFNOR (Association Française de Normalisation), 2001. Enumeration of *Listeria monocytogenes* and *Listeria* spp. AFNOR BRD 07/05-09/01.

Allerberger, F., Wagner, M., 2010. Listeriosis: a resurgent foodborne infection. Clin. Microbiol. Infect. 16, 16–23. https://doi.org/10.1111/j.1469-0691.2009.03109.x.

Amato, E., Panteghini, C., Parisi, A., Huedo, P., Gori, M., Tilola, M., Losio, N., Pontello, M.M., 2015, October. A microbial subtyping approach for the identification of food potentially involved in listeriosis in northern Italy. In: EFSA's 2nd Scientific Conference - Shaping the Future of Food Safety, Together, Parma, Italy.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P., SPAdes, A., 2012. A new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Buchanan, R.L., Gorris, L.G.M., Hayman, M.M., Jackson, T.C., Whiting, R.C., 2017. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control 75, 1–13. https://doi.org/10.1016/j.foodcont.2016.12.016.

Burnett, E., Kucerova, Z., Freeman, M., Kathariou, S., Chen, J., Smikle, M., 2022. Whole-Genome Sequencing reveals multiple subpopulations of dominant and persistent lineage I isolates of *Listeria monocytogenes* in two meat processing facilities during 2011-2015. Microorganisms 10, 1070. https://doi.org/10.3390/microorganisms10051070.

Centorotola, G., Guidi, F., D'Aurizio, G., Salini, R., Di Domenico, M., Ottaviani, D., Petruzzelli, A., Fisichella, S., Duranti, A., Tonucci, F., Acciari, V. A., Torresi, M., Pomilio, F., & Blasi, G. (2021). Intensive environmental surveillance plan for *Listeria monocytogenes* in food producing plants and retail stores of Central Italy: prevalence and genetic diversity. Foods, 10, article 1944. doi:https://doi.org/10.3390/foods10081944.

Chen, M., Cheng, J., Zhang, J., Chen, Y., Zeng, H., Xue, L., Lei, T., Pang, R., Wu, S., Wu, H., Zhang, S., Wei, X., Zhang, Y., Ding, Y., Wu, Q., 2019. Isolation, potential virulence, and population diversity of *Listeria monocytogenes* from meat and meat products in China. Front. Microbiol. 10, 946 https://doi.org/10.3389/fmicb.2019.00946.

Chenal-Francisque, V., Lopez, J., Cantinelli, T., Caro, V., Tran, C., Leclercq, A., Lecuit, M., Brisse, S., 2011. Worldwide distribution of major clones of *Listeria monocytogenes*. Emerg. Infect. Dis. 17, 1110–1112. https://doi.org/10.3201/eid/1706.101778.

Cito, F., Di Pasquale, A., Cammà, C., Cito, P., 2018. The Italian information system for the collection and analysis of complete genome sequence of pathogens isolated from animal, food and environment. Int. J. Infect. Dis. 73, 296–297. https://doi.org/10.1016/j.ijid.2018.04.4090.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J. Clin. Microbiol. 42, 3819–3822. https://doi.org/10.1128/JCM.42.8.3819-3822.2004.

Doumith, M., Jacquet, C., Gerner-Smidt, P., Graves, L.M., Loncarevic, S., Mathisen, T., Morvan, A., Salcedo, C., Torpdahl, M., Vazquez, J.A., Martin, P., 2005. Multicenter validation of a multiplex PCR assay for differentiating the major *Listeria monocytogenes* serovars 1/2a, 1/2b, 1/2c, and 4b: toward an international standard. J. Food Prot. 68, 2648–2650. https://doi.org/10.4315/0362-028x-68.12.2648.

Duranti, A., Sabbatucci, M., Blasi, G., Acciari, V.A., Ancora, M., Bella, A., Busani, L., Centorame, P., Cammà, C., Conti, F., De Medici, D., Di Domenico, M., Di Marzio, V., Filippini, G., Fiore, A., Fisichella, S., Gattuso, A., Gianfranceschi, M., Graziani, C., Guidi, F., Marcacci, M., Marfoglia, C., Neri, D., Orsini, M., Ottaviani, D., Petruzzelli, A., Pezzotti, P., Rizzo, C., Ruolo, A., Scavia, G., Scuota, S., Tagliavento, G., Tibaldi, A., Tonucci, F., Torresi, M., Migliorati, G., Pomilio, F., 2018. A severe outbreak of listeriosis in central Italy with a rare pulsotype associated with processed pork products. J. Med. Microbiol. 67, 1351–1360. https://doi.org/10.1099/jmm.0.00078.

EC – European Commission, 2005. European commission regulation (EC) no 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Off. J. Eur. Union L 338, 1–26.

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021a. The European Union one health 2019 Zoonoses report. EFSA J. 19 (2), 6406. https://doi.org/10.2903/j.efsa.2021.6406.

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021b. The European Union one health 2020 Zoonoses report. EFSA J. 19 (12), 6971. https://doi.org/10.2903/j.efsa.2021.6971.

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2022. The European Union one health 2021 zoonoses report. EFSA J. 20 (12), 7666. https://doi.org/10.2903/j.efsa.2022.7666.

EFSA Panel on Biological Hazards, 2018. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA J. 16 (1), 5134. https://doi.org/10.2903/j.efsa.2018.5134.

EURL Lm (EU Reference Laboratory for Listeria monocytogenes), 2021. Technical guidance document on challenge tests and durability studies for assessing shelf-life of ready-to-eat foods related to *Listeria monocytogenes*. Version 4. Retrieved from: https://food.ec.europa.eu/system/files/2021-07/biosafety_fh_mc_tech-guide-doc_listeria-in-rte-foods_en_0.pdf. Accessed August 3, 2023.

EURL LM (European Reference Laboratory for Listeria monocytogenes, 2013. Development of a set of *Listeria monocytogenes* strains for conducting challenge tests. Version 0 – 20/12/2013. Retrieved from: https://sitesv2.anses.fr/en/system/files/private/LIS-Cr-201317R.pdf. Accessed August 3, 2023.

Fadda, S., Chambon, C., Champomier-Vergès, M.C., Talon, R., Vignolo, G., 2008. *Lactobacillus* role during conditioning of refrigerated and vacuum-packaged Argentinean meat. Meat Sci. 79, 603–610. https://doi.org/10.1016/j.meatsci.2007.04.003.

FAO/WHO (Food and Agriculture Organization of the United Nations, World Health Organization), 2004. Risk assessment of Listeria monocytogenes in Ready-To-Eat foods. In: Technical Report. Microbiological risk Assessment Series 5. FAO.

FAO-WHO (Food and Agriculture Organization-World Health Organization), 2001. Joint FAO/WHO expert consultation on risk assessment of microbiological hazards in food. Risk characterization of Salmonella spp. in eggs and broiler chickens and *Listeria monocytogenes* in ready-to-eat foods. FAO Food and Nutrition Paper n° 72 (pp. 26–27). FAO.

Fernández-Martínez, N.F., Ruiz-Montero, R., Briones, E., Baños, E., García San Miguel Rodríguez-Alarcón, L., Chaves, J.A., Abad, R., Varela, C., on behalf of the LISMOAN team, Lorusso, N., 2022. Listeriosis outbreak caused by contaminated stuffed pork, Andalusia, Spain, July to October 2019. Euro Surveillance, 27, Article 2200279. https://doi.org/10.2807/1560-7917.ES.2022.27.43.2200279.

Ferreira, F., Gray, M., Wiedmann, M., Boor, K.J., 2004. Comparative Genomic Analysis of the sigB Operon in Listeria monocytogenes and in Other Gram-Positive Bacteria. Curr. Microbiol. 48, 39–46.

Ferreira, V., Wiedmann, M., Teixeira, P., Stasiewicz, M.J., 2014. *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. J. Food Prot. 77, 150–170. https://doi.org/10.4315/0362-028X.JFP-13-150.

Guidi, F., Orsini, M., Chiaverini, A., Torresi, M., Centorame, P., Acciari, V.A., Salini, R., Palombo, B., Brandi, G., Amagliani, G., Schiavano, G.F., Massacci, F.R., Fisichella, S., Domenico, M.D., Ancora, M., Pasquale, A.D., Duranti, A., Cammà, C., Pomilio, F., Blasi, G., 2021. Hypo- and hyper-virulent *Listeria monocytogenes* clones persisting in two different food processing plants of Central Italy. Microorganisms 9, 376. https://doi.org/10.3390/microorganisms9020376.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

Harter, E., Wagner, E.M., Zaiser, A., Halecker, S., Wagner, M., Rychli, K., 2017. Stress survival islet 2, predominantly present in *Listeria monocytogenes* strains of sequence type 121, is involved in the alkaline and oxidative stress responses. Appl. Environ. Microbiol. 83, e00827-17 https://doi.org/10.1128/AEM.00827-17.

Henri, C., Felix, B., Guillier, L., Leekitcharoenphon, P., Michelon, D., Mariet, J.F., Aarestrup, F.M., Mistou, M.Y., Hendriksen, R.S., Roussel, S., 2016. Population genetic structure of *Listeria monocytogenes* strains as determined by pulsed-field gel electrophoresis and multilocus sequence typing. Appl. Environ. Microbiol. 82, 5720–5728. https://doi.org/10.1128/AEM.00583-16.

Henriques, A.R., Cristino, J.M., Fraqueza, M.J., 2017. Genetic characterization of *Listeria monocytogenes* isolates from industrial and retail ready-to-eat meat-based foods and their relationship with clinical strains from human listeriosis in Portugal. J. Food Prot. 80, 551–560. https://doi.org/10.4315/0362-028X.JFP-16-310.

Huang, Y.-T., Ko, W.-C., Chan, Y.-J., Lu, J.-J., Tsai, H.-Y., Liao, C.-H., Sheng, W.-H., Teng, L.-J., Hsueh, P.-R., 2015. Disease burden of invasive listeriosis and molecular characterization of clinical isolates in Taiwan, 2000-2013. PloS One 10, e0141241. https://doi.org/10.1371/journal.pone.0141241.

Huffman, R., Butts, J., 2008. Lessons learned in the meat industry: control of *Listeria* in RTE meat and poultry products. Available at: http://www.meatami.com/ht/a/GetDocumentAction/i/, 42115.

Kérouanton, A., Marault, M., Petit, L., Grout, J., Dao, T.T., Brisabois, A., 2010. Evaluation of a multiplex PCR assay as an alternative method for *Listeria monocytogenes* serotyping. J. Microbiol. Methods 80, 134–137. https://doi.org/10.1016/j.mimet.2009.11.008.

Lakicevic, B., Nastasijevic, I., Raseta, M., 2015. Sources of *Listeria monocytogenes* contamination in retail establishments. Procedia Food Science 5, 160–163.

Maćkiw, E., Stasiak, M., Kowalska, J., Kucharek, K., Korsak, D., Postupolski, J., 2020. Occurrence and characteristics of *Listeria monocytogenes* in ready-to-eat meat products in Poland. J. Food Prot. 83, 1002–1009. https://doi.org/10.4315/JFP-19-525.

Magagna, G., Gori, M., Russini, V., De Angelis, V., Spinelli, E., Filipello, V., Tranquillo, V. M., De Marchis, M.L., Bossù, T., Fappani, C., Tanzi, E., Finazzi, G., 2023. Evaluation of the virulence potential of *Listeria monocytogenes* through the characterization of the truncated forms of internalin A. Int. J. Mol. Sci. 24, 10141 https://doi.org/10.3390/ijms241210141.

Malley, T.J.V., Butts, J., Wiedmann, M., 2015. Seek and destroy process: *Listeria monocytogenes* process controls in the ready-to-eat meat and poultry industry. J. Food Prot. 78, 436–445. https://doi.org/10.4315/0362-028X.JFP-13-507.

Maury, M.M., Bracq-Dieye, H., Huang, L., Vales, G., Lavina, M., Thouvenot, P., Disson, O., Leclercq, A., Brisse, S., Lecuit, M., 2019. Hypervirulent *Listeria monocytogenes* clones' adaption to mammalian gut accounts for their association with dairy products. Nat. Commun. 10, 2488 https://doi.org/10.1038/s41467-019-10380-0.

Moura, A., Criscuolo, A., Pouseele, H., Maury, M.M., Leclercq, A., Tarr, C., Björkman, Jonas T., J. T., Dallman, T., Reimer, A., Enouf, V., Larsonneur, E., Carleton, H., Bracq-Dieye, H., Katz, L.S., Jones, L., Touchon, M., Tourdjman, M., Walker, M., Stroika, S., Cantinelli, T., Chenal-Francisque, V., Kucerova, Z., Rocha, E. P.C., Nadon, C., Grant, K., Nielsen, E.M., Pot, B., Gerner-Smidt, P., Lecuit, M., Brisse, S., 2016. Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. Nat. Microbiol. 2, 16185 https://doi.org/10.1038/nmicrobiol.2016.185.

Palma, F., Brauge, T., Radomski, N., Mallet, L., Felten, A., Mistou, M.-Y., Brisabois, A., Guillier, L., Midelet-Bourdin, G., 2020. Dynamics of mobile genetic elements of *Listeria monocytogenes* persisting in ready-to-eat seafood processing plants in France. BMC Genomics 21, 130. https://doi.org/10.1186/s12864-020-6544-x.

Pérez-Rodríguez, F., Carrasco, E., Bover-Cid, S., Jofré, A., Valero, A., 2017. Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: activity 2, a quantitative risk characterization on *L. monocytogenes* in RTE foods; starting from the retail stage. EFSA J. 14 (7), 1252E. https://doi.org/10.2903/sp.efsa.2017.EN-1252.

Pichler, J., Much, P., Kasper, S., Fretz, R., Auer, B., Kathan, J., Mann, M., Huhulescu, S., Ruppitsch, W., Pietzka, A., Silberbauer, K., Neumann, C., Gschiel, E., de Martin, A., Schuetz, A., Gindl, J., Neugschwandtner, E., Allerberger, F., 2009. An outbreak of febrile gastroenteritis associated with jellied pork contaminated with *Listeria monocytoge-nes*. Wien. Klin. Wochenschr. 121, 81–88. https://doi.org/10.1007/s00508-009-1137-3.

Portmann, A.C., Fournier, C., Gimonet, J., Ngom-Bru, C., Barretto, C., Baert, L., 2018. A validation approach of an end-to-end whole genome sequencing workflow for source tracking of *Listeria monocytogenes* and *Salmonella enterica*. Front. Microbiol. 9, 446 https://doi.org/10.3389/fmicb.2018.00446.

Pradhan, A.K., Ivanek, R., Gröhn, Y.T., Bukowski, R., Geornaras, I., Sofos, J.N., Wiedmann, M., 2010. Quantitative risk assessment of listeriosis-associated deaths due to *Listeria monocytogenes* contamination of deli meats originating from manufacture and retail. J. Food Prot. 73, 620–630. https://doi.org/10.4315/0362-028x-73_4_620.

Sévellec, Y., Torresi, M., Félix, B., Palma, F., Centorotola, G., Bilei, S., Senese, M., Terracciano, G., Leblanc, J.C., Pomilio, F., Roussel, S., 2020. First report on the finding of *Listeria mnocytogenes* ST121 Strain in a dolphin brain. Pathogens 9, 802. https://doi.org/10.3390/pathogens9100802.

Stella, S., Ripamonti, B., Vandoni, S., Bernardi, C., Sgoifo Rossi, C.A., 2013. Microbiological and physicochemical quality evaluation of vacuum-packed argentine beef imported into Italy. J. Food Qual. 36, 253–262. https://doi.org/10.1111/jfq.12038.

Stoller, A., Stevens, M.J.A., Stephan, R., Guldimann, C., 2019. Characteristics of *Listeria monocytogenes* strains persisting in a meat processing facility over a 4-year period. Pathogens 8, 32. https://doi.org/10.3390/pathogens8010032.

Suo, Y., Qu, Y., Bai, Y., Zhou, X., Lin, T., Wang, J., Chen, W., Zhou, C., 2022. Genetic diversity of *Listeria monocytogenes* isolates from farm products in Shanghai, China. Food Quality and Safety 6, 1–8. https://doi.org/10.1093/fqsafe/fyac040.

Timme, R. E., Wolfgang, W. J., Balkey, M. Venkata, S. L. G., Randolph, R. Allard, M. & Strain, E. (2020). Optimizing open data to support one health: best practices to ensure interoperability of genomic data from bacterial pathogens. One Health Outlook, 2(1), article 20. doi:https://doi.org/10.1186/s42522-020-00026-3.

Torresi, M., Chiaverini, A., Acciari, V.A., Centorame, P., Centorotola, G., Guidi, F., Russini, V., Pomilio, F., 2021. Genotyping of *Listeria monocytogenes* strains in Italian food-processing environments. Eur. J. Public Health 31(S3), 3, ckab165.624. https://doi.org/10.1093/eurpub/ckab165.624.

Tsaloumi, S., Aspridou, Z., Tsigarida, E., Gaitis, F., Garofalakis, G., Barberis, K., Tzoumanika, F., Dandoulaki, M., Skiadas, R., Koutsoumanis, K., 2021. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat (RTE) cooked meat products sliced at retail stores in Greece. Food Microbiol. 99, 103800 https://doi.org/10.1016/j.fm.2021.103800.

US-FDA (United States Food and Drug Administration, Center for Food Safety and Applied Nutrition; U.S. Department of Agriculture, Food Safety and Inspection Service; and Centers for Disease Control and Prevention), 2003. Quantitative assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Retrieved from: https://www.fda.gov/food/cfsan-risk-safety-assessments/quantitative-assessment-relative-risk-public-health-foodborne-listeria-monocytogenes-among-selected. (Accessed 3 August 2023).

Uyttendaele, M., De Troy, P., Debevere, J., 1999. Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. Int. J. Food Microbiol. 53, 75–80.

Uyttendaele, M., Busschaert, P., Valero, A., Geeraerd, A.H., Vermeulen, A., Jacxsens, L., Goh, K.K., De Loy, A., Van Impe, J.F., Devlieghere, F., 2009. Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. Int. J. Food Microbiol. 133, 94–104.

de Valk, H., Vaillant, V., Jacquet, C., Rocourt, J., Le Querrec, F., Stainer, F., Quelquejeu, N., Pierre, O., Pierre, V., Deseclos, J.C., Goulet, V., 2001. Two consecutive nationwide outbreaks of Listeriosis in France, October 1999-February 2000. Am. J. Epidemiol. 154, 944–950. https://doi.org/10.1093/aje/154.10.944.

Vilchis-Rangel, R.E., Espinoza-Mellado, M.D.R., Salinas-Jaramillo, I.J., Martinez-Peña, M.D., Rodas-Suárez, O.R., 2019. Association of *Listeria monocytogenes* LIPI-1 and LIPI-3 marker llsX with invasiveness. Curr. Microbiol. 76, 637–643. https://doi.org/10.1007/s00284-019-01671-2.

Walker, S.J., Archer, P., Banks, J.G., 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. 68, 157–162. https://doi.org/10.1111/j.1365-2672.1990.tb02561.x.

Wingett, S. W., & Andrews, S. (2018). FastQ screen: a tool for multi-genome mapping and quality control. F1000Research, 7, article 1338. Doi:10.12688/f1000research.15931.1.

Zhou, Z., Alikhan, N.F., Sergeant, M.J., Luhmann, N., Vaz, C., Francisco, A.P., Carriço, J. A., Achtman, M., 2018. Grapetree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Res. 28, 1395–1404. https://doi.org/10.1101/gr.232397.117.