International Journal of Food Microbiology 350 (2021) 109247

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Characterisation of *Listeria monocytogenes* food-associated isolates to assess environmental fitness and virulence potential

Jessica A. Gray [a,b,c,*], P. Scott Chandry [b], Mandeep Kaur [d], Chawalit Kocharunchitt [c], John P. Bowman [c], Edward M. Fox [b,e]

[a] CSIRO Agriculture and Food, Coopers Plains, QLD, Australia
[b] CSIRO Agriculture and Food, Werribee, VIC, Australia
[c] Food Safety Centre, Tasmanian Institute of Agriculture, School of Land and Food, University of Tasmania, Hobart, TAS, Australia
[d] Biosciences and Food Technology, School of Science, RMIT University, VIC, Australia
[e] Department of Applied Sciences, Northumbria University, Newcastle upon Tyne NE1 8ST, United Kingdom

ARTICLE INFO

Keywords:
Disinfectant
Cadmium
Pathogenicity
Antimicrobial
Plasmid
Transposon

ABSTRACT

The ability of *Listeria monocytogenes* isolates to survive within the food production environment (FPE), as well as virulence, varies greatly between strains. There are specific genetic determinants that have been identified which can strongly influence a strains ability to survive in the FPE and/or within human hosts. In this study, we assessed the FPE fitness and virulence potential, including efficacy of selected hygiene or treatment intervention, against 52 *L. monocytogenes* strains isolated from various food and food environment sources. Phenotypic tests were performed to determine the minimum inhibitory concentration of cadmium chloride and benzalkonium chloride and the sensitivities to five clinically relevant antibiotics. A genomic analysis was also performed to identify resistance genes correlating to the observed phenotypic resistance profiles, along with genetic determinants of interest which may elude to the FPE fitness and virulence potential. A transposon element containing a novel cadmium resistance gene, *cadA7*, a Tn*916* variant insert in the hypervariable *Listeria* genomic island 1 region and an LGI2 variant were identified. Resistance to cadmium and disinfectants was prevalent among isolates in this study, although no resistance to clinically important antimicrobials was observed. Potential hypervirulent strains containing full length *inlA*, LIPI-1 and LIPI-3 were also identified in this study. Cumulatively, the results of this study show a vast array of FPE survival and pathogenicity potential among food production-associated isolates, which may be of concern for food processing operators and clinicians regarding *L. monocytogenes* strains colonising and persisting within the FPE, and subsequently contaminating food products then causing disease in at risk population groups.

1. Introduction

Listeria monocytogenes is a saprophyte and a human pathogen. *L. monocytogenes* can cause the severe disease, listeriosis, in at risk populations that includes the elderly, immuno-compromised, pregnant women and neonates with an associated mortality rate of almost 30% (Havelaar et al., 2015; Todd and Notermans, 2011). *L. monocytogenes* can enter the food production environment (FPE) through multiple vectors, such as the introduction of contaminated raw ingredients, or the ingress of staff or pests carrying the bacterium, which can then progress to the colonisation of the production environment and subsequent cross contamination of food products, particularly ready to eat (RTE) items (Camargo et al., 2017). Although not all strains which enter the FPE will go on to cause listeriosis, there are a variety of genetic and phenotypic traits/mechanisms which can indicate a strains ability to survive or cause disease. An understanding of the various factors influencing colonisation, survival and pathogenicity is thus important.

Within the FPE there are intrinsic (food-related) and extrinsic (intentionally applied to reduce microbial contamination and spoilage) stress factors utilised to control *L. monocytogenes* strains including high osmolarity, temperature and pH, disinfectants, sanitisers and episodes of desiccation (Jordan et al., 2018). *L. monocytogenes*' ability to survive various processing/hurdle technologies influences its ability to colonise and persist in the FPE, making it an important foodborne pathogen.

* Corresponding author at: CSIRO Agriculture and Food, Coopers Plains, QLD, Australia.
E-mail address: Jess.Gray@csiro.au (J.A. Gray).

https://doi.org/10.1016/j.ijfoodmicro.2021.109247
Received 13 February 2021; Received in revised form 3 May 2021; Accepted 13 May 2021
Available online 17 May 2021

Survival throughout the FPE can result in cross contamination of products like RTE foods, which are considered high risk products due to the lack of further cooking prior to consumption, and common vehicles for listeriosis outbreaks (Camargo et al., 2017; Jordan et al., 2018). Importantly, it has been suggested conditions present within the FPE may promote the development of survival strategies like cross protection and interconnectedness between tolerance or resistance to multiple stressors (Bucur et al., 2018). At refrigeration temperatures, or lower ambient temperatures used in many FPEs, an increase in tolerance of associated *L. monocytogenes* strains to cold temperatures, osmotic and oxidative stressors has been noted (Manso et al., 2020; Schmid et al., 2009). Recently identified genomic islands, stress survival islet (SSI)-1, and SSI-2, are responsible for tolerance to acid, salt, bile, gastric, alkaline and/or oxidative stress, further highlighting the diverse genomic arsenal which supports niche adaptation, survival and persistence of *L. monocytogenes* in the FPE (Harter et al., 2017; Ryan et al., 2010). In addition, the presence of plasmids and prophage elements also increases an isolates fitness within the FPE (Schmitz-Esser et al., 2015; Verghese et al., 2011).

An understanding of the pathogenic potential of *L. monocytogenes* strains isolated from the FPE, as well as their resistance to antibiotics, is also important. The listerial infection cycle is the result of several essential virulence factors, predominately a six gene virulence cluster also known as *Listeria* pathogenic island 1 (LIPI-1) (Hadjilouka et al., 2016; Karthikeyan et al., 2015; Luo et al., 2013; Osman et al., 2020; Poimenidou et al., 2018). In addition, there are other important genes involved in virulence, including *inlA* and *inlB*, which are required for initial invasion (Autret et al., 2001). However, not all isolates in the FPE contain functional virulence genes, with mutations in key virulence genes like *prfA* or *inlA* resulting in a reduced pathogenic potential (Miner et al., 2008; Nightingale et al., 2008). Determining the presence and the degree of diversity can provide an overview of the pathogenicity potential of transient or persistent isolates within the FPE (Poimenidou et al., 2018). Differences in virulence is an important public health concern as highly virulent strains can be associated with outbreaks and severity of illness, and therefore the sensitivity to clinically relevant antibiotics is also required. Importantly, resistance in *L. monocytogenes* isolates have been increasingly reported, along with the presence of genetic determinants being identified for various classes of antibiotics, some of clinical relevance (Grayo et al., 2008; Rakic-Martinez et al., 2011; Wilson et al., 2018); therefore continued surveillance of antibiotic sensitivity is required. Typically, the pathogenic potential of clinical isolates is looked at, with less studies considering food-isolated *L. monocytogenes* ability to both cause disease, in addition to the carriage of genes that may support their survival within the FPE. The aim of this study was to characterise *L. monocytogenes* isolates from food and food-environment sources using phenotypic and genomic methods to determine their ability to survive within the food production environment, their potential to cause infection and their susceptibility to frequently used antibiotics in the treatment of listeriosis.

2. Materials and methods

2.1. Bacterial strains, isolation and molecular characterisation

A total of 52 *L. monocytogenes* isolates from 12 sequence types (ST, up to five isolates per ST) representative of multi-locus sequence types commonly associated with the food chain were chosen from a variety of sources (dairy, meat, vegetable, mixed food and environment; Supplementary Table 1) across a span of 18 years (1998 to 2016). Isolates selected each possessed unique pulsed field gel electrophoresis pulsotypes to increase strain variance (data not shown). Isolates were stored in a −80 °C freezer, and resuscitated on Brain Heart Infusion (BHI, Oxoid, Australia) agar at 37 °C for 24 h, prior to experimental manipulations.

2.2. Phenotypic characterisation

2.2.1. Antimicrobial sensitivity

The sensitivity of *L. monocytogenes* strains to five antibiotics used for the treatment of listeriosis was determined on Muller Hinton Agar (Oxoid, Australia) supplemented with 5% (v/v) defibrinated sheep blood (MHSBA; Thermo Fisher Scientific, Australia). Ciprofloxacin, trimethoprim/sulfamethoxazole and penicillin G (0.002–32 μg/mL) along with gentamicin and amoxicillin (0.016–256 μg/mL), were assessed. Bacterial suspensions adjusted to 0.5 McFarland in maximum recovery diluent (MRD) were inoculated onto MHSBA using a sterile swab in three directions and incubated at 37 °C for 24–48 h. The minimum inhibitory concentration (MIC) for each antibiotic was assessed using Etest strips (Biomerieux, Australia) and MIC values were interpreted according to breakpoints provided by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2014, 2016) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2019) or the literature (Noll et al., 2018) for *L. monocytogenes* where available; or staphylococcal species MICs were used in the absence of breakpoints from the aforementioned sources. The MIC value for susceptibility was defined as ≤1 μg/mL for amoxicillin, ≤1 μg/mL for gentamicin and ciprofloxacin, ≤2 μg/mL for penicillin and ≤0.064 μg/mL for trimethoprim/sulfamethoxazole. The control strains used were *S. aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619.

2.2.2. Disinfectant and heavy metal sensitivity

Benzalkonium chloride (BC) (Sigma Aldrich, Australia) was used to determine sensitivity of *L. monocytogenes* strains to an important industrial quaternary ammonium compound disinfectant, using a broth microdilution method with the following modifications: briefly, *L. monocytogenes* strains were grown overnight in Mueller Hinton broth (MHB) and diluted to ~10^3 CFU/mL, then 190 μL was inoculated into 96 well microtitre plate. For each BC stock concentration, 10 μL was added to the microtitre plates to achieve final concentrations of 50, 40, 30, 20, 10, 5, 2.5, 2, 1.5, 1 and 0.5 μg/mL. All plates were incubated at 37 °C for 24 h. Growth was monitored immediately following inoculation (T0) and again at 24 h (T24) at OD_{600} using EnSpire™ multilabel plate reader 2300 (PerkinElmer, Singapore). The T24 reading was subtracted from T0 to determine if isolates were capable of growth. The bacteriostatic or bactericidal effect of BC was tested for any isolate with growth under OD_{600} 0.1 with 10 μL spotted onto Brain Heart Infusion agar (BHIA) and incubated at 37 °C. After 24 h plates were checked for growth or no growth. A minimum of two biological replicates were performed.

Cadmium chloride ($CdCl_2$) was used to determine *L. monocytogenes* sensitivity to the heavy metal cadmium, using a previously optimized method (Ratani et al., 2012). Mueller Hinton agar (Oxoid, Australia) was supplemented with $CdCl_2$ (Sigma Aldrich, Australia) at the following final concentrations: 10, 35, 40, 70, 140 and 150 μg/mL. Isolates were grown overnight on BHIA and a 1 μL loopful was inoculated into 2 mL phosphate-buffered saline (PBS) and vortexed until completely suspended. The PBS and isolate solution were streaked with a cotton swab onto the $CdCl_2$ plates in three directions and incubated at 37 °C. Plates were visually assessed for growth or no growth after 48 h. Two biological replicates were performed.

2.3. Genomic characterisation

Genomic characterisation was predominately performed in Geneious (2020). Genes of interest were downloaded from NCBI with searches performed in Geneious using Megablast or tblastn, with positive results for hits displaying >85% query coverage and pairwise identity. A phylogenetic tree was created based upon raw reads using Snippy and Snippy-core (Seemann, 2015) in Galaxy Australia (Jalili et al., 2020) utilising the genbank file of isolate 7943 as the reference genome and reconstructed with RAxML (v8.2.4) (Stamatakis, 2014), utilising substitution model 'GTRCAT' and the remainder with default parameters.

2.3.1. Genetic determinants of virulence potential and FPE stress survival

The LIPI-1 virulence cluster and a selection of genetic determinants identified in the literature were chosen to assess the potential of the isolates to survive various stress conditions encountered within the FPE, and the potential to cause disease should a contaminated food product be consumed (Table 1). The protein or gene was downloaded from NCBI and a BLAST search of the genetic determinants occurred in Geneious utilising the above criteria. EasyFig 2.2.5 (Sullivan et al., 2011) was used to visualise gene comparisons.

2.3.2. Antimicrobial and virulence gene databases

Mass screening of acquired antimicrobial and virulence genes were performed using Abricate in Galaxy (v1.0.1) (Seemann, 2016) against the associated databases NCBI AMRFinder Plus (Feldgarden et al., 2019), CARD (Alcock et al., 2020; Jia et al., 2017), ARG-ANNOT (Gupta et al., 2014), Resfinder (Zankari et al., 2012) and VFDB (Chen et al., 2016).

2.3.3. Mobile Genetic Elements characterisation

Plasmids were identified using PlasmidFinder 2.1 against the Gram-Positive database (Carattoli et al., 2014). Prophage elements were identified using the online platform PHASTER (Arndt et al., 2016; Zhou et al., 2011). Draft nucleotide sequences were utilised for both analyses. Confirmation of plasmid and prophage results was performed in Geneious (2020), through contig interrogation and read-mapping. Comparison of closed plasmids was visualised using BRIG (Alikhan et al., 2011), with the following combinations: ST8 plasmids with pLM1686 as the reference plus an additional section from p7922 from this study, ST121 and ST321 utilising pLM6179 for reference and ST3, ST9, ST155 and ST204 were compared to pN1-011A and pR479a plasmids.

2.3.4. Data availability

Draft genome sequences for strains from this project have been deposited in the NCBI genome database or sequence read archive under BioProjects: PRJNA725037, PRJNA320339, PRJNA377767, PRJNA295145, PRJNA295464, PRJNA692370 and PRJNA422580; accession numbers are displayed in Supplementary Table 1.

Table 1
Plasmids identified among isolates in this study.

Isolate	MLST	Plasmid		
		Closed/open	Size (bp)	GC (%)
7514	3	Open	59,826	35.2
7547	3	Open	32,307	36.1
7553	3	Closed	4176	34.1
7583	3	Open	56,146	36
7922	8	Closed	88,290	36.5
8112	8	Closed	79,144	36.7
8120	8	Open	85,394	36.9
8124	8	Closed	79,180	36.7
8117	9	Closed	25,550	36.5
8118	9	Closed	25,550	36.5
8119	9	Closed	25,550	36.5
8123	9	Open	49,141	36.3
8129	9	Open	49,281	35.8
7425	121	Closed	62,207	36.5
7475	121	Open	60,666	36.7
7495	121	Open	62,191	36.5
7987	121	Closed	60,923	36.6
8113	121	Closed	62,207	36.5
7533	155	Open	64,751	38.1
7920	155	Closed	77,756	37.5
7921	155	Closed	80,184	37.4
7488	204	Open	48,687	37.4
7919	204	Closed	38,191	37.3
7929	204	Open	91,345	37.7
7943	321	Open	66,904	36.5
8126	321	Open	60,124	36.7

3. Results

3.1. Genomic composition of L. monocytogenes isolates

An overview of the genomic composition of the 52 *L. monocytogenes* isolates included in this study is shown in Supplementary Table 2. The draft genome sizes ranged between 2.61 and 3.08 Mb, with the GC percentage between 37.7 and 38.1%. The number of coding DNA sequences ranged from 2668 to 3165.

3.2. Cadmium chloride and benzalkonium chloride phenotypes

The sensitivity of the 52 *L. monocytogenes* strains to various concentrations of BC and $CdCl_2$ is shown in Fig. 1. When assessed against BC only two isolates (7544 and 7546) were unable to grow at the lowest concentration (0.5 μg/mL), however when subsequently spotted onto BHI agar they were able to produce colonies indicating BC at 0.5 μg/mL had a bacteriostatic effect on these two isolates. Assessment against varying concentrations of $CdCl_2$ resulted in 10 isolates unable to grow at the lowest concentration with the remainder of the isolates growing at various concentrations between 10 and 140 μg/mL, however not at the highest concentration (150 μg/mL). Isolate 7920 contained a $CdCl_2$ resistant gene, however it was only able to grow to 10 μg/mL. There were also nine isolates which had no *cadA* genes but were able to grow at 10 μg/mL.

3.3. Antimicrobial susceptibility

Five antibiotics used for the treatment of listeriosis were tested against the *L. monocytogenes* isolates (Fig. 1). All the *L. monocytogenes* isolates displayed sensitivity to the antibiotics tested in this study (amoxicillin, gentamicin, penicillin, ciprofloxacin and trimethoprim/sulfamethoxazole).

3.4. Food production stress determinants

All isolates were assessed for the presence of genetic determinants relating to various stress conditions experienced within the food production environment, used as a mitigation strategy to reduce growth and/or survival of *L. monocytogenes*. These stress conditions included thermal treatment, low temperatures, acidification, oxidation, osmotic stress, the use of bacteriocins or nisin, and high hydrostatic pressure (HHP). A screening database of genetic determinants associated with each condition identified within the literature was selected to determine the potential of strains to survive within the FPE. The genetic determinants selected for heat, acid, cold, osmotic, bacteriocin/nisin and HHP stresses were present in all isolates (Supplementary Table 3). The stress survival islets (SSI) were also assessed, with SSI-1 present in 34 of the 52 isolates (65.4%) from ST3, 7, 8, 9, 12, 155, 204 and 321 (Fig. 1). The five isolates from ST121 were positive for SSI-2 (9.6%), and an SSI genotype harbouring an *LMOf2365_0481* gene homolog was present in 13 of the 52 isolates (25.0%) from ST1, 2 and 101.

3.5. Cadmium and disinfectant genes

The *L. monocytogenes* isolates were analysed for the presence of cadmium genes *cadA1C-A6C* (Fig. 1). There were 19 isolates which had no *cadA* genes present; the *cadA1* gene was present in 20 isolates, *cadA2* was represented in five isolates, four isolates had *cadA4* and five isolates had *cadA5* present. No isolates had the *cadA3* or *cadA6* gene. Isolate 7929 had two *cadA* genes present, *cadA2* and *cadA4*. Two *cadA* genes were also present in isolate 7533, *cadA2* and interestingly, it also contained a transposon with a novel *L. monocytogenes* cadmium resistance gene, referred to here as *cadA7*. A nucleotide BLAST search of NCBI nucleotide database identified four other *L. monocytogenes* strains also contain this transposon and the novel *cadA7* gene homolog. This

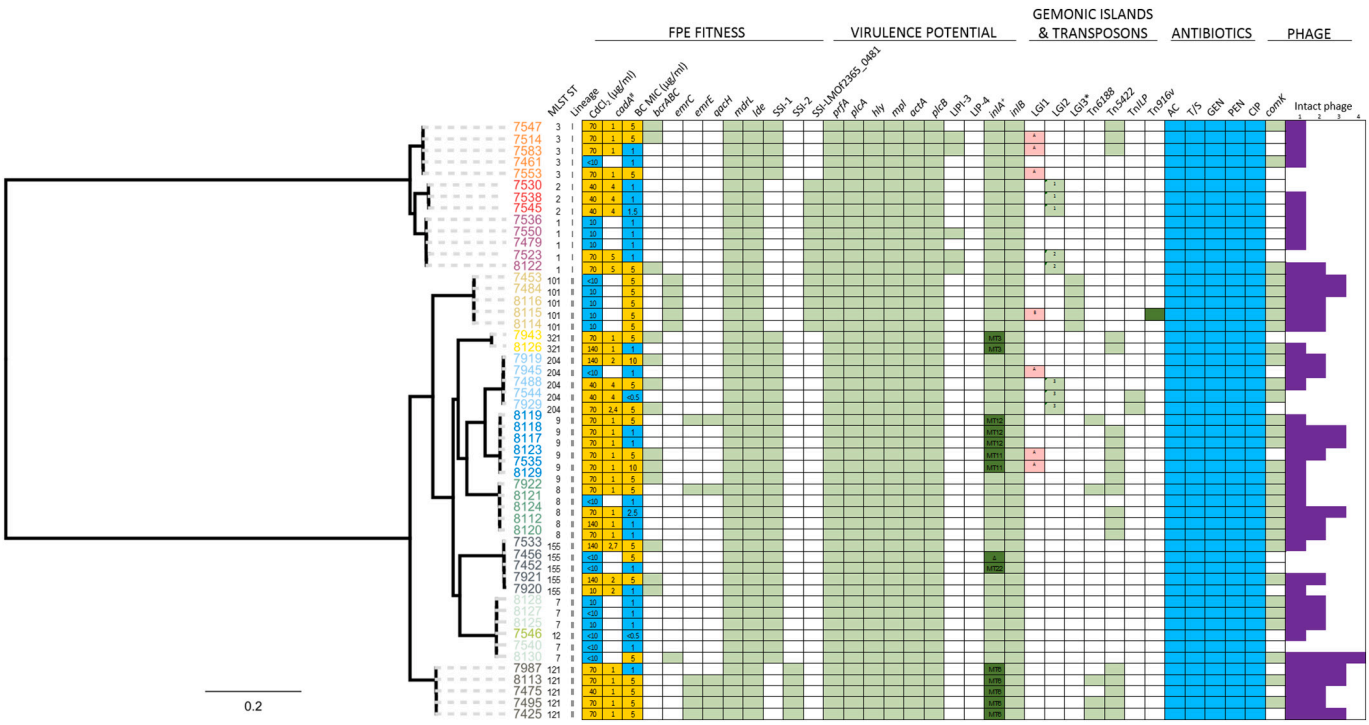

Fig. 1. Phylogeny and genetic determinants of 52 *L. monocytogenes* isolates relating to survival within the FPE, virulent potential and therapeutic treatment potential. Character designations are as follows: #, numbers designated *cadA* gene type; ^, pLi0048 – elements of the pLi100 are present however we were unable to close or identify full plasmid. This plasmid is known to carry cadmium resistant genes; +, *inlA* PMSC type; Δ, 70 AA deletion; A, phage insert in the *lmo1703-lmo1702* region; B, transposon insert in the *lmo1703-lmo1702* region (*Tn916* variant); 1, LGI2 insert within the *EGD-e LMO2257* gene; 2, LGI2 variant; 3, LGI2 insert in the *yfbR* gene; *, LGI3 lacking the *cadA1C* cassette; yellow, resistant phenotype; light green, gene is present; dark green, gene is present – does not match wildtype; light red, LGI1 is absent however there is alternative genes present within the *lmo1703-lmo1702* region; blue, sensitive phenotype; orange, *comK* phage is present; purple, number of intact phage regions present. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

transposon was also identified in Enterobacteriaceae strains suggesting direct or indirect horizontal gene transfer occurring between *Enterococcus* and *Listeria* (Fig. 2). No other isolates within this study contained the *cadA7* gene. Amino acid sequence identity of the published cadmium genes and the novel *cadA7* gene was determined, with *cadA7* sharing the highest amino acid percentage identity with *cadA2*, 75.74% (Supplementary Fig. 1); however, when compared to *E. faecalis* ATCC 29212 genome the novel *cadA7* displayed 100% coverage and 99.95% nucleotide identity.

A variety of genes and mechanisms providing resistance to disinfectants were assessed against the isolates in this study (Fig. 1). All isolates were found to contain the *mdrL* and *lde* efflux pumps. The *bcrABC* cassette was present in 13 isolates covering ST1, 3, 9, 155, 204 and 321. Six isolates in total were positive for *ermB* and *qacH* from ST8, 9 and 121. The *emrC* gene was present in six isolates, one isolate from ST7 and all the isolates from ST101. The ST101 isolates were the only whole ST group in which all contained the same disinfectant resistance gene.

3.6. Plasmids, prophages and transposons

Plasmid replicons were identified in 26 of the 52 isolates with PlasmidFinder. The identified regions were further interrogated in the draft genomes. A total of 13 closed plasmids and 12 draft open plasmids were identified (Table 1). Plasmids were present in ST3, ST8, ST9, ST121, ST155, ST204 and ST321 isolates (Fig. 3). ST121 was the only group in which plasmids were found in all five isolates and displayed a 95.9% pairwise identity with pLM6179, however only three of these plasmids were closed following sequence analysis. Genes shared across the plasmids, and not restricted to a single ST, included heavy metal and disinfectant resistance genes including the *bcrABC* operon *cadAC* operon, and genes for copper, zinc and arsenic resistance; stress response genes including UV damage repair protein, oxidative and heat stress

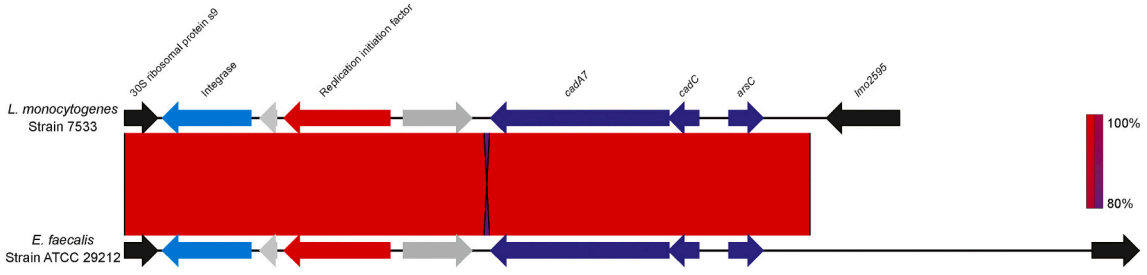

Fig. 2. The novel *cadA7* gene. Transposon identified in isolate 7533 inserted between 30S ribosomal protein S9 and *lmo2595*, compared to *E. faecalis* ATCC 29212. Integrase genes are in light blue, replication genes are in red, heavy metal resistance genes are in purple. Hypothetical genes or those with an unknown function are shaded grey. Nucleotide sequence identity of transposon where shared, ranged from 80 to 100% as depicted by the percentage homology bar. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

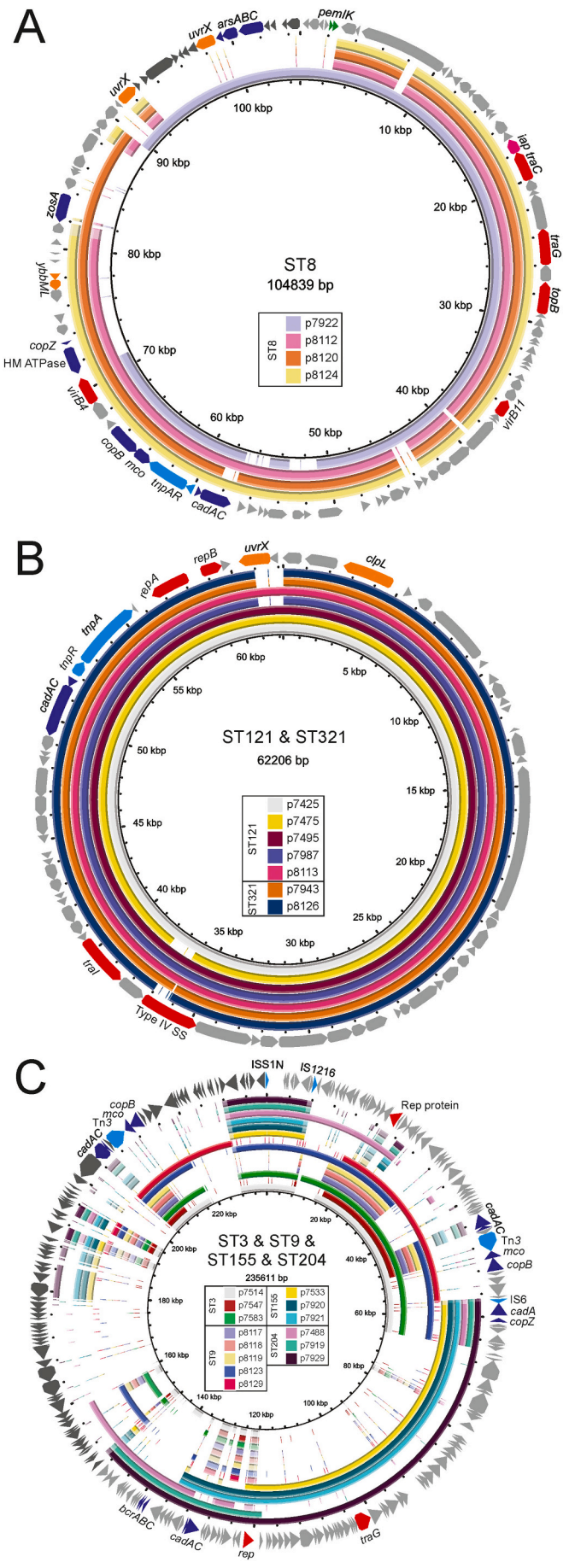

Fig. 3. BRIG comparison of plasmids identified within 26 isolates within this study. Each ring represents the plasmids of the individual isolates. The plasmid pangenome reference contains all the unique genetic features of the closest related plasmid identified in NCBI and segments of plasmids from the respective pST groups from this study which are not represented in NCBI, combined into a single contig reference (outside ring). (A) pST8 utilises the genome of pLM1686 (MK134858) (light grey) and p7922 (dark grey) as reference pangenome. (B) pST121 and pST321 utilises the p6179 as reference genome. (C) pST3, pST9, pST155 and pST204 utilises the genome of pN1-011A (light grey) and pR479a (dark grey) as reference pangenomes. Annotated genes are colour coded to represent genetic markers as follows: red – replication, light blue – transposases, dark blue – heavy metals, orange – stress response, pink – invasion associated, green – toxin/antitoxin and grey – hypothetical proteins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

response genes; invasion related genes; toxin/anti-toxin genes; genes involved in DNA replication, translation, recombination and conjugation; transposon genes; however most genes were hypothetical proteins.

Phaster identified 52 intact phage regions across 43 isolates. Nine isolates had no phage regions, most isolates had a single phage region, two regions were identified in 15 isolates and three and four regions identified in four and one isolate respectively. An additional analysis of the *comK* phage insertion site identified 21 isolates with a full length *comK* gene and 31 isolates with a *comK* prophage disruption (Fig. 1). Transposon elements were identified in 27 of the 52 isolates. Transposon Tn*6188* was present in five isolates, Tn*5422* was identified in 20 isolates, Tn*ILP* was present in two isolates and Tn*yfbR* was identified in six strains. Seven isolates contained two transposons.

3.7. Virulence determinants and genomic islands

The *Listeria* pathogenicity and genomic islands were assessed against the panel of isolates (Fig. 1). The LIPI-1 virulence cluster was present in all isolates, along with the *inlB* gene. The LIPI-3 element was found in three isolates (7523, 7550 and 8122) from ST1 and two isolates (7514 and 7583) from ST3. No isolates harboured LIPI-4; this island has only been identified in CC4 isolates, which were not included in this study. The LGI2 was present in eight isolates, in either one of two insertion locations; within the *LMOSA2140* (homolog of *LMOf2365_2257*) gene originally identified in the strain ScottA (Lee et al., 2013), or within the *yfbR* gene (Fox et al., 2016). The three isolates with the *LMOSA2140* LGI2 insertion region were from ST2 and three ST204 strains contained the *yfbR* LGI2 insert. Interestingly, an LGI2 variant was also identified from two ST1 isolates within a transmembrane protein that displays distant homology to *ydbT* gene. This LGI2 variant harbours an additional gene, a *metC* homolog, within the LGI2 region (Fig. 4). The recently reported *Listeria* genomic island 3 was partially identified in the five ST101 isolates only; however, they were missing the 6248 bp region containing the *cadAC* homolog, recombinase and Tn*3* family transposase.

3.8. Internalin A (inlA) analysis

The *inlA* gene was assessed for the presence of mutations resulting in premature stop codons (PMSCs), truncation or a full length *inlA* gene (Fig. 1 and Supplementary Fig. 2). Thirty-eight of the isolates contained a full length *inlA* gene. Thirteen isolates contained PMSCs. All ST121 isolates (7425, 7475, 7495, 7987 and 8113) contained mutation type 6 at AA 492, as previously described (Van Stelten et al., 2010). Isolates 8123 and 7535 contained a PMSC at AA 685, resulting in mutation type 11. Both these isolates are ST9, from which this mutation type has been previously associated with (Van Stelten et al., 2010). Mutation type 12, the result of a PMSC at AA 576 was identified in three isolates from ST9 (8117, 8118 and 8119) and mutation type 3, the result of a PMSC at AA 700, was identified in isolates 8126 and 7943 from ST321. A novel

(caption on next column)

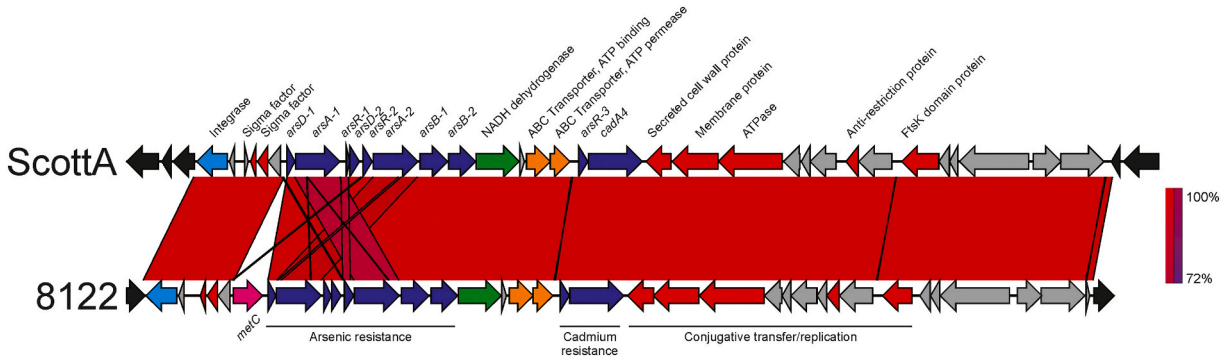

Fig. 4. LGI2 variant identified in isolate 8122. A variant of LGI2 inserted in a transmembrane protein within isolate 8122 and 7523. Annotated genes are colour coded to represent genetic markers as follows: black – flanking genes, light blue – integrase, dark blue – heavy metal and antimicrobial resistance, orange – metabolism and transport, red - transposon system and regulatory genes, pink - virulence, green – stress resistance, grey – hypothetical proteins. Sequence identity where shared, ranged from 72 to 100% as determined by the percentage homology bar. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PMSC was identified in isolate 7452, the result of a frame shift mutation from an AG insertion at nucleotide position 183 producing an *inlA* gene of 67 AA in length. Isolate 7456 contained an in-frame 70 AA deletion within the B-repeat region (B- repeat region 2 and 3).

3.9. *Listeria genomic island 1 analysis*

None of the isolates harboured the *Listeria* genomic island 1 (Fig. 1). To confirm this, we manually inspected the hypervariable region between *lmo1703-lmo1702* locus, an RNA methyltransferase gene and the fosfomycin resistance gene *fosX* respectively, for inserts in all isolates. A phage (ϕRNA-MT) insert was present in six of the isolates (7514, 7535, 7553, 7583, 7945 and 8123) and a novel insert was harboured in isolate 8115. This novel insert was not present in any of the other isolates. A BLAST search resulted in a 64% query coverage and 90.73% nucleotide identity with Tn*916* from *Bacillus subtilis* (which has over 98% identity with Tn*6198* of *L. monocytogenes* TTH-2007) and a 64% query coverage and 91% identity with an integrated chromosomal element ICESpnIC1 identified in *St. pneumoniae* isolate 9611+04103 (Fig. 5). This Tn*916* variant insert between *lmo1703* and *lmo1702* in isolate 8115 is approximately 23,275 bp in length, has a GC content of 37.5% and contains a Clp protease ATP-binding subunit *clpA*, as well as coding sequences for FtsK/SpoIIIE family protein, a Tn*916* transcriptional regulator, an anti-restriction protein, lipoprotein, a *XRE* family transcriptional regulator, an efflux *ABC* transporter, and a site-specific recombinase with the remaining genes being hypothetical proteins.

3.10. *Antimicrobial resistance determinants*

The *L. monocytogenes* isolates were analysed for the presence or absence of a variety of antimicrobial genes associated with conferring resistance to frequently used antibiotics in the treatment of listeriosis or other diseases (Supplementary Table 4). Resistance genes from the following selected antibiotics classes: trimethoprim, tetracycline except

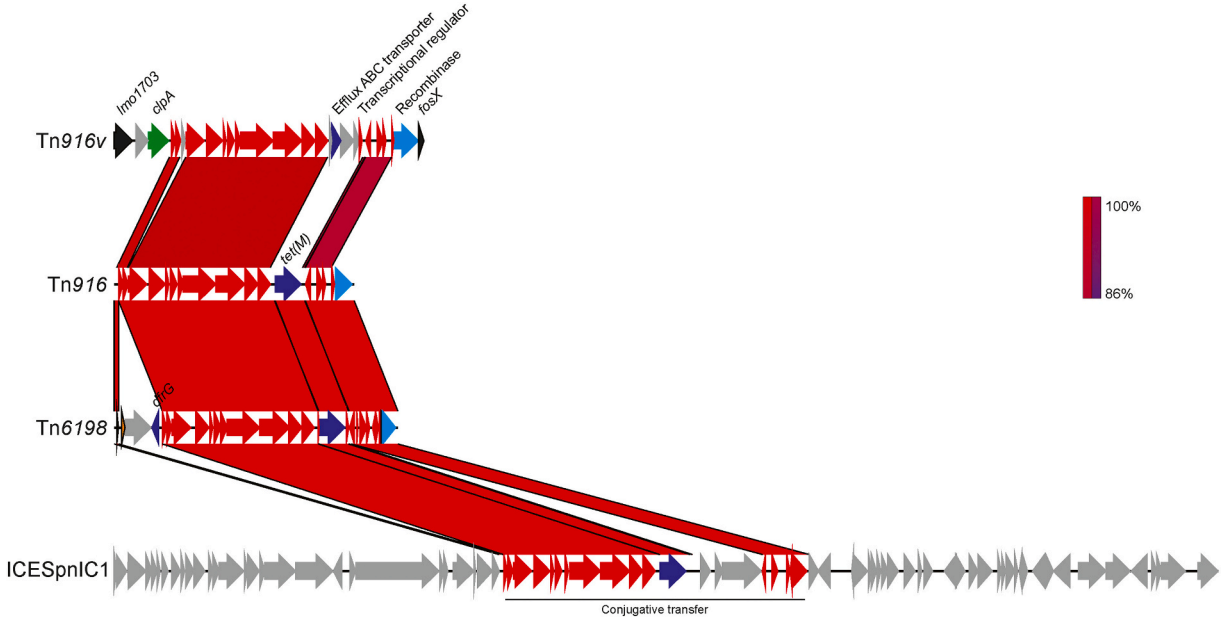

Fig. 5. Novel insert identified in isolate 8115. The insert was identified in the hypervariable region of isolate 8115 between genes *lmo1703-lmo1702*, compared to Tn916, Tn6198 and *S. pneumoniae* 9611+04103 ICESpnIC1. Sequence identity where shared, ranged from 78 to 100% as determined by the percentage homology bar. EGD-e flanking genes, *lmo1703* and *lmo1702* are shown in black; red genes are transposon systems and regulatory genes, light blue – integrase, dark blue – heavy metal/antimicrobial resistance, green – stress response and grey – hypothetical proteins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for the *tetA*-like resistance protein, vancomycin, erythomycin, amoxicillin and aminoglyosides along with the penicillin *mecC* protein were not detected in any isolates. The AMR genes selected relating to flurquinolones (second generation), penicillin (except *mecC*), sulfonamide, fosfomycin, lincomycin, fusidine, quinolone and cephalosporins were present within all isolates. There were no AMR genes or class specific to an isolate, lineage or ST. Potential resistance mechanism beyond known AMR genes were not examined.

4. Discussion

This study utilised phenotypic and genotypic analyses of whole genome sequences to assess the potential of *L. monocytogenes* to survive within the FPE, cause disease and provide insights into antimicrobial resistance relevant to control in the FPE, or to treatment of human infection.

4.1. Virulence potential

Clonal complex 1 (CC1) and 2 (CC2) are well established as being associated with clinical infections (Maury et al., 2016; Yin et al., 2019), and therefore the presence of genomic regions relating to hypervirulence in food isolates is of interest. In this study ST1 and ST2 isolates did not contain the SSI-1 or SSI-2 islands, however, the SSI-alternative, *LMOf2365_0481* homolog, was present in all isolates. A study by Harter et al. (2017) reported the presence of this SSI-alternative to be common among clinical strains, however its function at this stage is undetermined. While CC1 and CC2 did not contain SSI-1 or SSI-2, the isolates did contain all but one of the stress resistance-associated genes screened, with a role in heat, cold, acid, osmotic, oxidative or nisin stress response, suggesting that they are capable of surviving within the FPE, or in food. Horlbog et al. (2018) found CC1 strains were able to recover more quickly after salt stress, suggesting these strains could be able to proliferate faster within food environments. All isolates of CC1 and CC2 contained full length *inlA* genes; three of the CC1 isolates contained LIPI-3, suggestive of increased virulence potential.

Within Australia, CC3 and CC204 are widely distributed (Jennison et al., 2017), with these strains analysed in this study all containing LIPI-1, full length *inlA*, SSI-1, and all the various processing related stress genes. Neither CC3 nor CC204 contained the LGI1, however, three of the CC3 strains and one of the CC204 strains contained a phage insert in the LGI1 hypervariable position between *lmo1703* and *lmo1702*. Interestingly, two of the CC3 isolates contained the phage insert instead of LGI1. Increased bacterial colonisation and hypervirulence has been reported in isolates which encode a full length *inlA*, LIPI-1 and LIPI-3 (Maury et al., 2016; Yin et al., 2019), suggesting the isolates in this study which contain these have increased virulence potential, and with the addition of SSI-1 may also have increased FPE fitness. The CC204 strains which were negative for LGI1 and the ϕRNA-MT phage insert, contained LGI2. *Listeria* genomic island 2 contains cadmium and arsenic resistance genes, providing increased environmental survival potential; interestingly, also in both this study and Lee et al. (2017), LGI2 was common in CC2 strains, which are also prevalent in human cases. Although LGI2 requires further characterisation, it is prevalent in clonal complexes linked to hypervirulence and has been suggested to contribute to virulence (Lee et al., 2017).

Premature stop codons and mutations in the *inlA* gene resulting in secretion of *inlA* instead of being attached to the bacterial cell wall have been associated with reduced invasion and virulence ability (Ferreira da Silva et al., 2017; Gelbíčová et al., 2015; Van Stelten et al., 2016). In this study, 13 isolates were identified to carry one of five types of mutations, suggesting these isolates may have a reduced virulence potential. We identified a novel PMSC at 67 AA resulting from an AG insertion at position 183 bp producing a frameshift, referred to as mutation type 22. This mutation occurs within the signal cap region of the *inlA* protein. Mutation types 4 and 15 occurring at AA positions 9 and 77 respectively have been shown to affect invasion ability (Van Stelten et al., 2010), indicating mutation type 22 has the potential to also have reduced invasiveness, however, in vitro cell invasion assays and/or mouse model virulence assays will be required to confirm this theory. In addition, a 70 AA deletion within the B-repeat region was identified in isolate 7456. Deletion of the B-repeat region between AA 517 and 707 by Lecuit et al. (1997) resulted in similar invasiveness level to those with the WT *EGD-e inlA* protein, suggesting this deletion within the B-repeat region does not contribute to a strains ability to invade cells. All ST121 isolates contained the type 6 mutation and ST321 isolates contained mutation type 3. In addition, all but two *inlA* mutant isolates had cadmium resistance, contained either SSI-1 or SSI-2, and harboured plasmids. All of these isolates harboured LIPI-1. The majority of *inlA* mutants are commonly associated with the FPE and food isolates (Nightingale et al., 2005; Van Stelten et al., 2016), therefore the presence of these genes in the *inlA* mutant isolates are suggestive of increased survival within the FPE.

4.2. FPE survival potential

Agricultural practices and industrial pollution have resulted in increased levels of various heavy metals in the environment, and as such bacteria require resistance determinants in order to tolerate these substances, particularly heavy metals which are not required for cellular processes. Cadmium resistant determinants are widely distributed and are commonly associated with *L. monocytogenes* strains repeatedly isolated from food sources (Parsons et al., 2017). In this study, 32 isolates were capable of growing at levels above 40 μg/mL $CdCl_2$, which was associated with the presence of at least one cadmium resistant determinant, with similar results observed in previous studies (Haubert et al., 2019; Lee et al., 2013; Mullapudi et al., 2010; Ratani et al., 2012; Xu et al., 2019). Interestingly, in this study we did not identify the *cadA3* resistant determinant which is present as an integrating chromosomal element in a variable genomic region, that in other strains may contain diverse cassettes like LIPI-3 (Parsons et al., 2019).

The novel *cadA7*, identified in this study, contained all three key motifs, DKTGT, CPC and CTNCA, characteristic of the *cadA* protein family (Bal et al., 2003; Parsons et al., 2017). Parsons et al. (2017) identified an amino substitution in the CTNCA → CANCA motif in *cadA4* suggesting this substitution most likely, in conjunction with other elements, influencing *cadA4*'s reduced tolerance of 35 μg/mL to cadmium. In this study, the three key motifs of the novel *cadA7* matched the *cadA1-cadA3* sequences, which are purportedly associated with cadmium resistance of 140 μg/mL or higher, potentially suggesting *cadA7* may confer similar levels of resistance (Parsons et al., 2017). The single isolate harbouring *cadA7* in this study also contained *cadA2*; as such, further research is required to establish the resistance level conferred by *cadA7*, and to determine if it has a potential role in virulence.

The presence of disinfectant resistant genes in this study was associated with resistance to BC (≥5 μg/mL) in all but two isolates, with 50% of the isolates displaying a MIC of 5 μg/mL or higher. The capability of isolates to grow at higher levels of disinfectants like BC is being increasingly reported (Mullapudi et al., 2008; Møretrø et al., 2017). In addition, the *L. monocytogenes* strains' tolerance to disinfectants has been correlated with cadmium resistance and increased survival within the FPE being associated with subinhibitory levels of disinfectants (Martinez-Suarez et al., 2016; Mullapudi et al., 2008; Ortiz et al., 2014). Therefore, an evaluation of the level of resistance of cadmium and BC is important to understand the survival potential *L. monocytogenes* may have in the FPE.

Genomic islands have the potential to contain genes to improve the fitness of an isolate, while also being implicated in potential horizontal gene transfer (Palma et al., 2020); therefore, the presence of these islands might lead to increased FPE survival or pathogenic potential. Of the genomic islands identified in *L. monocytogenes*, LGI1 and LGI3 have been associated with survival and persistence in the FPE (Kovacevic et al., 2016; Palma et al., 2020), with LGI2 potentially providing

increase survival and persistence within the FPE as well as virulence potential (Lee et al., 2013; Lee et al., 2017).

In this study, LGI2 was the only full-length island present in six isolates, inserted within one of two genes, *LMOSA2140* or *yfbR*. This has the potential to provide increased virulence and environmental fitness. In addition, an LGI2 variant was identified in two ST1 isolates within a transmembrane protein that displays distant homology to the *ydbT* gene from *Bacillus*. The *ydbT* in *Bacillus subtilis* strains has been reported to provide resistance to bacteriocins produced by *B. amyloliquefaciens*, an important function particularly in natural environmental reservoirs like soil (Butcher and Helmann, 2006). The LGI2 variant shows high homology with LGI2 and maintains the arsenic and cadmium resistant determinants along with various metabolism, transport, stress resistance, transposon and regulatory genes. However, the LGI2 variant contains an additional cystathionine β-lyase (*metC*) gene. A previous study demonstrated that disruption of *metC* in *Salmonella*, reduced strain virulence in a mouse model (Ejim et al., 2004). This suggests the LGI2 variant may play a role in virulence as well as survival within food and the food environment; however, this needs to be further confirmed experimentally.

In this study, the LGI3 variant which lacks the *cadA1C* cassette was identified in all ST (CC) 101 isolates. The LGI3 element was first identified in CC101 isolates by Palma et al. (2020) and found to harbour a *cadA1C* cassette; however, a search of the NCBI genome database identified a smaller LGI3 variant lacking the *cadA1C* cassette in the *L. monocytogenes* strain ATCC 51775 (ST222). In comparison, the CC101 isolates from this study also contained the LGI3 variant, suggesting CC101 strains may display either LGI3 genotype.

Instead of LGI1, six isolates contained a phage insert and one isolate contained a Tn*916* variant insert, in the associated insertion locus. This Tn*916* variant shares similarity with Tn*916* and Tn*6198*; however, it lacks the tetracycline (*tetM*) and the trimethoprim (*dfrG*) resistant genes. Interestingly, an efflux ABC transporter is present on the Tn*916* variant, which shares homology to efflux systems. Further experimental work is required to determine its function in this transposon, and the ability of this transposon to transfer to other *Listeria* strains, or other bacterial species.

4.3. Therapeutic treatment potential

Traditionally, listeriosis is treated with a β-lactam (penicillin, ampicillin or amoxicillin) either alone or in combination with an aminoglycoside, typically gentamicin (Grayo et al., 2008; Knudsen et al., 2013; Olaimat et al., 2018; Temple and Nahata, 2000) or trimethoprim and sulfamethoxazole combination for patients with a β-lactam sensitivity(Bertrand et al., 2016; Wilson et al., 2018). While in this study all the isolates were sensitive to the five clinically relevant antibiotics tested, there has been reports in the literature of resistance to gentamicin, penicillin, ciprofloxacin and trimethoprim/sulfamethoxazole along with a variety of other antibiotics and importantly reports of resistance to multiple antibiotic classes (Arslan and Özdemir, 2020; Kuan et al., 2017; Obaidat et al., 2015; Obaidat and Stringer, 2019; Welekidan et al., 2019). Our study provides a timely contribution to the current state of AMR in *L. monocytogenes* and does not highlight any resistance concern among food isolates in this study.

In this study, we identified a novel cadmium gene, *cadA7* as part of a transposon insert, a variant of LGI2, as well as a novel insert in the hypervariable region LGI1, in the latter sharing similarity to a Tn*916* transposon. The identification of these novel genes and inserts contributes to our understanding of the *L. monocytogenes* pangenome, in particular to elements relating to survival ability and pathogenic potential. The isolates analysed in this study showed potential to survive and persist within the FPE, with all isolates containing one of the SSIs, various genes relating to stressors present in the FPE to reduce bacteria, in addition to a high portion of strains containing cadmium or disinfectant resistance genes. Hypervirulent strains of *L. monocytogenes* have been previously reported, with some isolates from CC1 and CC3 in this study harbouring genes associated with this virulence status, suggesting a concern to public health.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2021.109247.

CRediT authorship contribution statement

JG, PSC and EF conceived and designed the study. JG performed the experiments. Data was analysed by JG, EF, PSC, JPB, MK and CK. JG and EF drafted the manuscript. All authors corrected and approved the manuscript.

Declaration of competing interest

None.

Acknowledgments

This work is supported by University of Tasmania and CSIRO joint funding. JG is a recipient of University of Tasmania and CSIRO PhD scholarship.

References

Alcock, B.P., Raphenya, A.R., Lau, T.T.Y., Tsang, K.K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A.L.V., Cheng, A.A., Liu, S., Min, S.Y., Miroshnichenko, A., Tran, H.K., Werfalli, R.E., Nasir, J.A., Oloni, M., Speicher, D.J., Florescu, A., Singh, B., Faltyn, M., Hernandez-Koutoucheva, A., Sharma, A.N., Bordeleau, E., Pawlowski, A.C., Zubyk, H.L., Dooley, D., Griffiths, E., Maguire, F., Winsor, G.L., Beiko, R.G., Brinkman, F.S.L., Hsiao, W.W.L., Domselaar, G.V., McArthur, A.G., 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res. 48, D517–D525.

Alikhan, N.F., Petty, N.K., Ben Zakour, N.L., Beatson, S.A., 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12, 402.

Arndt, D., Grant, J.R., Marcu, A., Sajed, T., Pon, A., Liang, Y., Wishart, D.S., 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res. 44, W16–W21.

Arslan, S., Özdemir, F., 2020. Prevalence and antimicrobial resistance of *Listeria* species and molecular characterization of *Listeria monocytogenes* isolated from retail ready-to-eat foods. FEMS Microbiol. Lett. 367, 1–9.

Autret, N., Dubail, I., Trieu-Cuot, P., Berche, P., Charbit, A., 2001. Identification of new genes involved in the virulence of *Listeria monocytogenes* by signature-tagged transposon mutagenesis. Infect. Immun. 69, 2054–2065.

Bal, N., Wu, C.C., Catty, P., Guillain, F., Mintz, E., 2003. Cd2+ and the N-terminal metal-binding domain protect the putative membranous CPC motif of the Cd2+-ATPase of *Listeria monocytogenes*. Biochem. J. 369, 681–685.

Bertrand, S., Ceyssens, P.J., Yde, M., Dierick, K., Boyen, F., Vanderpas, J., Vanhoof, R., Mattheus, W., 2016. Diversity of *Listeria monocytogenes* strains of clinical and food chain origins in Belgium between 1985 and 2014. PLoS One 11, 1–16.

Bucur, F.I., Grigore-Gurgu, L., Crauwels, P., Riedel, C.U., Nicolau, A.I., 2018. Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing environments. Front. Microbiol. 9, 1–18.

Butcher, B.G., Helmann, J.D., 2006. Identification of *Bacillus subtilis* sigma-dependent genes that provide intrinsic resistance to antimicrobial compounds produced by Bacilli. Mol. Microbiol. 60, 765–782.

Camargo, A.C., Woodward, J.J., Call, D.R., Nero, L.A., 2017. *Listeria monocytogenes* in food-processing facilities, food contamination, and human listeriosis: the Brazilian scenario. Foodborne Pathog. Dis. 14, 623–636.

Carattoli, A., Zankari, E., García-Fernández, A., Voldby Larsen, M., Lund, O., Villa, L., Møller Aarestrup, F., Hasman, H., 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob. Agents Chemother. 58, 3895–3903.

Chen, L., Zheng, D., Liu, B., Yang, J., Jin, Q., 2016. VFDB 2016: hierarchical and refined dataset for big data analysis - 10 years on. Nucleic Acids Res. 44, D694–D697.

CLSI, C.a.L.S.I, 2014. Performance Standards for Antimicrobial Susceptibility Testing Twenty-forth Informational Supplement. CLSI Document M100-S24. CLSI, Wayne, PA, USA.

CLSI, C.a.L.S.I, 2016. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolates or fastidious bacteria. In: CLSI Guideline M45, 3rd ed. CLSI, Wayne, PA, USA.

Ejim, L.J., D'Costa, V.M., Elowe, N.H., Loredo-Osti, J.C., Malo, D., Wright, G.D., 2004. Cystathionine beta-lyase is important for virulence of *Salmonella enterica serovar Typhimurium*. Infect. Immun. 72, 3310–3314.

EUCAST, E.C.o.A.S.T, 2019. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 9.0. EUCAST, Växjö, Sweden.

Feldgarden, M., Brover, V., Haft, D.H., Prasad, A.B., Slotta, D.J., Tolstoy, I., Tyson, G.H., Zhao, S., Hsu, C.H., McDermott, P.F., Tadesse, D.A., Morales, C., Simmons, M.,

Tillman, G., Wasilenko, J., Folster, J.P., Klimke, W., 2019. Validating the AMRFINder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob. Agents Chemother. 63, 1–19.
Ferreira da Silva, M., Ferreira, V., Magalhães, R., Almeida, G., Alves, A., Teixeira, P., 2017. Detection of premature stop codons leading to truncated internalin A among food and clinical strains of *Listeria monocytogenes*. Food Microbiol. 63, 6–11.
Fox, E.M., Allnutt, T., Bradbury, M.I., Fanning, S., Chandry, P.S., 2016. Comparative genomics of the Listeria monocytogenes ST204 subgroup. Front. Microbiol. 7.
Gelbíčová, T., Koláčková, I., Pantůček, R., Karpíšková, R., 2015. A novel mutation leading to a premature stop codon in *inlA* of *Listeria monocytogenes* isolated from neonatal listeriosis. New Microbiol. 38, 293–296.
Grayo, S., Join-Lambert, O., Desroches, M.C., Le Monnier, A., 2008. Comparison of the in vitro efficacies of moxifloxacin and amoxicillin against *Listeria monocytogenes*. Antimicrob. Agents Chemother. 52, 1697–1702.
Gupta, S.K., Padmanabhan, B.R., Diene, S.M., Lopez-Rojas, R., Kempf, M., Landraud, L., Rolain, J.-M., 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob. Agents Chemother. 58, 212–220.
Hadjilouka, A., Molfeta, C., Panagiotopoulou, O., Paramithiotis, S., Mataragas, M., Drosinos, E.H., 2016. Expression of *Listeria monocytogenes* key virulence genes during growth in liquid medium, on rocket and melon at 4, 10 and 30 °C. Food Microbiol. 55, 7–15.
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, 1–15.
Haubert, L., Zehetmeyr, M.L., da Silva, W.P., 2019. Resistance to benzalkonium chloride and cadmium chloride in *Listeria monocytogenes* isolates from food and food-processing environments in southern Brazil. Can. J. Microbiol. 65, 429–435.
Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N., Bellinger, D.C., de Silva, N.R., Gargouri, N., Speybroeck, N., Cawthorne, A., Mathers, C., Stein, C., Angulo, F.J., Devleesschauwer, B., Adegoke, G.O., Afshari, R., Alasfoor, D., Baines, J., Balakrishnan, K., Hamza, W.M.B., Black, R.E., Bolger, P.M., Chaicumpa, W., Cravioto, A., Döpfer, D., Ehiri, J.E., Fazil, A., Ferreccio, C., Fèvre, E. M., Hall, G., Kasuga, F., Keddy, K.H., Lanata, C.F., Lei, H., Liu, X., Manyindo, B., Nasinyama, G., Ongolo-Zogo, P., Pitt, J.I., Rokni, M.B., Sripa, B., van Leeuwen, R., Verger, P., Willingham, A.L., Zhou, X.N., Aspinall, W., Buchanan, R., Budke, C., Caipo, M.L., Carabin, H., Cole, D., Cooke, R.M., Crump, J.A., El-Jardali, F., Fischer-Walker, C., Fürst, T., Haagsma, J.A., Hall, A.J., Henao, O., Hoffmann, S., Jensen, H., Jessani, N., Koopmans, M.P.G., Levine, M.M., de Noordhout, C.M., Majowicz, S., McDonald, S.A., Pires, S., Scallan, E., Sripa, B., Thomas, M.K., Verhoef, L., Wu, F., Zeilmaker, M., 2015. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS Med. 12, 1–23.
Horlbog, J.A., Kent, D., Stephan, R., Guldimann, C., 2018. Surviving host - and food relevant stresses: phenotype of *Listeria monocytogenes* strains isolated from food and clinical sources. Sci. Rep. 8, 1–10.
Jalili, V., Afgan, E., Gu, Q., Clements, D., Blankenberg, D., Goecks, J., Taylor, J., Nekrutenko, A., 2020. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. Nucleic Acids Res. 48, W395–W402.
Jennison, A.V., Masson, J.J., Fang, N.X., Graham, R.M., Bradbury, M.I., Fegan, N., Gobius, K.S., Graham, T.M., Guglielmino, C.J., Brown, J.L., Fox, E.M., 2017. Analysis of the *Listeria monocytogenes* population structure among isolates from 1931 to 2015 in Australia. Front. Microbiol. 8, 1–13.
Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave, B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye, J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., Johnson, T.A., Brinkman, F.S., Wright, G.D., McArthur, A.G., 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 45, D566–d573.
Jordan, K., Hunt, K., Lourenco, A., Pennone, V., 2018. Listeria monocytogenes in the food processing environment. Curr. Clin. Microbiol. Rep. 5, 106–119.
Karthikeyan, R., Gunasekaran, P., Rajendhran, J., 2015. Molecular serotyping and pathogenic potential of *Listeria monocytogenes* isolated from milk and milk products in Tamil Nadu, India. Foodborne Pathog. Dis. 12, 522–528.
Knudsen, G.M., Ng, Y., Gram, L., 2013. Survival of bactericidal antibiotic treatment by a persister subpopulation of *Listeria monocytogenes*. Appl. Environ. Microbiol. 79, 7390–7397.
Kovacevic, J., Ziegler, J., Wałecka-Zacharska, E., Reimer, A., Kitts, D.D., Gilmour, M.W., 2016. Tolerance of *Listeria monocytogenes* to quaternary ammonium sanitizers is mediated by a novel efflux pump encoded by *emrE*. Appl. Environ. Microbiol. 82, 939–953.
Kuan, C.H., Rukayadi, Y., Ahmad, S.H., Wan Mohamed Radzi, C.W.J., Kuan, C.S., Yeo, S. K., Thung, T.Y., New, C.Y., Chang, W.S., Loo, Y.Y., Tan, C.W., Ramzi, O.S.B., Mohd Fadzil, S.N., Nordin, Y., Kwan, S.Y., Son, R., 2017. Antimicrobial resistance of *Listeria monocytogenes* and *Salmonella Enteritidis* isolated from vegetable farms and retail markets in Malaysia. Int. Food Res. J. 24, 1831–1839.
Lecuit, M., Ohayon, H., Braun, L., Mengaud, J., Cossart, P., 1997. Internalin of *Listeria monocytogenes* with an intact leucine-rich repeat region is sufficient to promote internalization. Infect. Immun. 65, 5309–5319.
Lee, S., Rakic-Martinez, M., Graves, L.M., Ward, T.J., Siletzky, R.M., Kathariou, S., 2013. Genetic determinants for cadmium and arsenic resistance among *Listeria monocytogenes* serotype 4b isolates from sporadic human listeriosis patients. Appl. Environ. Microbiol. 79, 2471–2476.
Lee, S., Ward, T.J., Jima, D.D., Parsons, C., Kathariou, S., 2017. The arsenic resistance-associated *Listeria* genomic island LGI2 exhibits sequence and integration site diversity and a propensity for three *Listeria monocytogenes* clones with enhanced virulence. Appl. Environ. Microbiol. 83, 1–12.
Luo, Q., Shang, J., Feng, X., Guo, X., Zhang, L., Zhou, Q., 2013. *PrfA* led to reduced biofilm formation and contributed to altered gene expression patterns in biofilm-forming *Listeria monocytogenes*. Curr. Microbiol. 67, 372–378.
Manso, B., Melero, B., Stessl, B., Jaime, I., Wagner, M., Rovira, J., Rodríguez-Lázaro, D., 2020. The response to oxidative stress in *Listeria monocytogenes* is temperature dependent. Microorganisms 8, 1–10.
Martinez-Suarez, J.V., Ortiz, S., Lopez-Alonso, V., 2016. Potential impact of the resistance to quaternary ammonium disinfectants on the persistence of *Listeria monocytogenes* in food processing environments. Front. Microbiol. 7, 638.
Maury, M.M., Tsai, Y.H., Charlier, C., Touchon, M., Chenal-Francisque, V., Leclercq, A., Criscuolo, A., Gaultier, C., Roussel, S., Brisabois, A., Disson, O., Rocha, E.P.C., Brisse, S., Lecuit, M., 2016. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat. Genet. 48, 308–313.
Miner, M.D., Porta, G.C., Freitag, N.E., 2008. Functional impact of mutational activation on the *Listeria monocytogenes* central virulence regulator *PrfA*. Microbiology 154, 3579–3589.
Møretrø, T., Schirmer, B.C.T., Heir, E., Fagerlund, A., Hjemli, P., Langsrud, S., 2017. Tolerance to quaternary ammonium compound disinfectants may enhance growth of *Listeria monocytogenes* in the food industry. Int. J. Food Microbiol. 241, 215–224.
Mullapudi, S., Siletzky, R.M., Kathariou, S., 2008. Heavy-metal and benzalkonium chloride resistance of *Listeria monocytogenes* isolates from the environment of Turkey-processing plants. Appl. Environ. Microbiol. 74, 1464–1468.
Mullapudi, S., Siletzky, R.M., Kathariou, S., 2010. Diverse cadmium resistance determinants in *Listeria monocytogenes* isolates from the Turkey processing plant environment. Appl. Environ. Microbiol. 76, 627–630.
Nightingale, K.K., Windham, K., Martin, K.E., Yeung, M., Wiedmann, M., 2005. Select *Listeria monocytogenes* subtypes commonly found in foods carry distinct nonsense mutations in *inlA*, leading to expression of truncated and secreted internalin A, and are associated with a reduced invasion phenotype for human intestinal epithelial cells. Appl. Environ. Microbiol. 71, 8764–8772.
Nightingale, K.K., Ivy, R.A., Ho, A.J., Fortes, E.D., Njaa, B.L., Peters, R.M., Wiedmann, M., 2008. *inlA* premature stop codons are common among *Listeria monocytogenes* isolates from foods and yield virulence-attenuated strains that confer protection against fully virulent strains. Appl. Environ. Microbiol. 74, 6570–6583.
Noll, M., Kleta, S., Al Dahouk, S., 2018. Antibiotic susceptibility of 259 *Listeria monocytogenes* strains isolated from food, food-processing plants and human samples in Germany. J. Infect. Public Health 11, 572–577.
Obaidat, M.M., Stringer, A.P., 2019. Prevalence, molecular characterization, and antimicrobial resistance profiles of *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli* O157:H7 on dairy cattle farms in Jordan. J. Dairy Sci. 102, 8710–8720.
Obaidat, M.M., Bani Salman, A.E., Lafi, S.Q., Al-Abboodi, A.R., 2015. Characterization of *Listeria monocytogenes* from three countries and antibiotic resistance differences among countries and *Listeria monocytogenes* serogroups. Lett. Appl. Microbiol. 60, 609–614.
Olaimat, A.N., Al-Holy, M.A., Shahbaz, H.M., Al-Nabulsi, A.A., Abu Ghoush, M.H., Osaili, T.M., Ayyash, M.M., Holley, R.A., 2018. Emergence of antibiotic resistance in *Listeria monocytogenes* isolated from food products: a comprehensive review. Compr. Rev. Food Sci. Food Saf. 17, 1277–1292.
Ortiz, S., López, V., Martínez-Suárez, J.V., 2014. The influence of subminimal inhibitory concentrations of benzalkonium chloride on biofilm formation by *Listeria monocytogenes*. Int. J. Food Microbiol. 189, 106–112.
Osman, K.M., Kappell, A.D., Fox, E.M., Orabi, A., Samir, A., 2020. Prevalence, pathogenicity, virulence, antibiotic resistance, and phylogenetic analysis of biofilmproducing *Listeria monocytogenes* isolated from different ecological niches in Egypt: food, humans, animals, and environment. Pathogens 9, 1–19.
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, 1–20.
Parsons, C., Lee, S., Jayeola, V., Kathariou, S., 2017. Novel cadmium resistance determinant in *Listeria monocytogenes*. Appl. Environ. Microbiol. 83, 1–16.
Parsons, C., Lee, S., Kathariou, S., 2019. Heavy metal resistance determinants of the foodborne pathogen Listeria monocytogenes. Genes 10, 1–13.
Poimenidou, S.V., Dalmasso, M., Papadimitriou, K., Fox, E.M., Skandamis, P.N., Jordan, K., 2018. Virulence gene sequencing highlights similarities and differences in sequences in *Listeria monocytogenes* serotype 1/2a and 4b strains of clinical and food origin from 3 different geographic locations. Front. Microbiol. 9, 1–15.
Rakic-Martinez, M., Drevets, D.A., Dutta, V., Katic, V., Kathariou, S., 2011. *Listeria monocytogenes* strains selected on ciprofloxacin or the disinfectant benzalkonium chloride exhibit reduced susceptibility to ciprofloxacin, gentamicin, benzalkonium chloride, and other toxic compounds. Appl. Environ. Microbiol. 77, 8714–8721.
Ratani, S.S., Siletzky, R.M., Dutta, V., Yildirim, S., Osborne, J.A., Lin, W., Hitchins, A.D., Ward, T.J., Kathariou, S., 2012. Heavy metal and disinfectant resistance of *Listeria monocytogenes* from foods and food processing plants. Appl. Environ. Microbiol. 78, 6938–6945.
Ryan, S., Begley, M., Hill, C., Gahan, C.G.M., 2010. A five-gene stress survival islet (SSI-1) that contributes to the growth of *Listeria monocytogenes* in suboptimal conditions. J. Appl. Microbiol. 109, 984–995.
Schmid, B., Klumpp, J., Raimann, E., Loessner, M.J., Stephan, R., Tasara, T., 2009. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. Appl. Environ. Microbiol. 75, 1621–1627.

Schmitz-Esser, S., Müller, A., Stessl, B., Wagner, M., 2015. Genomes of sequence type 121 *Listeria monocytogenes* strains harbor highly conserved plasmids and prophages. Front. Microbiol. 6, 1–10.
Seemann, T., 2015. snippy: Fast Bacterial Variant Calling From NGS Reads, v4.5.0 ed. Github.
Seemann, T., 2016. Abricate, 1.0.1 ed. Github.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Sullivan, M.J., Petty, N.K., Beatson, S.A., 2011. Easyfig: a genome comparison visualizer. Bioinformatics (Oxford, England) 27, 1009–1010.
Temple, M.E., Nahata, M.C., 2000. Treatment of listeriosis. Ann. Pharmacother. 34, 656–661.
Todd, E.C.D., Notermans, S., 2011. Surveillance of listeriosis and its causative pathogen, Listeria monocytogenes. Food Control 22, 1484–1490.
Van Stelten, A., Simpson, J.M., Ward, T.J., Nightingale, K.K., 2010. Revelation by single-nucleotide polymorphism genotyping that mutations leading to a premature stop codon in *inlA* are common among *Listeria monocytogenes* isolates from ready-to-eat foods but not human listeriosis cases. Appl. Environ. Microbiol. 76, 2783–2790.
Van Stelten, A., Roberts, A.R., Manuel, C.S., Nightingale, K.K., 2016. *Listeria monocytogenes* isolates carrying virulence-attenuating mutations in internalin A are commonly isolated from ready-to-eat food processing plant and retail environments. J. Food Prot. 79, 1733–1740.
Verghese, B., Lok, M., Wen, J., Alessandria, V., Chen, Y., Kathariou, S., Knabel, S., 2011. *comK* prophage junction fragments as markers for *Listeria monocytogenes* genotypes unique to individual meat and poultry processing plants and a model for rapid niche-specific adaptation, biofilm formation, and persistence. Appl. Environ. Microbiol. 77, 3279–3292.
Welekidan, L.N., Bahta, Y.W., Teklehaimanot, M.G., Abay, G.K., Wasihun, A.G., Dejene, T.A., Muthupandian, S., Mezgebo, T.A., Hagos, A.K., 2019. Prevalence and drug resistance pattern of *Listeria monocytogenes* among pregnant women in Tigray region, Northern Ethiopia: a cross-sectional study. BMC Res. Notes 12, 1–6.
Wilson, A., Gray, J., Chandry, P.S., Fox, E.M., 2018. Phenotypic and genotypic analysis of antimicrobial resistance among *Listeria monocytogenes* isolated from Australian food production chains. Genes 9, 80.
Xu, D., Deng, Y., Fan, R., Shi, L., Bai, J., Yan, H., 2019. Coresistance to benzalkonium chloride disinfectant and heavy metal ions in *Listeria monocytogenes* and *Listeria innocua* swine isolates from China. Foodborne Pathog. Dis. 16, 696–703.
Yin, Y., Yao, H., Doijad, S., Kong, S., Shen, Y., Cai, X., Tan, W., Wang, Y., Feng, Y., Ling, Z., Wang, G., Hu, Y., Lian, K., Sun, X., Liu, Y., Wang, C., Jiao, K., Liu, G., Song, R., Chen, X., Pan, Z., Loessner, M.J., Chakraborty, T., Jiao, X., 2019. A hybrid sub-lineage of *Listeria monocytogenes* comprising hypervirulent isolates. Nat. Commun. 10, 4283.
Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67, 2640–2644.
Zhou, Y., Liang, Y., Lynch, K.H., Dennis, J.J., Wishart, D.S., 2011. PHAST: a fast phage search tool. Nucleic Acids Res. 39, W347–W352.