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MINI-REVIEW

Genomic and phenotypic diversity of *Listeria monocytogenes* clonal complexes associated with human listeriosis

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Abstract

Listeria monocytogenes is a pathogen of significant concern in many ready to eat foods due to its ability to survive and multiply even under significant environmental stresses. Listeriosis in humans is a concern, especially to high-risk populations such as those who are immunocompromised or pregnant, due to the high rates of morbidity and mortality. Whole genome sequencing has become a routine part of assessing *L. monocytogenes* isolated from patients, and the frequency of different genetic subtypes associated with listeriosis is now being reported. The recent abundance of genome sequences for *L. monocytogenes* has provided a wealth of information regarding the variation in core and accessory genomic elements. Newly described accessory genomic regions have been linked to greater virulence capabilities as well as greater resistance to environmental stressors such as sanitizers commonly used in food processing facilities. This review will provide a summary of our current understanding of stress response and virulence phenotypes of *L. monocytogenes,* within the context of the genetic diversity of the pathogen.

Keywords *Listeria monocytogenes* · Listeriosis · Stress response · Genetic variation · Adaptation

Introduction

Listeria monocytogenes is a Gram positive, non-spore-forming bacterium that can be isolated from a number of environments, including soil, water, and vegetation. It is considered to be an environmental saprotroph (Swaminathan et al. 2007). This bacterium is also an opportunistic pathogen of humans and animals that is acquired by consumption of contaminated food. Compared to other foodborne pathogens, *L. monocytogenes* can thrive in numerous environments outside the host, such as soil and water, which are entry points for contamination of the food supply (Freitag et al. 2009). The robust stress adaptive capabilities of *L. monocytogenes* contribute to its ability to survive and grow in diverse, non-host environments. For example, *L. monocytogenes* can grow with salt concentrations as high as 1.9 M NaCl (Ribeiro et al. 2014), at pH 4.5 (Vermeulen et al. 2007), and is a psychrotroph, able to grow at temperatures as low as 2 °C (Swaminathan et al. 2007).

The population structure of *L. monocytogenes* consists of four lineages, each of which have multiple clonal complexes (Haase et al. 2014). Analyses of multi-locus sequence data indicate that *L. monocytogenes* has a low rate of homologous recombination (Ragon et al. 2008), though the rates of recombination differ between lineages 1 and 2, with recombination more prevalent in lineage 2 (den Bakker et al. 2008). Comparative genomics analyses identified that ~ 43% (2360) of the *L. monocytogenes* genome is composed of core genes, with the remaining ~ 57% (3109) composed of accessory genes (Tan et al. 2015). An assessment of the accessory genome of *L. monocytogenes* found a number of lineage-specific accessory genes (den Bakker et al. 2013). Variation in stress resistance (Adriao et al. 2008; Bergholz et al. 2010; Lianou et al. 2006) as well as virulence (Gray et al. 2004; Rakic Martinez et al. 2017) phenotypes has been observed among genotypes of *L. monocytogenes*, though direct associations between specific genetic elements and phenotype are not always clear. While in the USA and many other countries, *L. monocytogenes* regulation is based on finding any *L. monocytogenes* in foods and/or the food processing

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environment, it has become increasingly clear that all *L. monocytogenes* strains are not equally represented among clinical and food isolates, whether these isolates are from outbreaks or sporadic cases (Norton and Braden 2007; Ward et al. 2008). There is an overwhelming preponderance of serotypes 4b, 1/2a, and 1/2b among clinical and food isolates, clearly pointing to differences in ability to survive in foods and/or cause disease.

Recent studies capitalizing on the ease and rapidity of whole genome sequencing (WGS) have uncovered to a greater extent the genetic variation among clonal complexes of *L. monocytogenes* (Moura et al. 2016). The scope of this mini-review includes the changing landscapes of different clonal complexes around the globe, and new insights arising from this extensive WGS data combined with phenotypic assessments, establishing connections with variation in genetic information and phenotypes that influence survival of *L. monocytogenes* in extra-host and in host environments.

Newly emerging clones of *L. monocytogenes*

L. monocytogenes phylogenetic studies have made use of various typing methods, including multi-locus sequence typing (MLST) and multi-locus virulence genes sequence typing (MLVST) to evaluate the relationships between strains and to identify lineages and clonal groups that correlate with infection and/or source. Both these schemes use about 3000 nucleotides from six different genes for phylogenetic analyses. In 2008, Ragon et al. used MLST sequence types (ST) to group strains by clonal complex (CC), which are comprised of ST groups that had a recent common ancestor (Ragon et al. 2008). Subsequent studies have led to the identification of additional CCs, though for some lineages, such as lineages 1 and 2, few new CCs were found, suggesting that most of the clinically relevant CCs have been identified (Haase et al. 2014). Many of these CCs have been linked to epidemic clones (Cantinelli et al. 2013), responsible for multiple outbreaks of listeriosis, suggesting these strains may represent more fit pathogens, though whether that is due to virulence attributes, higher incidence in food processing environments or increased stress resistance properties is unclear. As such, determination of dominant CCs over time may provide clues about fitness as well as indicate alterations in selective pressures that may lead to changes in the CCs that are predominant.

Several studies have focused on the distribution of CCs and STs during different time periods and in different geographic regions. An evaluation of these studies individually, as well as holistically, provides clues to changes in the environment *L. monocytogenes* resides within. Data collected from ten studies, with redundant strains removed when identified, were evaluated and found that certain groups represent potentially emerging CCs (Fig. 1). It is apparent that historically dominant CCs, such as CC1 and CC2 are represented less frequently in recent years. Instead, previously uncommon CCs are rising in frequency, particularly CC5, CC6, CC9, and CC121.

Within these datasets are observations of other possible CCs that represent an emerging population. Haase et al. noted that CC101 was common in the 1950s but became less frequent until more recently when it expanded notably relative to other CCs (Haase et al. 2014). Additionally, Haase et al. noted that these isolates are typically associated with human clinical sources, a bias suggesting a higher pathogenic potential. However, an examination of CC101 from Australia (Jennison et al. 2017) and Europe (Bertrand et al. 2016), as well as the aggregated data presented here (Fig. 1), does not show the same expansion of this CC as seen by Haase et al. Similarly, the apparent expansion of CC121 may represent the impact of certain geographic regions. Evaluation of data from Australia suggests that CC1 and CC3 show no sign of decline and evidence of any emergent clones is less clear, though CC121 appears to be expanding in Australia as well as Europe. Studies in Asia are more limited but suggest that other CCs play a larger role in listeriosis in that region. Similarly, while not included in the aggregated data, an evaluation of clinical isolates from Canada showed a heavy predominance of CC8 (Knabel et al. 2012). While the aggregated dataset showed an increase in the merged CC (CC8 and CC16) since 2000, the percentage of CC8-CC16 (8%) observed is still lower than incidence of CC8 alone in the Canadian dataset (58%). The presence of differing dominant CCs in different geographic regions is expected as dietary choices and environmental factors are likely to impact the required survival adaptations necessary for *L. monocytogenes* transmission within different food matrices.

Besides phylogenetically defined groups, other subtypes may represent an emerging trend in listeriosis with their own risk factors to consider. Among the 13 serotypes of *L. monocytogenes*, over 95% of listeriosis cases are associated with serotype 1/2a, 1/2b, and 4b, with serotype 4b causing over half of these cases. This suggests that serotypes, which are broadly distinct phylogenetic groups, have differences in fitness depending on their evolutionary niche. Within these serotypes there may be subgroups representing emerging trends in listeriosis. One example is the recent series of outbreaks that have been linked to a variant of serotype 4b, termed 4bV or IVb-v1 (Burall et al. 2017a). This clade of *L. monocytogenes* was linked to a few atypical foods, specifically fresh produce, and presented atypical cases. The 4bV subtype of *L. monocytogenes* has been observed previously, with isolates dating back to 1959; however, these isolates historically had no epidemiological links and cases prior to 2014 were considered sporadic (Leclercq et al. 2011).

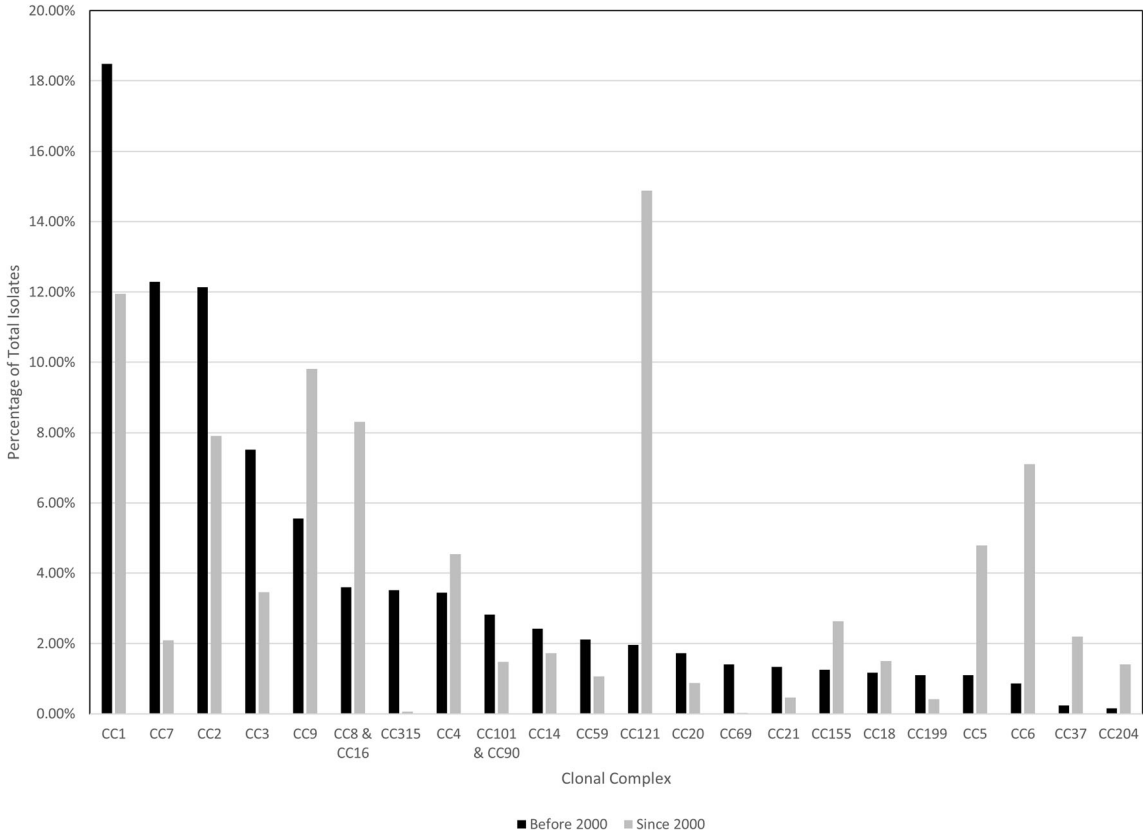

Fig. 1 Relative frequency of clonal complexes before and since 2000. Isolates from ten studies (Bertrand et al. 2016; Chenal-Francisque et al. 2011; Haase et al. 2014; Huang et al. 2015; Jennison et al. 2017; Jensen et al. 2016; Kwong et al. 2016; Maury et al. 2016; Ragon et al. 2008; Wang et al. 2012) were used to evaluate CC frequency before (black) and since (gray) 2000. Data has been ranked based on frequency prior to 2000

Evaluation of some of these isolates by WGS (Burall et al. 2017b; Laksanalamai et al. 2013) further supports the possibility of these isolates being part of unrecognized outbreaks, though due to the lack of epidemiological linkage, this possibility cannot be proven (Burall et al. 2017b; Lee et al. 2014). Prior to 2013, only about 40 4bV isolates had been reported (Lee et al. 2014) while nearly 300 have been collected subsequently. This higher number may be due to the large number of isolates associated with a few outbreaks, which further supports an emerging trend associated with these strains. Additionally, evaluation of the geographic sources of these 4bV strains found that the clade associated with these outbreaks has been linked to a specific region in California, while the other large clade of 4bV strains has been predominantly linked to isolates from the eastern half of the USA, again underscoring the possibility of geographic differences influencing strain association.

Critically lacking in these studies is evaluation of these trends from both a temporal and geographic perspective. While there is clear evidence for CC6 as an emerging group within Europe, its presence wasn't mentioned in either Australian study and was low in the Asian surveillance efforts. However, due to smaller sample sizes compared to European efforts, this may not be reflective of the overall population. Similarly, the 4bV expansion may represent the effects of a localized microcosm altered by local environmental factors, such as agricultural practices, within the overall global population dynamics. Whether these expansions represent persistent emerging global and/or regional trends or simply reflect a brief increase remains to be seen and underscores the need for on-going evaluation of CCs, 4bV, and other *L. monocytogenes* subgroups of relevance as they are identified for shifts in frequency globally and regionally, especially when considering the changing trends in listeriosis related to patient demographics (more elderly individuals compared to pregnant women), food, and serotypes (Cartwright et al. 2013; Goulet et al. 2008; Mammina et al. 2013; Munoz et al. 2012; Pontello et al. 2012).

L. monocytogenes clones and hyper virulence

The disease-causing potential of different *L. monocytogenes* strains has been difficult to measure because of the numerous variables associated with disease manifestation. These variables include, but are not limited to, small genetic

differences between strains, sources of these strains which could affect epigenetic make-up, physiology, and genetics of the host population, the amount of *L. monocytogenes* ingested, and consumers' health, as well as the treatment regimen they undergo. Nevertheless, the severity of different outbreaks and sporadic cases in terms of morbidity and mortality as well as the number of people affected and attack rate (number of people infected/number of people exposed) often point to some intrinsic difference in genomic composition of the strains associated with these illnesses. Recent efforts with WGS data have led to the identification of STs and CCs associated with outbreaks of invasive disease and sporadic cases (Hain et al. 2007; Orsi et al. 2011; Piffaretti et al. 1989). For example, CC1 (also known as Epidemic Clone 1) strains were associated with several major outbreaks in the USA from 1985 to 2000 (Kathariou 2002). Although the vast majority of listeriosis cases are restricted to the elderly, immunocompromised, and neonates, a few cases have been recorded where the infected individuals did not fall in these categories (Painter and Slutsker 2007). For example, in a recent outbreak involving caramel apples, three cases were found to be outside the "susceptible group" (Centers for Disease Control 2015). These cases and a few other recent outbreaks were caused by a clade of 4bV *L. monocytogenes* strains, indicating definite genetic differences from other 4b and 4bV strains (Burall et al. 2017a). However, absence of clinical data associated with the listeriosis cases as well as a reliable and sensitive ex vivo or animal model for virulence assessment also adds to the complexity of assessing the virulence potential of *L. monocytogenes* strains. Many animal models require a substantial number of laboratory grown bacterial cells (10^5–10^8 CFU) for any measurable end-point, which is not a true reflection of the number of cells that would be consumed. Maury et al. recently published a retrospective study in which they identified a few CCs correlated with cases of meningitis while other CCs were associated with cases of septicemia (Maury et al. 2016). They identified that the CCs involved in the meningitis cases had a more virulent phenotype than the strains associated with the septicemia cases. Interestingly, these meningitis-associated CC4 strains were also found to be more virulent in tissue culture and humanized mouse model of infection. In addtion to other genetic differences the so called "hyper virulent" CC4 strains contain an ~ 8 kb DNA fragment, termed LIPI-4 (*Listeria* pathogenicity island 4). At this point, the role of LIPI-4 genes, which are involved in carbohydrate metabolism, is debatable as this island was also found in *L. innocua* strain (Lecuit personal communication). It is still possible that LIPI-4, in addition to other virulence factors which are not present in *L. innocua*, could make 4b strains more virulent. Although this study was the first to identify the existence of such a pathogenicity island, its association with other listeriosis cases, whether they are meningitis, septicemia, or just self-limiting gastroenteritis requires further study.

Variation in virulence phenotypes

Given that certain clones of *L. monocytogenes* differ in their severity of disease based on clinical data, assessing variation in virulence can lead to identifying genetic elements that may be associated with virulence phenotypes. In addition to identifying LIPI-4 and hyper virulence of CC1, CC4, and CC6, Maury et al. demonstrated that strains of CCs more commonly isolated from foods, such as CC9 and CC121, were not as virulent in mice. This was represented by significantly higher loss of body weight and concentration of cells isolated from the spleen, liver, and mesenteric lymph nodes of mice infected with CC1, CC4, and CC6 strains compared to CC9 and CC121 strains (Maury et al. 2016). This work is one of the only studies assessing virulence phenotypes by clonal complex; other studies have measured variation in virulence by serotypes (Kuenne et al. 2013) or source of isolation (Rakic Martinez et al. 2017). While these studies have identified that serotype 4b strains or clinical isolates typically exhibit greater virulence, they have not identified any underlying genetic features that are associated with these differences (Kuenne et al. 2013; Rakic Martinez et al. 2017). Taken together, listeriosis severity based on outbreak data and in vitro and ex vivo virulence phenotypes provide evidence for variability in virulence properties of *L. monocytogenes*, likely linked to genetic differences among strains. Assessing virulence phenotypes among CCs or other WGS based subtypes will provide more insights into these genetic associations.

Variation in stress resistance phenotypes—food processing environment

L. monocytogenes is exposed to a number of environmental stressors both in foods and in the food processing environment. The ability of *L. monocytogenes* to adapt to these stresses facilitates its survival and transmission in the food supply. In food processing environments, persistence of *L. monocytogenes* has been associated with numerous outbreaks, as the processing environment was the most likely source of contamination for different foods (Angelo et al. 2017; McCollum et al. 2013). The ability of *L. monocytogenes* to survive and persist after cleaning and disinfection in food processing environments have been attributed to numerous factors, including colonization of niches and reservoirs out of disinfectant reach, biofilm formation, and activation of resistance mechanisms (Martinez-Suarez et al. 2016). Following prolonged exposure to sub-lethal concentrations of disinfectants, bacteria may develop increased resistance to the biocide in use (To et al., 2002). Increased resistance of bacteria in biofilms is a result of adaptation of bacteria to the environment within the biofilm, in addition to the intrinsic

factors which may vary between the strains (Bridier et al. 2011; Kastbjerg and Gram 2009).

Environmental adaptations of *L. monocytogenes* are also associated with resistance to heavy metals, most commonly cadmium (Cd) and arsenic (As) (McLauchlin et al. 2004). Recent reports indicated that resistance to cadmium may correlate with resistance to quaternary ammonium compounds (QAC) (Katharios-Lanwermeyer et al. 2012). QACs are water soluble, broad spectrum antibacterial agents most commonly used in the food processing industry. Benzalkonium chloride (BC) is the most frequently used QAC in food processing plants (McDonnell and Russell 1999). Reports of tolerance to BC among *L. monocytogenes* isolates from food processing facilities vary between 10 and 46% (Aase et al. 2000; Mereghetti et al. 2000; Mullapudi et al. 2008; Soumet et al. 2005). So far, only a few molecular mechanisms for tolerance to BC have been elucidated. The genetic elements responsible for BC tolerance include *bcrABC*, a resistance cassette on a putative transposon harbored by pLM80-like plasmids (Elhanafi et al. 2010), a novel transposon Tn*6188* (Muller et al. 2013), and small multidrug-resistant (SMR) efflux pump encoded by *emrE* carried by a novel genomic island LGI1 (Kovacevic et al. 2015).

The distribution of genetic elements associated with BC tolerance varies. Some elements, such as *bcrABC and Tn6188,* are widely distributed among strains as they are carried on mobile elements. The *bcrABC* resistance cassette has been identified in a strain related to the 1998/1999 hot dog-associated listeriosis outbreak in the USA (Elhanafi et al. 2010). The cassette is comprised of a transcriptional regulator *bcrA* and a multidrug resistance protein *transporter bcrBC.* This cassette is found to be widely disseminated among *L. monocytogenes* regardless of the serotype (Elhanafi et al. 2010). Its presence in nonpathogenic Listeria spp. indicates the possibility of these strains as reservoirs of BC and other resistance determinants for *L. monocytogenes* through the possibility of conjugative transfer (Katharios-Lanwermeyer et al. 2012). A novel transposon Tn*6188*, related to Tn*554* from *Staphylococcus aureus*, can contribute to *L. monocytogenes* resistance to *BC.* When present in *L. monocytogenes* strains, this transposon along with other Tn*554*-like transposons have been shown to contribute to higher minimum inhibitory concentration of BC and significantly higher expression of *qacH* (coding a protein responsible for export of BC) in the presence of BC (Muller et al. 2013*)*.

During the deadliest outbreak of listeriosis in Canada in 2008, a novel genomic island LGI1, specific to *L. monocytogenes* CC8 strains was discovered (Gilmour et al. 2010). Strains harboring this island belong to CC8, found to be predominant in listeriosis outbreaks and sporadic cases over two decades in Canada (Knabel et al. 2012; Kovacevic et al. 2015). LGI1 carries a small multidrug-resistant efflux pump encoded by *emrE* believed to be responsible for the increased tolerance to QACs. Expression of *emrE* gene in the presence of BC is upregulated raising the concern of possible adaptation and persistence of *Listeria* strains harboring this gene in the food processing environment (Kovacevic et al. 2015).

Resistance to heavy metals can correlate with resistance to QACs. Resistance to cadmium is frequent in *Listeria*, with several major outbreaks of listeriosis involving cadmium resistant *L. monocytogenes* isolates (Elhanafi et al. 2010). The same plasmid that carries *bcrABC* also harbors the cadmium efflux determinant *cadA2* and trans-conjugants acquire both determinants (Katharios-Lanwermeyer et al. 2012). In addition to *cadA2*, the resistance to Cd is also conferred by plasmid-borne *cadA1* and chromosomal *cadA3*—members of the *cadAC* efflux system (Lee et al. 2013). Recently, chromosomal *cadA4* has been identified in strains belonging to CC1, CC2, and CC6 (Lee et al. 2013). Although the presence of *cadA4* repressed virulence of *L. monocytogenes* in the *Galleria mellonella* insect model (Parsons et al. 2017), its effect on human listeriosis is not known.

Resistance to arsenic is chromosomally encoded with specific genes described recently (Harter et al. 2017; Lee et al. 2013). Harter et al. demonstrated the role of stress survival islet 2 (SSI-2) specific to the *L. monocytogenes* strains of sequence type (ST) 121 in pathogen survival under alkaline and oxidative stress caused by exposure to some commonly used disinfectants in the food processing environment (Harter et al. 2017). *L. monocytogenes* strains of serotype 4b harbor a 35-kb chromosomal *Listeria* genomic island 2 (LGI2) associated with arsenic resistance genes *arsA1* and *arsA2* (Lee et al. 2013). Lee et al. described LGI2 as a "floating island "with capacity to mobilize heavy metal resistance genes into different location in *L. monocytogenes*. They also speculated that due to the wide presence of this island among serotype 4b strains, especially CC1, CC2 and CC4, this island could have a role in *L. monocytogenes* virulence (Lee et al. 2017).

Variation in resistance to food-related stresses

Survival and growth capabilities of *L. monocytogenes* in foods, particularly in ready to eat (RTE) foods, are important from a food safety perspective. As *L. monocytogenes* is capable of growing at refrigerated temperature and has a high tolerance to salt, which are two main hurdles used to preserve foods, it is important to understand how *L. monocytogenes* cope with these stresses. Besides high salt and cold, foods containing other standard, generally recognized as safe (GRAS) preservatives have also been associated with human listeriosis. For this review, we will limit ourselves to *L. monocytogenes* response to low temperature, high salt,

low pH, and presence of a few GRAS compounds including nisin. For a more comprehensive review on microbial stress response and adaptations, please refer to recent reviews on the topic (Milillo et al. 2012; NicAogain and O'Byrne 2016).

Various salts including NaCl are commonly used to control bacterial growth in foods. This is most relevant for RTE foods with a long shelf life including cheese, a food often associated with listeriosis. Detailed studies involving various strains of *L. monocytogenes* in laboratory media as well as isolation of *L. monocytogenes* from various foods showed that *L. monocytogenes* can tolerate as high as 10% NaCl although the growth rate decreases as function of salt concentration (Swaminathan et al. 2007). *L. monocytogenes* salt tolerance varies considerably from strain to strain. A recent study by Hingston et al. showed a wide range of variability among strains representing various CCs, with CC2 and CC11 exhibiting greater salt tolerance (Hingston et al. 2017b). While the study by Hingston et al. is one of the few to assess salt stress phenotypes by CC, older studies have examined variation in salt stress phenotypes by genetic lineage or serotype (Ribeiro and Destro 2014). Growth under salt stress varies by lineage and is also dependent on growth temperature. Lineage 1 strains grew at a significantly faster rate under 6% NaCl compared to lineage 2 strains, but this difference was only evident at 37 °C, not at 7 °C (Bergholz et al. 2010).

The use of osmoprotectants or compatible solutes by *L. monocytogenes* to manage osmotic stress is well-described (Burgess et al. 2016). These compounds include glycine betaine, carnitine, proline, glycerol, and trehalose, which can be found in foods at varying levels. The ability of *L. monocytogenes* to utilize compatible solutes or osmoprotectants to manage salt stress in foods would not only depend upon the genetic composition of the strain but also on the presence of osmoprotectants in that food. For example, foods such as alfalfa sprouts, beetroot, spinach, clams, and chicken contain elevated levels of glycine betaine, proline betaine, or trigonelline, and it would be expected that *L. monocytogenes* would be able to tolerate higher levels of salt in these foods compared to others with lower levels, e.g., cream cheese, celery, or butter (de Zwart et al. 2003). In addition to mechanisms to utilize osmoprotectants, *L. monocytogenes* utilize other mechanisms to alleviate osmotic stress which are not as well characterized (Bergholz et al. 2012). The increase in transcription of these genes in high-salt-containing media indicates that these genes play important roles in *L. monocytogenes* salt tolerance. Random mutagenesis experiments have identified several genes which encode functions involving salt transport as important for survival under salt stress, though the function of many of these genes is poorly understood (Burall et al. 2012; Burall et al. 2015).

L. monocytogenes has been isolated from several low acid foods indicating its ability to tolerate low pH (Koutsoumanis et al. 2003). The ability to tolerate acid varies from strain to strain, and can be related to growth phase and prior exposure to low pH (Datta and Benjamin 1997; Davis et al. 1996). In general, *L. monocytogenes* growth has been found to be completely inhibited at pH 4.5 and below, although the recent findings following the *L. monocytogenes* outbreak linked to caramel apples and several in vitro studies indicated that *L. monocytogenes* survival and growth in apples may be a function of other factors including titratable acidity and temperature of storage (Glass et al. 2015; Salazar et al. 2016). One of the most well-studied acid tolerance systems in *L. monocytogenes* is the glutamate decarboxylase system. Strain to strain differences in acid resistance were linked to differences in accumulation of the decarboxylated glutamate (γ-amino butyric acid, GABA) intracellulary, as well as differences in regulation of the genes encoding the decarboxylases and transporters involved in this system (Feehily et al. 2014; Karatzas et al. 2012).

Many GRAS substances are commonly used to suppress *L. monocytogenes* growth in RTE foods. Nisin, a well-characterized GRAS bacteriocin, has been studied in detail for its role in the control of *L. monocytogenes* in food (Datta and Benjamin 1997). The effectiveness of nisin depends on pH, salt concentration, and storage temperature of the food. Variation in sensitivity among strains has been reported (Begley et al. 2010). Strains belonging to CC6 and CC7 were found to have significantly higher innate resistance to nisin compared to strains belonging to CC2, CC3, CC5, CC9, and CC11 (Malekmohammadi et al. 2017). Adaptation of *L. monocytogenes* to either low pH (van Schaik et al. 1999) or osmotic stress (Bergholz et al. 2013) can significantly increase nisin resistance, with the potential for limiting the efficacy of nisin in some foods (Bonnet and Montville 2005).

L. monocytogenes is one of the few foodborne pathogens which grows relatively well at cold temperature (4–10 °C) provided that other conditions, e.g., pH, nutrients, and water activity are favorable. Several mechanisms have been implicated in the *L. monocytogenes* response to cold, and several genes have been identified whose functions are associated with optimum cold tolerance (Tasara and Stephan 2006). Strains can vary in cold tolerance, and greater levels of cold tolerance have been linked to increased transcript levels of known cold tolerance genes, including *cspA* and *pgpH,* demonstrating an association between genetic factors and cold tolerance (Arguedas-Villa et al. 2010; Lianou et al. 2006). Hingston et al. compared cold tolerance capabilities across different CCs of *L. monocytogenes*, and while they did not find any significant differences among CCs, they did find that strains of serotype 1/2c had lower cold tolerance compared to other serotypes (Hingston et al. 2017b). As low temperature is a very important tool for food safety, a thorough understanding

of cold tolerance in *L. monocytogenes* will be extremely useful to develop new intervention strategies benefiting both public health and food industry.

L. monocytogenes can overcome many of the standard hurdles used to control microbes in the food industry. Development of increased tolerance to one hurdle may provide cross protection to another. As shown by genetic and phenotypic studies, genes which are involved in stress tolerance often have multiple overlapping functions thereby achieving a tolerance response to multiple stresses. Some of the genes contributing to the different stress responses have been identified using random or site-directed mutagenesis in commonly used reference strains (Begley et al. 2002; Burall et al. 2012; Hingston et al. 2015). The exact mechanisms by which these genes influence stress tolerance is yet to be determined.

Links between stress resistance and human listeriosis

Adaptation of *L. monocytogenes* to environmental stresses present in foods can influence their ability to survive subsequent stresses that may be encountered in the gastrointestinal tract of a potential host. For example, *L. monocytogenes* adapted to the pH and osmotic stresses present in different types of cheeses had significantly greater survival during subsequent challenge in a simulated gastric environment (Kapetanakou et al. 2017). Growth of *L. monocytogenes* in an oxygen-limited environment, similar to vacuum packaging of a food, significantly increased subsequent survival in a simulated gastric system (Sewell et al. 2015). It would be useful to assess a wider range of strains to determine if these phenomena are universally applicable.

As noted in the "Newly emerging clones of L. monocytogenes" section, certain subtypes of *L. monocytogenes* have been increasing in frequency, in particular CC6 in the Netherlands (Koopmans et al. 2017). Isolates subtyped as ST6 (belonging to CC6) were significantly associated with cases of listerial meningitis which had unfavorable outcomes (Koopmans et al. 2017). A genome-wide association analysis was conducted to identify genetic elements from these CC6 isolates that were associated with unfavorable outcomes, including mortality (Kremer et al. 2017). A novel phage, phiLMST6, and a novel plasmid, pLMST6, were both identified as strongly associated with ST6 isolates that were associated with more severe disease. A gene encoding a QAC efflux pump, *emrC*, was present on pLMST6. It is interest to note that additional isolates were surveyed for the presence of pLMST6, and it was found predominantly in CC6 isolates, though also occurred in one isolate each from CC9, CC8, and CC101 (Kremer et al. 2017). Isolates carrying pLMST6 had greater resistance to BC, as well as to gentamycin and amoxicillin (Kremer et al. 2017). This is the first study that provided links between an increased frequency of a given *L. monocytogenes* subtype and specific genetic elements that may contribute to its ability to survive in the environment and resist treatments in the host.

Future perspectives

With the significant increase in WGS data for *L. monocytogenes*, there is the opportunity to identify additional genetic elements, i.e., accessory genes that are associated with virulence and/or stress resistance phenotypes (Table 1). In addition to assessing these factors among CCs recently reported to be associated with more severe disease (including CC1, CC4, and CC6 (Kremer et al. 2017; Maury et al. 2016)), investigation of potential factors associated with the emergence of ST382 in the USA is also needed. Our understanding of the genes and regulatory elements involved in different stress responses has slowly expanded beyond that from the reference strains LO28, EGD, and EDGe, to include relevant outbreak- and food processing environment-associated strains (Burall et al. 2012; Hingston et al. 2017a; Liu et al. 2017; Stasiewicz et al. 2011). Genome-wide association studies have the potential to significantly transform our

Table 1 Genetic elements associated with stress or virulence phenotypes

Genetic element	Present in these groups	Phenotype	References
LIPI-3	CC1, CC4, CC6	Increased competitiveness in GI tract	(Moura et al. 2016; Quereda et al. 2017)
LIPI-4	CC4	Central nervous system and maternal/fetal infection	(Maury et al. 2016)
LGI 1	CC8	Increased tolerance to QACs	(Kovacevic et al. 2015)
LGI 2	CC1, CC2, CC4	Arsenic resistance	(Lee et al. 2017)
SSI-2	ST121	Alkaline and oxidative stress resistance	(Harter et al. 2017)
pLMST6	CC6	Increased resistance to BC, amoxicillin, gentamycin	(Kremer et al. 2017)
Tn6188	Varied	Resistance to BC	(Muller et al. 2013)

knowledge of the underlying genetic mechanisms leading to variation in stress response and virulence by utilizing the range of genetic diversity among *L. monocytogenes* strains.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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