

Current Trends in Foodborne Human Listeriosis

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Human listeriosis results from the ingestion of foods contaminated with *Listeria monocytogenes* (*Lm*). About 1600 listeriosis cases are reported every year in the USA with >95% hospitalization and 15–20% death. The proportions of persons with listeriosis hospitalized and who die are very similar in Europe with slightly higher rates in Scandinavian countries. The occurrence of disease requires adaptation, survival, and usually growth of *Lm* in foods before consumption by members of the susceptible population. Despite concerted efforts by the food safety community, the disease incidence has not changed significantly since 2001 and remains higher than the Healthy People 2020 target of 0.2 cases per 100,000 individuals.

In recent years, human listeriosis cases have been reported to involve non-typical foods, e.g. celery, cantaloupe, caramel apple, frozen vegetables and ice cream. In some outbreaks, a few infected individuals were considered outside the realm of the standard vulnerable population group. Our recent work with the outbreak associated with ice cream samples, indicated that a low-level contamination in a food that does not support growth can cause listeriosis in highly susceptible populations. Separately, using a combination of polymerase chain reaction (PCR)-based serotyping and whole genome sequencing (WGS)-based analyses; we have discovered that a genetic variant of the serotype 4b strain, called 4bV, was responsible for 3–4 recent outbreaks in the USA. Three of the four products associated with these outbreaks were grown in a small geographical region of the USA while the fourth was never linked to a specific grower, but rather a processing facility. These 4bV strains contain a 6.3kb DNA fragment normally associated with lineage II *Lm* strains. The significance of this DNA fragment in the serotype 4b background is currently being investigated. This article reviews current listeriosis outbreaks with an emphasis on the expansions in food niche, case demography and genotypes of *Lm*. The discussion raises important questions about *Lm* adaptation in different foods and environments and the role of certain genotypes in such adaptation and disease outcome.

Key words: human listeriosis, new food types, *Listeria monocytogenes*, new serotypes/genotypes

Introduction

Human listeriosis, caused by *Listeria monocytogenes* (*Lm*), is a major foodborne disease which causes severe invasive infections (meningitis and sepsis) and, in some instances, gastroenteritis. The febrile gastroenteritis is a self-limiting disease which has been reported to occur in healthy persons and presents with fever, watery diarrhea, nausea, headache, and pains in joints and muscles. Several

reported outbreaks of febrile gastroenteritis due to *Lm* have been reported in different continents¹). The invasive form of the disease, on the other hand, most often affects immunocompromised persons, including the elderly, neonates, and persons with underlying diseases associated with reduced immune function. During pregnancy, listeriosis most often presents in the mother as a mild febrile illness. In the USA, approximately 1600 cases of listeriosis are reported annually resulting in 250 deaths²). The high fatality rate (about 20%)

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Abbreviations: BLAST: Basic Local Alignment Search Tool, CFSAN: Center for Food Safety and Nutrition, *Lm*: *Listeria monocytogenes*, MPN: most probable number, MVLST: Multi Virulence Loci Sequence Typing, PCR: polymerase chain reaction, PFGE: Pulsed Field Gel Electrophoresis, WGS: Whole Genome Sequencing

and the hospitalization rate (>95%) make listeriosis one of the deadliest foodborne diseases. According to the Centers for Disease Control and Prevention, reported rates of human listeriosis in the USA have remained steady over the last few years, and if no changes occurs in the next few years, the rate will exceed the Healthy People 2020 target of 0.2 cases per 100,000 populations³), suggesting the need for additional information and approaches to prevent this infection.

Lm is a Gram-positive, micro-aerophilic, non-spore forming organism ubiquitously present in the environment and has been detected in most raw agricultural products and many dairy products, including cheeses and ice cream. The organism is known to be resistant to high salt and grows well at refrigerated temperatures provided other conditions are suitable⁴). The resistance to high salt and the ability to grow at refrigerated temperatures makes this organism hard to control by standard preservation and storage conditions. It is often assumed that because of these properties *Lm* has an advantage over other organisms, which are more inhibited than *Lm* in high salt condition and in refrigerated temperatures as *Lm* is more resistant to these conditions. The genus *Listeria* is currently comprised of 17 species⁵) of which *Lm* and *L. ivanovii* are the only pathogens, although human infection by *L. ivanovii* is rarely reported⁵). Based on somatic and flagellar antigens, *Lm* can be grouped into 13 serotypes although the clear majority of infections are caused by serotypes 1/2a, 1/2b and 4b^{6,7}). Because of the complexity involved in classical serotyping by antigen-antibody reaction, several DNA based methods have been developed which depend on the presence/absence of genetic sequences. Using a PCR-based method, Doumith et al.⁸) first reported a sero-grouping approach which resulted in formation of five serogroups- 1/2a-3a, 1/2b-3b-7, 1/2c-3c, 4b-4d-4e and non-typeable, which don't fall into any of the other groups. Burall et al.⁹) combined this method and a simple slide agglutination method to further divide the first three groups into 1/2a, 1/2b, 1/2c, 3a, 3b-7 and 3c. This PCR-based method also resulted in uncovering a subgroup of 4b, called 4b variant, 4bV¹⁰) or IVb-v1¹¹). The 4bV group contains a 6.3kb DNA fragment, not found in typical 4b strains but instead is found to be associated with 1/2a-3a and 1/2c-3c strains of *Lm*. Although the gene composition and organization of this 6.3kb DNA and putative annotated functions have been elucidated, a limited effort to unravel its biological significance has been unsuccessful¹⁰). Although all *Lm* strains are considered pathogenic, it is not clear why certain serotypes are over-represented among clinical isolates and why certain serotypes are over-represented among food and environmental isolates. Assessment of the virulence potential of *Lm* strains using several *in vitro* and *in vivo* models has not been very successful in

discerning any strain-to-strain or serotype-to-serotype differences in virulence potential although there are distinct genetic footprints associates with the different serotypes¹²). The lack of a sensitive model for virulence assessment has also hampered our attempt to obtain a realistic picture of the infectious dose and the effect of food matrices on *Listeria* virulence¹³).

Lm contains a single chromosome of about 3MB with the occasional presence of some plasmids and/or a few phage sequences. The relatively small genome length, 3MB vs 4.5 MB for *E. coli* and *Salmonella*, may be related to a lack of diversity (synteny) of the genome, although a recent publication by Chen et al.¹⁴) questions that notion. The major virulence genes of *Lm* and *L. ivanovii* are in a 10kb pathogenicity island (LPI) while few others are scattered around the chromosome. Two global transcription regulators, *prfA* for virulence genes and *sigB* for stress related functions, have been well characterized as have several of their regulatory targets. However, the significance of virulence genes in overall disease outcome and their impact on infectious dose along with the significance of *sigB*-controlled genes in environmental adaptation are little understood. From a food contamination and preservation standpoint, the genetic controls underlying salt and acid tolerance, growth at refrigerated temperature, ability to form biofilm and how *Lm* escapes standard sanitary processes, remains very poorly understood.

A close look at the major invasive listeriosis outbreaks (Table 1) shows some important trends associated with food and serotype distribution starting with the coleslaw outbreak in Canada in 1980–1981. Starting from early 1980s and the next 20 years or so, dairy and meat products constituted the major food groups and serotype 4b was the major serotype. This pattern, however, changed considerably as we have witnessed many more outbreaks involving strains of serogroup 1¹⁵). Additionally, in the last few years we have witnessed many more outbreaks involving fresh produce and the potentially increased involvement of serotype 4bV strains. Understanding these trends would be useful in formulating policy guidelines which might reduce future outbreaks. A brief discussion of a few of these recent outbreaks highlights how policy guidelines could be affected by this increased understanding.

Listeriosis Outbreak Related to Ice Cream

In the early 2015, a listeriosis outbreak USA was reported involving 5 hospitalized patients in Kansas City. All the patients were reported to have drunk milkshakes made with individual pre-packaged ice cream servings. Three of the five patients died with listeriosis, as well as severe

Table 1. Major invasive listeriosis outbreaks*.

Year	Location	Cases (% Mortality)	Food	Serotype
1980–81	Canada	41 (34)	Coleslaw	4b
1983	Massachusetts	49 (29)	Pasteurized Milk	4b
1984	Switzerland	57 (32)	Soft Cheese	4b
1985	California	142 (34)	Jalisco Cheese	4b
1987–89	UK	823	Pate	4b
1992	France	279 (30)	Rillettes (Pork)	4b
1998–99	USA	101 (21)	Hot Dog, Deli Meats	4b
1998–99	Finland	25 (24)	Butter	3a
2000	USA	29 (7)	Deli Turkey Meat	1/2a
2008	Canada	57 (40)	Deli Meat	1/2a
2011	USA	147 (33)	Cantaloupe	1/2a, 1/2b
2012	USA	22 (25)	Ricotta Salata Cheese	1/2a
2013	USA	6 (33)	Soft Cheese	4b
2014	USA	8 (12)	Soft Cheese	1/2b
2014	USA	35 (9)	Caramel Apple	4b, 4bV
2010–2015	USA	10 (30)	Ice Cream	1/2b
2015	USA	30 (10)	Soft Cheese	Unknown
2016	USA	19 (5)	Packaged Greens	4b-V
2016	USA	9 (33)	Frozen Vegetables	1/2a

* <https://www.cdc.gov/listeria/outbreaks/index.html>

underlying health issues, and, except for one, all patients had isolates which were very closely related by Whole Genome Sequencing (WGS), though there were three different Pulsed Field Gel Electrophoresis (PFGE) patterns associated with these four cases. These four clinical isolates were found to be closely related by PFGE and WGS with the isolates obtained from unopened ice cream products made by a company in their Texas facility. Subsequently, a sampling of ice cream produced at other facilities within the company found *Lm* in another facility that was found to match 5 more cases in a retroactive analysis of listeriosis cases by PFGE and WGS dating back to January 2010. Investigation of the facility in Texas resulted in collection of large number of samples produced at that facility for further analysis to better understand the extent of contamination, the period of contamination, and the number of viable *Lm* present in these contaminated products. As ice cream is a no-growth food and *Listeria* survives well in ice cream under standard storage conditions¹⁶, it was assumed that the enumeration of the products involved in the

Kansas City cases would provide fairly accurate information as to the number of organisms ingested by these patients. **Table 2** summarizes the results of enumeration from three different ice cream products of which product C was the only one implicated in the Kansas City outbreak; however, all three products were produced on the same production line in the same facility. The data showed that all three products have a very low level of consistent contamination, the highest being in product C (99%) followed by product A (77%) and product B (46%)^{17,18}. The enumeration was performed using a most probable number (MPN) method with a lower limit of detection of 0.03 MPN/g, as well as a subset being evaluated by direct colony count. The data obtained with the product C was utilized to assess the dose response relationship as the *Lm* numbers in the enumerated samples were assumed to be representative of the numbers present at the time of exposure, especially given the consistency of the level of contamination observed^{17,19}. The analysis revealed that although the product was distributed widely

Table 2. Ice cream samples, produced in one production line, enumerated to determine *Listeria monocytogenes* levels of contamination.

Product Type	Samples analyzed	Production dates	Percent contamination	Median contamination levels (MPN/g)	Reference
A	344	May 2014-January 2015	77	0.07–3.8	Burall et al. ¹⁸⁾
B	95	December 2014-January 2015	46	0.02–0.64	Burall et al. ¹⁸⁾
C*	2320	November 2014-March 2015	99	0.15–7.1	Chen et al. ¹⁷⁾

*Product was linked to the cases in Kansas City, USA

and consumed by a broad cross section of the population in the USA, the absence of other reported listeriosis cases confirmed the previous assessments that the probability of developing listeriosis by the vast majority of the population from ingestion of low numbers of *Lm* is very small. However, the study indicated that the infectious dose, the minimum number of viable *Lm* to cause disease, could be very low for certain immunocompromised people, requiring additional risk assessment considerations to avoid such incidents.

Recent Listeriosis Outbreaks Related to Produce

Although the first major listeriosis outbreak in North America, was associated with cabbage²⁰⁾ in 1980, in the following years, dairy and meat products became the main food vehicles responsible for most listeriosis outbreaks reported in the USA and elsewhere. Between 1998 and 2008, 24 listeriosis outbreaks were reported, and ready-to-eat meat products caused most of the outbreaks¹⁵⁾. Serotype 4b was the single most prevalent serotype. However, among more recent outbreaks (**Table 3**), ready-to-eat meats have played a less prominent role as vehicles of infection, while dairy products have remained a prominent cause; importantly, produce-related outbreaks have been reported with increasing frequency in recent years. These outbreaks also indicate that other serotypes, e.g. 1/2a, 1/2b and a few recent cases of 4bV, also contribute to these outbreaks. It is interesting to note that with the implementation of PFGE- and WGS-based typing systems, we are discovering more outbreaks and discovering them earlier, resulting in fewer cases than the outbreaks before the PulseNet era. Recently, Burall et al.²¹⁾ described a series of outbreaks involving caramel apple, cheese, stone fruit and bagged salad and showed that all these outbreaks/incidents were caused by serotype 4bV, though the caramel apple outbreak also involved cases caused by serotype 4b strains. The most interesting is the caramel apple outbreak²²⁾, which caused 35 illnesses and seven deaths encompassing 12 states (**Table 3**). The outbreak was caused by two different related serotypes, 4b and 4bV, a feature

also observed during 2011 cantaloupe outbreak where 1/2a and 1/2b were involved²³⁾. The caramel apple outbreak also included three cases, two caused by 4bV strains, between 3–15 years of age without any recognized causes of underlying immune compromise and one male between 18–25 years of age with no disclosed pre-disposing conditions. This was very unusual given the demographics of listeriosis cases are comprised mostly of elderly and immunocompromised patients¹⁵⁾. A series of WGS analysis of the 4bV isolates from these outbreaks by JSpecies, Center for Food Safety and Nutrition (CFSAN) WGS Pipeline and Basic Local Alignment Search Tool (BLAST) analysis also revealed that these isolates belonged to a distinct clade and were genetically well separated from 4b and 4bV strains reported from other outbreaks, including 4b isolates recovered from the caramel apple-associated outbreak²¹⁾. All three different analytical tools resulted in a very similar conclusion although the Single Nucleotide Polymorphism (SNP) analyses provided much more resolution in the analysis. Another significant finding from that work²¹⁾ was the observation with Multi Virulence Loci Sequence Typing (MVLST) analysis, utilizing a limited set of defined virulence gene sequences, that selective pressures on a subset of genes may have played a significant role in our ability to understand the strain-to-strain variability and thus the evolution of newer traits. Furthermore, the analysis reported by Burall et al.²¹⁾ clearly showed that these highly related 4bV strains may have originated from a small geographical region in the USA. Whether this was due to shared agricultural practices, supplies, and/or equipment leading to cross-contamination or due to the natural presence of this strain throughout this geographical region is not clear at this point. It is, however, very clear that these 4bV strains, because of their involvement in these recent outbreaks, may represent an emergent clade and must be monitored carefully and studied in-depth to understand their role in listeriosis disease burden.

Conclusions

Although improved food production practices and educa-

Table 3. Multistate Outbreaks in the USA since 2011.

Year	Food	Cases	States	Hospitalization	Death
2011	Cantaloupe	147	28	143	33
2012	Cheese	22	14	20	4
2013	Cheese	6	5	6	1
2014	Cheese	8	2	7	1
2014	Cheese	5	4	4	1
2014	Bean sprouts	5	2	5	2
2014	Caramel apple	35	12	34	7
2015	Ice cream*	10	4	10	3
2015	Soft cheese	30	10	28	3
2016	Packaged salad	19	9	19	1
2016	Raw milk	2	2	2	1
2016	Frozen vegetables	9	4	9	3
2017	Soft raw milk cheese	8	4	8	2

* Number includes cases from a retrospective study

tion campaigns are likely to have reduced the incidence of listeriosis in the USA in recent decades, recent outbreaks have provided important insights into this pathogen. Increasing outbreaks/cases related to fresh produce and continuing dairy-related cases indicate much work remains to bring about further reductions in incidence. The 2015 ice cream outbreak showed that a non-growth ready-to-eat food with very low numbers of *Lm* can pose a substantial threat to subsets of immunocompromised persons, reinforcing the concept that the infectious dose for *Lm* should be considered as a function of the population exposed rather than a single number for all²⁴). This emphasizes the value of enumerating outbreak-related samples as they provide valuable information regarding infectious doses in different exposed populations. Better *in vivo* models, e.g. *Galleria mellonella*²⁵), together with improved understanding of role of food matrices in the infection process may further help to prevent human listeriosis. The ice cream outbreak also highlighted that *Lm* in the ready-to-eat food processing environment must be strictly monitored, and all efforts should be taken to eliminate the burden of *Lm* in production environments.

The recent outbreaks involving a clade of 4bV strains indicate that this genotype may be an emerging clade of *Lm*. Although we know this clade is genetically different from the other 4b and 4bV strains associated with different listeriosis outbreak, their biological and physiological significance in terms of disease-causing potential and environmental sur-

vival are not clear. Further research is needed to understand these properties. It is, however, clear that continuous monitoring of outbreak isolates and analysis by WGS is needed to identify the emergence of new clones or clades of *Lm* that may present increased risks to public health.

Conflict of interest

The authors have no conflict of interest.

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