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# Occurrence and characterization of *Listeria* spp. in ready-to-eat retail foods from Vancouver, British Columbia

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The occurrence of *Listeria* spp. and *Listeria monocytogenes* in retail RTE meat and fish products in Vancouver, British Columbia (B.C.) was investigated. To assess potential consumer health risk, recovered *L. monocytogenes* isolates were subjected to genotypic and phenotypic characterization. Conventional methods were used to recover *Listeria* spp. from deli meat ($n = 40$) and fish ($n = 40$) samples collected from 17 stores. *Listeria* spp. were recovered only from fish samples (20%); 5% harboured *Listeria innocua*, 5% had *L. monocytogenes* and 10% contained *Listeria welshimeri*. *L. monocytogenes* isolates serotyped as 1/2a and 1/2b, possessed dissimilar PFGE patterns, and had full-length InlA. Three 1/2a clonal isolates encoded the 50 kb genomic island, LGI1. Antimicrobial resistance (AMR) profiling showed all *Listeria* spp. possessed resistance to cefoxitin and nalidixic acid. *L. monocytogenes* were resistant to clindamycin, two were resistant to streptomycin, and one to amikacin. Reduced susceptibility to ciprofloxacin was seen in all *L. monocytogenes*, *L. innocua* and three *L. welshimeri* isolates. Reduced susceptibility to amikacin and chloramphenicol was also observed in one *L. monocytogenes* and three *L. welshimeri* isolates, respectively. Recovery of *L. monocytogenes* in fish samples possessing AMR, full-length InlA, LGI1, and serotypes frequently associated with listeriosis suggest B.C. consumers are exposed to high-risk strains.

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## 1. Introduction

*Listeria monocytogenes* is an organism commonly associated with food-processing environments and ready-to-eat (RTE) foods. Although an infrequent cause of foodborne disease, it is linked to disproportionately high levels of morbidity and mortality (Clark et al., 2010; Weatherill, 2009). Its presence in RTE products is particularly troublesome for vulnerable populations. This group of people, including pregnant women and their foetuses, the really young and the elderly, is particularly susceptible to invasive listeriosis, with mortality rates ranging between 20 and 40% (Clark et al., 2010; Public Health Agency of Canada, 2010). Evidence suggests the risk to vulnerable populations may be even higher if virulent strains of *L. monocytogenes* in RTE foods are encountered (Chen et al., 2006; Gilmour et al., 2010).

In Canada, eight listeriosis outbreaks have been reported over the years and have been linked to a variety of RTE foods (Clark et al., 2010; Health Canada, 2011). The most notable, however, was the 2008 nationwide outbreak associated with contaminated deli meats that originated from a single food-processing facility (Public Health Agency of Canada, 2010), and resulted in 57 invasive listeriosis cases and 23 deaths (Weatherill, 2009). The originating source of contamination was suspected to be a large commercial slicer harbouring *L. monocytogenes* (Weatherill, 2009). The facility's environmental sampling records showed the intermittent presence of *L. monocytogenes* on two processing lines for almost a year prior to the outbreak. Similar scenarios have been reported in other listeriosis outbreaks where *L. monocytogenes* in the processing environment led to contamination of RTE products (CDC 2002; Mead et al., 2006; Olsen et al., 2005). It is well established that food product contamination is associated with food-processing environments harbouring *L. monocytogenes* and subsequent post-processing transfer to finished products (Lappi et al., 2004; Lundén et al., 2002; Olsen et al., 2005; Tompkin, 2002). Numerous studies have focused on the prevalence of *Listeria* spp. in production environments and contamination patterns in these facilities (Barros et al., 2007; Chasseignaux et al., 2002, 2001; Eklund et al., 1995; Norton et al., 2001). Strains of *L. monocytogenes* capable of persisting in food-processing environments for up to 12 years and intermittently contaminating products have been reported (Holah et al., 2004; Lundén et al., 2002; Olsen et al., 2005; Senczek et al., 2000). Retail establishments of RTE foods, however, have received

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less attention and consequently fewer data examining prevalence are available.

Canadian data on the presence of *L. monocytogenes* in retail RTE foods have varied across studies (Bohaychuk et al., 2006; Dillon et al., 1994; Farber, 1991, 2000). As a result, contradicting messages have been conveyed regarding the safety of Canadian RTE foods. In 1991, Farber (Farber, 1991) reported results of a limited sampling survey of wholesale and retail seafood products originating from Canada and other countries. Based on the low recovery of *L. monocytogenes* in shrimp and smoked salmon, they concluded the observed levels did not represent a serious health hazard. In 1994, however, a study examining *Listeria* spp. contamination of retail RTE fish in Newfoundland found 18.3% (11/60) of cod samples were contaminated with *L. monocytogenes* (Dillon et al., 1994). In contrast, a report on government seafood testing in 2000 revealed *L. monocytogenes* contamination in 0.3–0.88% of imported products and its absence in domestic products (Farber, 2000). Similarly, a low prevalence of *L. monocytogenes* in raw and RTE meats from retail was observed in Alberta (Bohaychuk et al., 2006).

Looking at other Canadian provinces, and particularly B.C., limited data exist on the occurrence of *Listeria* spp. in RTE products and the associated risks of listeriosis linked to consumption of contaminated RTE foods. A recent survey of food-processing facilities reported inadequate sanitation and food safety practices in a number of B.C.'s fish processing facilities, which lead to *L. monocytogenes*-contamination of a variety of RTE fish products across the province (Kovačević et al., 2012). The survey, however, did not look at the contamination levels at retail. From this study, a number of concerns were raised about the nature of RTE fish products sold in bulk, their handling at retail and high potential for cross-contamination, and the subsequent difficulties in tracing and recalls of such products contaminated with *L. monocytogenes* (Kovačević et al., 2012).

Regardless of the size, retail establishments that sell foods within B.C. are not required to test products or food handling areas for *Listeria* spp. However, these establishments are inspected at least once per year and their foods tested as part of the provincial Food Quality Check Sampling Program (BCCDC, 2010). This program is primarily educational, with bacteriological tests (e.g. indicator organisms) used as sanitation checks to inform inspectors and producers of the effectiveness of current food handling practices, but does not include testing for *Listeria* spp. or other foodborne pathogens. Foods produced within B.C. for retail outside of province are inspected by the federally regulating Canadian Food Inspection Agency (CFIA), and are subject to more intensive microbiological monitoring programs, including testing for *Listeria* spp. (CFIA, 2011).

Generally, food testing for *Listeria* spp. occurs at the food-processing level, providing little information on the microbial quality and safety of food at retail. This is a concern because *L. monocytogenes* populations can increase during shipping and prolonged storage, particularly if RTE foods are stored at temperatures above 4 °C (Farber et al., 2000; Glass and Doyle, 1989). Additional handling of RTE foods at the retail, such as slicing, weighing, and packaging, may increase the potential for cross-contamination (Lin et al., 2006). The current Canadian policy on *Listeria* allows 100 CFU/g of *L. monocytogenes* in RTE foods in which proliferation of the organism to levels above this before the end of the product's shelf-life is not possible. However, extensive microbial challenge of retail products is required to determine listerial growth potential. Failure to control the proliferation of *L. monocytogenes* using extrinsic and intrinsic mitigation strategies may allow for unacceptable levels of the organism in products, particularly towards the end of the shelf-life.

Considering the lack of data on the occurrence of *Listeria* spp. and *L. monocytogenes* in RTE foods at the retail level in B.C., and the inconsistent reports on consumer health risks associated with *L. monocytogenes*-contamination of RTE foods, the goals of this study were two-fold: (1) test RTE meat and fish products from retail establishments for the presence of *Listeria* spp.; and, (2) investigate possible health risks associated with recovered *L. monocytogenes* through genotypic and phenotypic characterization.

## 2. Materials and methods

### 2.1. Sample collection

Ready-to-eat meat and fish products were purchased from seven large chain retail establishments and 10 smaller retailers in the Metro Vancouver area (B.C., Canada) in September and October 2010. Overall, 80 samples were collected: 40 deli meats and 40 RTE fish products. Meat samples included: beer sausage, bologna, cervelat and genoa salami, cheese loaf, chicken and turkey breast, cooked ham, corned beef, meat macaroni loaf, mortadella, variety pack sausages, and different types of pepperoni (e.g. beef, chicken, turkey). Fish samples consisted of different flavoured, candied and/or smoked fish jerky, nuggets, and pepperoni samples, as well as lox, sockeye sticks, smoked steelhead trout, and tuna. Samples (approximately 50 g) were purchased as sliced/weighed deli products or in manufacture-sealed packaging. Samples were transported to the laboratory in coolers on the day of purchase, and tested prior to best before/expiry date.

### 2.2. Isolation of *Listeria* spp.

Samples were analyzed according to Health Canada's MFLP-74 enumeration (Pagotto et al., 2002) and MFHPB-30 two-step enrichment (Pagotto et al., 2001) methods. Confirmation of *Listeria* spp. was based on Gram stain, catalase and oxidase reactions, and motility at room temperature. Isolates were speciated by standard biotyping (Microgen *Listeria* ID, Microgen Bioproducts Ltd., Camberley, Surrey, U.K. and API *Listeria*, BioMerieux, Marcy l'Etoile, France).

### 2.3. Serotyping and genetic fingerprinting

Isolates were serotyped by slide agglutination and antisera prepared according to Seeliger and Höhne (1979) at the Canadian National Microbiology Laboratory. Genetic fingerprinting based on pulsed-field gel electrophoresis (PFGE) was performed according to PulseNet standardized protocol at the Canadian Listeriosis Reference Service Laboratory using restriction enzymes *Asc*I and *Apa*I (Gilmour et al., 2010). PFGE patterns were assigned after comparison to the PulseNet Canada database.

### 2.4. Screening for *Listeria* genomic island and internalin A profiling

Conventional polymerase chain reactions (PCR) were used to screen for the presence of the 50 kb *Listeria* genomic island (LGI1) and amplification of the 2.4 kb *inlA* gene. Briefly, LGI1 screening was performed using two sets of primers (Table 1). DNA was isolated from overnight cultures grown on Tryptic Soy Agar (Difco, Becton Dickinson Diagnostics, Mississauga, ON, Canada). A single colony was resuspended in 100 µl of 1 × Tris–EDTA buffer, heated at 90 °C for 10 min, cooled on ice for 2 min, and centrifuged (16,000 × *g* for 5 min). PCR reactions (25 µL) using 5 U of AmpliTaq Gold 360 DNA polymerase (Applied Biosystems, Life Technologies, Carlsbad, California, USA), 0.4 µM of respective primers, 200 µM dNTPs (Invitrogen Canada Inc., Burlington, ON), and template DNA (1 µL) were cycled as follows: 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 20 s; followed by 72 °C for 5 min.

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