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Prevalence and persistence of *Listeria monocytogenes* in premises and products of small food business operators in Northern Ireland

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ABSTRACT

Listeriosis is a foodborne disease, with a high mortality rate, that predominantly affects the elderly. Under European Union legislation, EC 2073/2005, food business operators are encouraged to undertake sampling to ensure that the food processing environment, and required to ensure that food products, are free of *Listeria monocytogenes*. To determine the prevalence of *L. monocytogenes* in smaller food processing facilities in Northern Ireland, 24 companies submitted six processing environment swabs and two food samples every two months for eighteen months (July 2015 to November 2016) for *L. monocytogenes* examination. The prevalence of *L. monocytogenes* was 4.6% in food samples, and 6.3% in processing environment swabs. Over the duration of the study, 96 isolates of *L. monocytogenes* were obtained, one from each positive sample, except for two meat samples that had >100 cfu/g, where two isolates were obtained from each sample. No seasonality in occurrence of *L. monocytogenes* was seen for food isolates but significantly higher numbers of positive processing environment swabs were found in the warmer months of May, July and September ($p = .007$). Pulsed Field Gel Electrophoresis (PFGE) analysis revealed the presence of 27 pulsotypes; 9 pulsotypes were shared between different facilities and 9 were persistent. Based on a Combase predictive growth model, 77.5% ($n = 130$) of the foods tested were predicted to support the growth of *L. monocytogenes*. All of the isolates carried the pathogenicity genes *inl*A and *act*A and 71.4% carried *qac*H, which confers resistance to quaternary ammonium compounds which are frequently used in sanitizers. Whole genome sequencing of the isolates allowed multilocus sequence typing to be undertaken. The data indicated that the sequence types identified included those with disease-causing ability, highlighting the disease-causing potential of the isolates.

1. Introduction

Clinical invasive infection by *Listeria monocytogenes*, listeriosis, is rare in healthy humans. However, there are subsections of the population that are vulnerable to invasive infection, including the immuno-compromised, the elderly and pregnant women (CDC, 2017). From a public health perspective, finding ways to reduce exposure of vulnerable consumers to *L. monocytogenes* in ready-to-eat (RTE) foods is important. Despite this, the number of cases of listeriosis reported in the EU has increased. In 2015, the number of confirmed human cases of listeriosis reported in the European Union was 2206 (0.46 cases per 100,000 population), which was similar to 2014. In 2015, nineteen member states reported 270 deaths due to listeriosis, which was the highest annual number of deaths reported since 2008 (EFSA & ECDC, 2016). In a systematic review of the literature, De Noordhout et al. (2014) estimated the case fatality rate was 23.5%. The susceptibility of older people is of special concern in the UK due to its ageing population (Harper, 2016).

Since *L. monocytogenes* is a ubiquitous environmental bacterium (Farber & Peterkin, 1991; Hellberg & Chu, 2016; Montero et al., 2015), food processing environments are at a continuous risk of colonisation by *L. monocytogenes*. For many RTE products the main,

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but not sole contamination mechanism, is by transfer of *L. monocytogenes* strains from raw materials into niches in the plant environment and subsequent transfer from these niches into final products (Tompkin, 2002). Such cross-contamination has been shown in many studies (Bolocan et al., 2015; Leong et al., 2017; Muhterem-Uyar et al., 2015; Stessl et al., 2014), although cross-contamination from the food to the processing environment cannot be ruled out. Effective cleaning (the removal of soil) followed by sanitising (the destruction of microorganisms), or the application of heat can remove *L. monocytogenes* from processing environments (Murugesan, Kucerova, Knabel, & Laborde, 2015; Zottola & Sasahara, 1994). However, any failings in these procedures can result in contamination of products that have been rendered *Listeria*-free by the critical control point of cooking (Currie et al., 2015; Leong et al., 2017; Swaminathan & Gerner-Smidt, 2007). Accordingly, the European Union has legislated to ensure the safety of RTE products that support the growth of *L. monocytogenes* (EU, 2005). For foods supporting growth of *L. monocytogenes* (apart from foods for infants or special dietary purposes, where complete absence is required), absence is required when the product leaves the manufacturer, unless the manufacturer can demonstrate that the numbers will be <100 cfu/g at the end of the shelf life. Foods not supporting growth must have <100 cfu/g at the end of the shelf life.

Since RTE food processing environments are recognised as a significant source of *L. monocytogenes* contamination (Beno et al., 2016; Tompkin, 2002), it is important for food business operators (FBOs) to have an appropriate surveillance programme to monitor and control the risk of *L. monocytogenes* contamination of the final product. Environmental monitoring programmes are recommended in the EU (EU, 2005) and required in some food sectors in the United States of America (FDA, 2011) and contribute to the identification and tracking of *L. monocytogenes* along the food chain, and within food processing facilities. Such programmes can have an impact on avoiding cross-contamination to food (Ho, Lappi, & Wiedman, 2007). Applying genetic fingerprinting, such as pulsed field gel electrophoresis (PFGE) or whole genome sequencing (WGS) can assist studies on isolate characterisation and tracking (Dalmasso & Jordan, 2015; Schmid et al., 2014; Stasiewicz, Oliver, Wiedmann, & den Bakker, 2015), especially with regard to determining persistence of *L. monocytogenes* in food processing facilities.

Whilst large scale food processors will be aware of strategies and interventions to exclude *L. monocytogenes,* small to medium sized enterprises (SMEs) may require assistance. These are defined in European Union document 2003/361/EC as, inter alia, having fewer than 250 employees, but in the current project most of the SMEs involved had <20 employees. In the Republic of Ireland (RoI), a research project on assessment of *L. monocytogenes* was considered to have contributed to a reduction of *L. monocytogenes* in food and food processing environments, leading to a decreased risk to public health (Leong et al., 2017).

The aim of this study was to assess the occurrence and persistence of *L. monocytogenes* in 24 RTE food processing facilities in Northern Ireland over an eighteen-month period (July 2015 to November 2016). Regular monitoring of the processing environments and products of the RTE food manufacturers, with molecular characterisation of the *L. monocytogenes* strains isolated was undertaken.

2. Materials and methods

2.1. L. monocytogenes monitoring program

Staff of the College of Food, Agriculture & Rural Enterprise (CAFRE), Cookstown, led the recruitment of FBOs to be involved in the programme. In total, 24 companies participated in this study with staff attending a half day workshop for training in sampling procedures. All participating FBOs provided sketch plans of their premises and marked the environmental sites to be sampled during the programme. At the workshop the FBOs received detailed instructions which included information on how to take swab samples, which areas to sample, and on the packaging and shipment of the samples to the laboratory. This was designed to ensure consistent sampling by all participants. In addition a video of the appropriate sampling procedures was made and placed on YouTube for subsequent access by participants to ensure uniform sampling. For swab samples, all FBOs were asked to take samples from three specific areas: a drain in the main processing hall, an area of floor (1 m^2) and a storage shelf. Because of the variation in layout and type of the facilities, the area to swab for the remaining samples was to be chosen by the FBO from anywhere in the food processing environment, and the location noted. Cutting areas, walls, other drains and pooled water were suggested as optimum locations. For food samples, FBOs were instructed to send two food samples which were at the stage of being ready to be sent from the processing facility. All sampling took place during normal production conditions. Management practices were assessed by means of a detailed questionnaire submitted to all participants.

From July 2015 to November 2016, a total of 24 food processing facilities from seven food sectors (cooked meat, horticultural products, sandwich, baked goods, salads, seafood and dairy [Table 1]) were analysed bimonthly for the presence of *L. monocytogenes*. All of these food processing facilities produced RTE food products, and were distributed throughout Northern Ireland (NI). Sampling packs, which consisted of a polystyrene box (DS Smith, UK) containing six pre-moistened 3M sponge-stick swabs (Technopath, Ireland), a sterile liquid container (VWR, Ireland), two sterile bags (VWR, Ireland), two cable ties, and two ice packs, were sent to all participating food processing facilities two weeks prior to the assigned sampling date.

2.2. Microbiological analyses

All microbiological media were supplied by Oxoid, (Basingstoke,

Table 1
Occurrence of *L. monocytogenes* by category of food product produced by the food business.

Food category	Total samples	*L. monocytogenes* positive	% Samples Positive
Cooked meat	225	27	12.0
Horticultural products[a]	398	26	9.1
Sandwich	286	24	8.4
Baked goods	128	10	7.8
Salads	136	5	3.7
Seafood	144	2	1.4
Dairy	281	0	0.0
Total	1598	94	5.8

[a] Includes mushrooms, vegetables and fruits.