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 multi-locus 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.

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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.

UK), unless otherwise stated. Sampling kits were dispatched to FBOs two weeks before the target sampling date, and samples were sent from FBOs by courier on the day of sampling, to arrive at AFBI the next morning, where they were analysed immediately (less than 24 h after sampling). The methods used were detection of *Listeria* spp. (including *L. monocytogenes*), BS EN ISO 11290−1:1996/ Amd 1:2004, and enumeration of *Listeria* spp. (including *L. monocytogenes*) as described in BS EN ISO 11290−2:1998/Amd 1: 2004, except that only agar *Listeria* according to Ottavani and Agosti (ALOA) was used. Briefly, for food samples, for the enumeration of *Listeria* spp. 25 g of sample were added to 225 ml of Fraser broth base (CM0895 without selective supplements), blended for 2 min (Colworth 400, Seward Limited, Worthing, UK) then allowed to stand for 1 h. The samples were then plated (0.1 ml) onto plates of ALOA agar and incubated (37 °C for 48 h), with examination after 24 h and 48 h. Plates with less than 150 typical colonies were counted. Where plates yielded presumptive *L. monocytogenes,* five colonies were purified and confirmed, using API *Listeria* (bio-Mérieux UK Limited, Basingstoke, RG22 6HY), and the final count obtained by multiplying the presumptive count by the percentage of confirmed *L. monocytogenes* colonies. For swabs, 90 ml of Fraser broth base with half strength supplements was added to the swab in the bag in which it was transported to the laboratory, followed by incubation and sub-culture as described above.

2.3. Pulsed field gel electrophoresis

The PFGE analysis was carried out using the International Standard PulseNet protocol (PulseNetUSA, 2013). *Listeria mono-cytogenes* isolates were grown overnight in Brain Hearth Infusion (BHI) agar at 37 °C, subsequently a suspension in 10 mM Tris:1 mM EDTA buffer, pH 8.0 (TE) was prepared with an approximate OD_{610} of 1.0. To 400 μL of cell suspension was added 20 mg/ml of Lyso-zyme which was incubated at 55 °C for 20 min. After incubation, 20 μl of Proteinase K stock (20 mg/ml) was added, followed by 400 μl of SeaKem Gold agarose 1%. Plugs were prepared by dispensing the mix into plug moulds. Solid plugs were then lysed for 2 h in 5 ml of cell lysis buffer (50 mM Tris, 50mMEDTA, pH8.0 + 1% Sarcosyl) supplemented with 25 μl of 20 mg/ml pro-teinase K solution. The plugs were then washed twice in distilled water and four times in TE buffer.

DNA was digested with 10 U/μl of the restriction enzyme *AscI* FastDigest (Fisher Scientific, Ireland) and 50 U/μl of the restriction enzyme *ApaI* FastDigest (Fisher Scientific, Ireland); the restricted DNA was run in a 1% SeaKem Gold agarose gel for 21 h as described in the PulseNet protocol, on a CHEF-DR III (Bio-Rad). After staining with 1 μg/ml ethidium bromide solution, the gels were observed with the Alpha Imager (Alpha Innotech, DE). Analysis of the gels was performed with BioNumerics v7.0 software (Applied Maths) using and UPGMA (unweighted pair group method with averages) and the Pearson coefficient with 1% tolerance.

2.4. Whole genome sequencing

DNA was extracted from all isolates using a PureLink Genomic DNA Kit (Thermofisher Scientific, Paisley, UK) as per the manufac-turer's instructions. The whole genome sequencing of all *L. monocytogenes* isolates was performed as previously described (Ugarte-Ruiz et al., 2015) using Illumina MiSeq 2 × 250 bp paired-end sequencing. To analyse the data quality, FastQC was used (Andrews, 2016). To trim and crop the sequencing reads, Trimmo-matic was used with the following parameters: (v0.32) 'leading' and 'trailing' setting of 3, a 'slidingwindow' setting of 4:20 and a 'minlength' of 36 nucleotides (Bolger, Lohse, & Usadel, 2014). BWA-MEM (v0.7.7-r441) was used to map the reads using the genome

sequence of *L. monocytogenes* EGD (HG421741) as described by Li & Durbin (2010) . VelvetOptimiser (v2.2.5) using n50 optimization was used to perform assembly (Gladman & Seeman, 2012; Zerbino & Birney, 2008). The reference strain *L. monocytogenes* EGD (HG421741) was used to complete contigs using ABACAS (v1.3.1) (Assefa, Keane, Otto, Newbold, & Berriman, 2009). Multi locus sequence type (MLST) was determined using PubMLST (https://github.com/tseemann/mlst). Genome annotation was provided by using Prokka (Seemann, 2014). To read the genomes, and assess them for presence of internalin A, *act*A, *Listeria* pathogenicity islands 3 and 4, the stress survival islet SSI-1, *bcr*ABC and *qac*H, Artemis and ACT software were used (Carver, Harris, Berriman, Parkhill, & McQuillan, 2012).

2.5. Measurement of pH and a_w

A subset of food samples (n = 130) was analysed to determine the pH, using the methodology of BS 4401-9-1975, ISO 2917-1974, and water activity (a_w) determined according to British Standard method BS ISI 21807:2004, using a Rotronic HygroLab 3 (Rotronic Instruments [UK] Ltd, Crawley, RH10 9EE, UK). These represented two samples of each of the products submitted by FBOs.

2.6. Statistical analyses

All statistical analyses were undertaken by the Biometrics & Information Systems Branch, AFBI, using Genstat Release 18.1 for Windows (VSN International Ltd, Hemel Hempstead, HP2 4TP, UK). One-way analysis of variance was used to study temporal differ-ences with $P < 0.05$ indicating significance.

3. Results

3.1. Occurrence of L. monocytogenes

Overall, the 24 FBOs submitted 1598 samples for analysis. These consisted of 1203 swabs and 395 food samples. Seventy-six swabs (6.3%) and eighteen food samples (4.6%) yielded *L. monocytogenes*, with 5.9% of samples being positive overall. All the food samples had <100 cfu/g, except for two samples of cooked meat, one that had >2000 cfu/g and one that had 140 cfu/g. *L. monocytogenes* were not isolated from twelve of the premises tested; four produced dairy products and the eight others a variety of products, including hot smoked salmon, pasta sauces, pâtés and ready meals, baked goods, cooked chicken, and salad and vegetable based products. The overall prevalence of *L. monocytogenes* at the different facilities is shown in Table 1, while the type of positive sample, is shown in Table 2. Overall, 96 *L. monocytogenes* isolates were obtained during an eighteen-month sampling schedule, one from each positive sample, except for two meat samples that had >100 cfu/g, where two isolates were obtained from each sample. Study of the number of positive samples with time (data not shown) suggested that there were differences in the prevalence of *L. monocytogenes* be-tween the seasons of summer and winter (summer = May, July, September; winter = November, January, March). No statistically significant difference was found between the numbers of positive food samples found in summer and winter however, for processing environment swabs, significantly more positive samples were found during the summer months, p =0.007.

Of the positive samples, 47.8% were in drains and on floors, while 19.1% were from food samples. The remainder were from other processing environment sites, for example trolleys, tables and walls.

Table 2

Food premises yielding *Listeria monocytogenes* from the processing environment and/or products. To maintain anonymity, the premises listed have been designated in terms of the type of principal products.

Products from food premises	Number of isolates from each food premises	
	Processing environment swabs	Food product
Cooked Meats A[a]: pulled chicken, turkey and beef	17	2
Cooked Meats B: pork from fresh and cured meat	5	5[b]
Sandwiches A: sandwiches, rolls and wraps	10	2
Sandwiches B: sandwiches, salads, wraps and snacks	7	0
Sandwiches C: sandwiches, pasta and salad bowls, coleslaw, potato salad	0	4
Sandwiches D: sandwiches	1	0
Processed mushrooms	12	7
Baked goods: cakes, pies, sausage rolls	10	0
Salads: green and pasta salads, chicken tuna and egg mixes	5	0
RTE raw fruit pieces in consumer packs	5	0
RTE processed fish/shellfish	2	0
RTE vegetable products	2	0

[a] Where more than one company manufactured a product type they have been designated successively, as A, B etc.
[b] Three food samples were positive and two of these samples yielded *L. monocytogenes* isolates from both enrichment cultures and enumeration plates.

3.2. Analysis of the isolates by pulsed field gel electrophoresis

The isolates were analysed by pulsed field gel electrophoresis (PFGE); two isolates that were not recoverable were not included in the PFGE analysis, leaving PFGE analysis on 94 isolates. A total of 27 distinguishable pulsotypes were identified, based on > 90% similarity. Fig. 1 shows a minimum spanning tree, representing the PFGE profiles of all the isolates. The different colors represent different food categories. Within a circle, each segment represents an isolate, while the circle represents a pulsotype where the isolates show >90% similarity. The length of the line between the circles represents the distance of the relationship between the pulsotypes/isolates. Pulsotypes with only one isolate are represented by a circle with no segments. Of the 27 pulsotypes, there were 10 with a single isolate and 8 with more than 4 isolates. Nine of the pulsotypes had isolates from more than one food sector, indicating possible cross contamination between food sectors (Table 3 gives more detail on pulsotypes shared across food sectors).

For each of the companies which were positive for

L. monocytogenes, Table 3 shows the number of pulsotypes, number of persistent pulsotypes and the number of pulsotypes shared between different food sectors (widespread pulsotype). A persistent pulsotype was defined as repeated identification of an isolate of the same pulsotype over a period longer than 6 months. Of the 27 pulsotypes identified, 21 of these were identified in 3 of the companies tested. This shows the diversity of the isolates obtained, but also shows a degree of similarity in the isolates from the different companies (Fig. 2).

Persistent pulsotypes were identified at 6 of the 12 facilities. Of the 27 pulsotypes, 9 were persistent. Five pulsotypes showed a cross-contamination scenario in 3 of the companies, where indistinguishable pulsotypes were found on food and in the processing environment.

The PFGE profiles obtained in this study were compared with a database of strain PFGE profiles obtained in a similar study in the RoI. There were no similarities at the level of >90% between the isolates from this study and isolates obtained previously.

Comparison between the pulsotypes obtained in this study and about 2500 PFGE profiles in a database of international isolates at
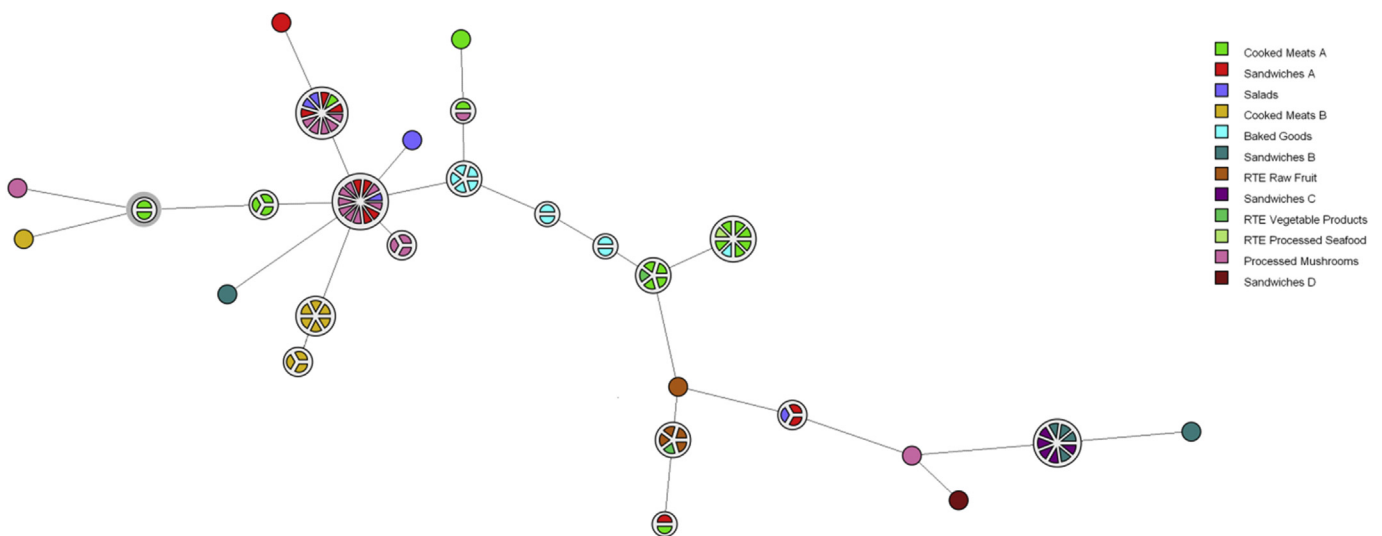

Fig. 1. Minimum spanning tree summarising the data from the PFGE profiles of the 94 isolates tested. The different colours represent different food companies. Within a circle, each segment represents an isolate, while the circle represents a pulsotype where the isolates show >90% similarity. The length of the line between the circles represents the distance of the relationship between the pulsotypes/isolates. Pulsotypes with only one isolate are represented by a circle with no segments. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Pulsotypes obtained from different food premises. To maintain anonymity premises listed have been designated in terms of their principal products.

Products of Food Premises	Number of pulsotypes	Number of persistent pulsotypes	Number of pulsotypes shared with other companies (persistent isolates shared)
Cooked Meats A[a]	10	2	4 (1)
Sandwiches A	5	3	5 (3)
Salads	4	0	4
Cooked Meats B	2	1	0
Baked goods	4	3	1
Sandwiches B	3	0	1
RTE raw fruit	2	0	1
Sandwiches C	1	1	1
RTE vegetable products	2	0	2
RTE processed fish/shellfish	1	0	1
Processed Mushrooms	6	1	4 (1)
Sandwiches D	1	0	1

[a] Where more than one company manufactured a product type they have been designated successively, as A, B etc.
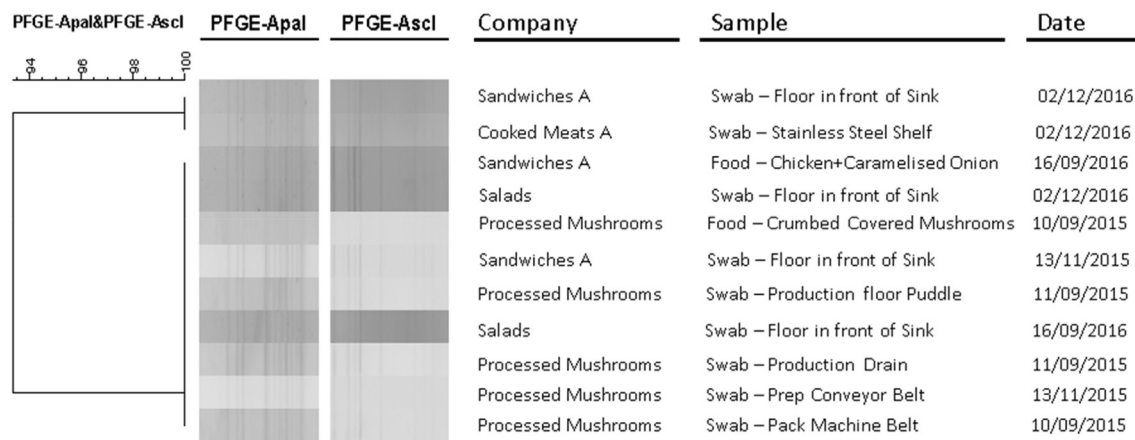
Fig. 2. PFGE profiles showing similarity between *L. monocytogenes* isolates obtained from different food premises.

Teagasc, Moorepark, Ireland, showed that of the 27 pulsotypes obtained in this study, 10 were comparable with international isolates. These included similarities with strains from Ireland, Austria, Romania, Czech Republic, Turkey and Australia (data not shown).

Seven of the pulsotypes from the current project were >90% similar to mushroom production chain isolates from a project in the RoI. Fig. 3 shows an example of one of these.

A comparison between the pulsotypes from this study and those of clinical isolates from the ROI was made. Seven of the pulsotypes identified in this project were similar to pulsotypes from clinical isolates at >90% (data not shown).

### 3.3. Potential for growth of L. monocytogenes on the food products

The pH and a_w data were used as the input into the Combase *L. monocytogenes* computer growth prediction model (http://browser.combase.cc) to obtain an estimate of the mean generation time at three storage temperatures: 4, 6, and 8 °C. Combase predicted that 19 of the 130 samples would not support the growth of *L. monocytogenes* due either to their pH being below 4.40 (14 samples), or their a_w being below 0.934 (5 samples). The pH and a_w for the remaining 111 samples which were predicted to support growth is shown in Supplementary Fig. 1. Over 80% of the 111 samples had a pH greater than 5.5 and over 86% had a a_w greater than 0.97. To assess, and compare, the potential of each foodstuff to support the growth of *L. monocytogenes,* an arbitrary growth parameter was chosen. This was the ability of the food to support ten generations of growth, equivalent to approximately 1000-fold

growth, in seven days or less. This corresponded to growth from 1 cell per 10 g of food to the limit of 100 cfu/g, and was intended to be a parameter comprehensible to the FBOs (data not shown). At 4 °C, growth predictions showed that none of the foods supported 10 generations of growth in one week. However, at 6 °C growth could exceed this target in 55.0% of the foods, and at 8 °C this rose to 77.5% of the foods.

3.4. Whole genome sequencing of the isolates

All of the *L. monocytogenes* isolates were subjected to WGS and 91 sequences were obtained; the quality of the DNA did not allow WGS to be completed for 5 strains. The genome sequences were then analysed for the presence of four virulence-associated genes: internalin A, *inl*A; actin assembly protein, *act*A; *Listeria* pathogenicity island 3, LIPI-3 and *Listeria* pathogenicity island 4, LIPI-4. Three genes indicative of stress tolerance were also studied; stress survival islet, SSI-1; a resistance cassette, which contributes to resistance to quaternary ammonium compounds, *bcr*ABC; and the quaternary ammonium compound-resistance gene, *qac*H (Fox, Allnutt, Bradbury, Fanning, & Chandry, 2016). All 91 isolates carried functional genes for *inl*A and *act*A and 65 (71.4%) carried *qac*H. None carried LIPI-3, LIPI-4, or *bcr*ABC.

These whole genome sequences were analysed to determine the MLST of the isolates, a feature frequently used for clinical characterisation of isolates. Twelve MLSTs were found (Supplementary Fig. 1), and in nine FBOs some MLSTs were isolated on more than one occasion (Table 4).
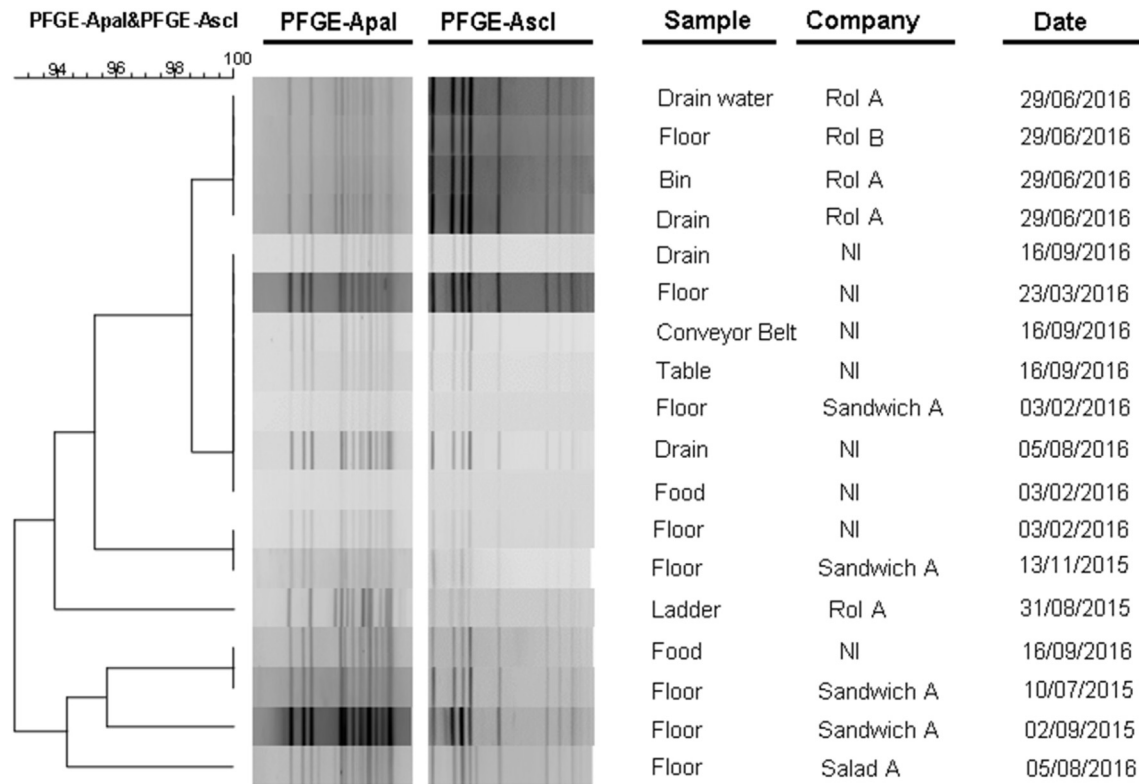
Fig. 3. PFGE profiles of *L. monocytogenes* from mushroom producers in the Republic of Ireland (RoI) (A and B) compared with food producers in Northern Ireland (NI): a mushroom producer (NI), sandwich maker (Sandwich A) and a salad producer (salad A). Dates are those on which isolates were stored. The scale shows % similarity.

Table 4
Sequence types (ST) isolated from individual premises on more than one occasion. To maintain anonymity, the premises listed have been designated in terms of the type of principal products.

Products from food premises	Total number of sequence types (ST)	Number of recurrent ST	Recurrent ST
Cooked Meats A[a]: pulled chicken, turkey and beef	8	4	ST204
Cooked Meats B: pork from fresh and cured meat	7	1	ST204
Sandwiches A: sandwiches, rolls and wraps	4	1	ST204
Sandwiches B: sandwiches, salads, wraps and snacks	4	1	ST6
Sandwiches C: sandwiches, pasta and salad bowls, coleslaw, potato salad	3	1	ST6
Processed mushrooms	8	2	ST8, ST204
Baked goods: cakes, pies, sausage rolls	6	2	ST6, ST20, ST121, ST21, ST121
Salads: green and pasta salads, chicken tuna and egg mixes	4	0	
RTE[b] raw fruit pieces in consumer packs	2	1	ST155

[a] Where more than one company manufactured a product type, they have been designated successively, as A, B etc.
[b] RTE- Ready to Eat.

4. Discussion

The results of this study show that 50% of the 24 food processing facilities where sampling took place were free of *L. monocytogenes* in the food and the food processing environment for the 18-month duration of the sampling. It is significant that *L. monocytogenes* was not detected in any of the 5 of the dairy processing companies where sampling took place (all the companies used pasteurised milk, with the main products being ice cream, yoghurt and cheese). Sandwich production companies and horticultural product production facilities were the food sectors where the highest number of facilities were positive — 4 of 4 and 3 of 4, respectively. In a similar study in the RoI, 10 of 54 facilities were completely free of *L. monocytogenes* for the 3-year duration of that study (Leong et al., 2017). Correlation of data on management practices at facilities that were positive and negative for *L. monocytogenes* did not identify any practices that would lead to an increase or decrease in occurrence of *L. monocytogenes* (data not shown). From a food safety perspective, a *L. monocytogenes*-free processing environment will have a reduced risk of *L. monocytogenes* contamination of the food produced.

All of the positive samples came from 12 facilities, 4 of 4 sandwich producing companies, 3 of 4 horticultural product production facilities, 2 of 3 meat companies, 1 of 2 baked goods companies and 1 of 1 seafood company. It should be noted that two samples of meat products sampled prior to distribution were found to exceed 100 cfu/g, and were therefore not fit for sale. In these cases, the FBOs were informed and appropriate action was taken. All other food samples (n = 393) had <100 cfu/g. In this study, sandwiches had significant *Listeria* contamination, as was shown by (Cossu et al., 2016), and they have been implicated in cases of listeriosis (Silk, McCoy, Iwamoto, & Griffin, 2014). This show the importance

of anti-listeria controls in sandwich producing facilities.

The overall occurrence in food and in the processing environment was 4.6% and 6.3%, respectively. Comparing the results with other published surveys is not applicable as, 1) different methodologies (for sampling and analysis) are frequently used, 2) in many surveys, one facility is surveyed on several occasions or several facilities are surveyed on one occasion, 3) some surveys are targeted at facilities that have been shown to be positive. In extensive surveys, using similar methodologies, Leong et al. (2017) found that 3.8% of 4667 processing environment samples and 4.2% of 1202 food samples were positive, while Beno et al. (2016) found 1.4% of 4430 processing environment samples positive. Muhterem-Uyar et al. (2015) reported a higher occurrence in meat processing facilities than in dairy processing facilities (32.0% and 8.8%, respectively), attributing the higher occurrence in meat processing facilities to cross-contamination from carcasses. In addition, not all the meat processing facilities produced ready-to-eat food.

The locations most commonly associated with *L. monocytogenes* contamination were areas such as floors and drains (Kells & Gilmour, 2004; Leong, Alvarez-Ordonez, & Jordan, 2014; Ruckerl et al., 2014; Schoder, Rossmanith, Glaser, & Wagner, 2012). This prevalence was not consistent over the 18-month period of the study as processing environment samples were more frequently positive during the warmer months of May to September (p = .007). This is in contrast to other studies such as that undertaken in the RoI (Leong et al., 2017), where no seasonality was found. Weather, such as warm or wet conditions, can affect the prevalence of *L. monocytogenes* (Hellberg & Chu, 2016). However, in this study, no obvious cause for the seasonality observed was determined. In contrast, the statistical analyses showed no seasonal effects with regard to prevalence in the food samples. Seasonality of contamination has been reported in dairy products (Meyer-Broseta, Diot, Bastian, Rivière, & Cerf, 2003), but no dairy products were positive in this study.

The ninety-four isolates genetically characterised by PFGE resulted in 27 distinguishable pulsotypes; 33% of these pulsotypes showed persistence at the processing facility. Nine of the pulsotypes were found in different food sectors. If more extensive sampling and analysis were undertaken, it is possible that other pulsotypes would be identified as persistent. The persistence of *L. monocytogenes* indicates either repeated contamination events or the resistance of *L. monocytogenes* to the hygiene procedures in the facility (for review see Carpentier & Cerf, 2011). Persistence of *L. monocytogenes* in the food processing environment presents a risk of cross contamination to the food being produced. In order to address such persistence, more stringent hygiene measures, or hygiene barriers on access to the facility (including for raw materials) are necessary. Such measures can reduce cross contamination to the final product and contribute to the prevention of public health issues (Awofisayo-Okuyelu et al., 2016; McCollum et al., 2013; Montero et al., 2015).

The results of the project show the diversity of the isolates obtained (Fig. 1), but also shows a degree of similarity in the isolates from the different companies (Fig. 2). For example, the PFGE profile of an isolate from the floor in front of a sink at one facility was >90% similar to a food isolate from the same facility and isolates from processing environment and food samples from other facilities (Fig. 2). Further studies analysing the WGS data in more detail may help to resolve this issue and determine how similar the isolates are.

The presence of the same *L. monocytogenes* pulsotype in different companies (widespread occurrence) shows the possibility of cross contamination between different production units and sometimes different food sectors. This can happen when services are shared, like transporters, raw materials, couriers, etc. In the current study, there were no known links between the companies where the same pulsotype was found in different facilities, except for one case where the same company operates two facilities. Widespread contamination was detected in the mushroom industry. In Ireland, the mushroom industry is a very 'cross border' industry, where companies have premises in both NI and RoI. This could explain the occurrence of seven common pulsotypes that were commonly found in both NI and the RoI. Alternatively, there are global clones of *L. monocytogenes* (Chenal-Francisque et al., 2013), indicating that it is possible for the same pulsotype to be found in different facilities that have no apparent epidemiological link.

Cross contamination was shown at four facilities, where *L. monocytogenes* isolates from processing environment swabs had the same pulsotype as food samples in the same facility. PFGE does not discriminate between the two possible scenarios, namely if the contamination went from the food to the environment or vice-versa. Further analyses would be required to differentiate these scenarios. Persistence of *L. monocytogenes* in the food processing environment presents a risk of cross contamination to the food being produced. Cross contamination from the processing environment has been previously reported (Ivanek, Grohn, Wiedmann, & Wells, 2004; Leong et al., 2017; McCollum et al., 2013) and has been implicated in outbreaks of listeriosis (Currie et al., 2015).

In a comparison between the pulsotypes identified in this study and those obtained in other countries, 10 common pulsotypes were identified. Again, this shows the existence of global clones of *L. monocytogenes*, not necessarily connected to epidemiological data (Chenal-Francisque et al., 2013). The relationship between these clones is unclear, and further studies would be required to clarify this. For example, a comparison of whole genome sequences can highlight the presence of single nucleotide polymorphisms (SNPs) between strains with indistinguishable PFGE profiles. This is especially important where PFGE similarities are shown in the comparison with clinical isolates, but in the absence of epidemiological data no link can be made between isolates from food and disease-causing isolates.

Since *L. monocytogenes* was present in half of the food processing facilities participating in this study, there is potential for cross-contamination to food products. Thus, it is relevant to determine if these foods support the growth of *L. monocytogenes*. Although not foolproof, predictive microbiology can give an indication of growth potential. Nineteen of the 130 food products tested (using Combase predictions based on pH and water activity) were predicted not to support the growth of *L. monocytogenes*; these were mainly low pH products such as yoghurts, cheese, fruit products and coleslaw, which all had a pH below 4.4. While the pH of cheese can increase post-production due to the actions of the endogenous microflora (Schoder, Skandamis, & Wagner, 2013), this was unlikely to occur with the cheese products studied, hence they would be not support growth during their normal shelf life.

The remaining 111 food samples, i.e. 85%, all had pH and a_w values which would support the growth of *L. monocytogenes* at 4 °C, 6 °C and 8 °C, according to the ComBase model. While 4 °C is the intended temperature of a domestic refrigerator, a UK-wide microbiological study found that the mean temperature of pre-packed meats on retail display was 6.8 °C, with 71.3% of samples above the industry guideline of 5 °C, and 32.7% being stored above 8 °C (Madden, 2014). Hence, the temperatures of 4 and 8 °C, and the intermediate temperature of 6 °C, were chosen for the growth studies. Growth in the foodstuffs over a seven-day period was determined using the arbitrary criterion of calculating the time for 10 generations of microbial growth (approximately a 1000-fold increase in numbers), using the generation time predicted by ComBase. This allowed a simple metric to be communicated to

FBOs. At 4 °C none of the 111 foodstuffs were predicted to support such growth. However, increasing the temperature to 6 °C resulted in 55% of the samples failing the test, while 77.5% failed at 8 °C. This shows the importance of temperature in controlling the growth of *L. monocytogenes*, and the importance of proper control over the temperature of domestic refrigerators. In order to help them comply with Commission Regulation (*EC*) No 2073/2005, this information was communicated to participants. Additionally, guidance for FBOs undertaking challenge studies to determine the ability of foods to support growth of *L. monocytogenes* in the food matrix has been published (Beaufort, Bergis, Lardeux, Polet, & Botteldoorn, 2014). Thus, for most of the food products analysed in this study, an initial contamination of one *L. monocytogenes* per 10 g could exceed the regulatory level of *L. monocytogenes* cited in Commission Regulation (EC) No 2073/2005 (100 cfu/g) within a week, if they were subjected to abuse temperatures found in retail premises.

The Gastrointestinal Bacteria Reference Unit, National Infection Service, Public Health England, supplied data on clinical MLSTs from the UK (April 2016−March 2017), where 10 MLSTs were found (STs 1, 5, 6, 8, 20, 21, 121, 155, 204 and 394). ST204 was the most common sequence type identified in this study, comprising 30 isolates. This MLST was isolated from a number of different food processing facilities, and was found for the duration of the project. All of the clinical-associated MLSTs were found in the isolates from this study, where they comprised 98% of the isolates. Furthermore, the National *Salmonella, Shigella & Listeria* Reference Laboratory in the RoI reported that eight of the nine most common MLSTs isolated during this study were also found as clinical isolates. However, based on a study of fifteen ST204 isolates, this MLST was reported as being mainly an environmental isolate (Fox et al., 2016). It should be noted that not all isolates with a given MLST are genetically identical. For example, 86% of the ST204 isolates carried the *qac*H gene, and were therefore different from the 14% which did not carry it.

Overall, most of the isolates for which WGS data was obtained (71%) carried the *qac*H gene, which confers resistance to quaternary ammonium salts, the basis of many sanitizers used in the food industry (Sidhu, Sørum, & Holck, 2004). The high rate of carriage of *qac*H by ST204 isolates found in this study contrasts with its absence in the 15 isolates described in the report of Fox et al. (2016), and may reflect selection for this property in food processing environments. Resistance to quaternary ammonium salts may also be a contributory factor to the high prevalence of this MLST found in this study.

Study of the WGS data showed that all of the isolates obtained carried the virulence genes *inl*A and *act*A (Fox et al., 2016). However, determining the true pathogenic abilities of the isolates was beyond the scope of this study; further work on the WGS data and on invasion assays would be required to further investigate pathogenic properties of the isolates (Chen et al., 2016).

Overall, the results obtained showed that most participating FBOs can produce RTE food free from *L. monocytogenes*, and many were able to keep their production facility free from this pathogen. Hence, most FBOs appear to have determined effective control strategies and practical interventions. However, WGS data suggests some FBOs have persistent strains, and that many of the isolates could be potential pathogens. Therefore, there is a need for FBOs to continually assess control strategies and practical interventions for the exclusion of *L. monocytogenes*.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; RM, MH, KJ, VP, OG, NC.

Drafting the work or revising it critically for important intellectual content; RM, MH, KJ, OG, NC.

Final approval of the version to be published; RM, MH, KJ, VP, OG, NC.

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. RM, MH, KJ, VP, OG, NC.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.foodcont.2017.12.020.

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