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Process environment sampling can help to reduce the occurrence of *Listeria monocytogenes* in food processing facilities

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The occurrence and persistence of *Listeria monocytogenes* strains in food processing environments pose a risk of cross-contamination to food. The control of these strains is thus essential to ensure food safety. In the present study, 205 samples were collected from a food processing facility between May 2012 to February 2013 and analysed for the presence of *L. monocytogenes* by the ISO11290 standard method. *L. monocytogenes* isolates were differentiated using pulsed field gel electrophoresis. Up to 55% of the samples were positive for *L. monocytogenes* until October 2012. Advice was given on the implementation of corrective actions regarding cleaning and disinfection procedures and workflows. This resulted in a decrease in the number of positive samples, reflecting the reduction of *L. monocytogenes* in the processing environment. Eight pulsotypes were found in the food processing facility environment, mainly on non-food contact surfaces. One type was identified as persistent as it was isolated on each sampling occasion and constituted more than 71% of the isolates collected. It was the only type found in the processing environment after the implementation of corrective actions. This work demonstrates that processing environment sampling plans are effective to assess hygiene and implement corrective actions. This contributes to prevention of contamination events and consequently to assuring the safety of the food product.

Keywords: environment sample; *Listeria monocytogenes*; persistence; PFGE; sampling plan

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Introduction

Listeria monocytogenes is an important human pathogenic bacterium and is the causative agent of listeriosis that can afflict the elderly, neonates and immunocompromised adults (Swaminathan and Gerner-Smidt 2007). Cross-contamination from the environment to food has been reported (Nakama *et al.* 1998; Lin *et al.* 2006) and can lead in some cases to listeriosis outbreaks (Winter *et al.* 2009; Gaulin, Ramsay and Bekal 2012). *L. monocytogenes* has been isolated from a wide variety of ready-to-eat foods, such as meat, fish and dairy products (Lianou and Sofos 2007). The production of safe food is the responsibility of the food business operator, and is based on food safety systems like the hazard analysis of critical control points (HACCP) system and the implementation of general preventive measures such as good hygiene and manufacturing practices. It has been shown that *L. monocytogenes* can survive improper cleaning and disinfection processes and that exposure to sublethal concentration of disinfectants supports the survival and growth of these bacteria (Pricope *et al.* 2013). *L. monocytogenes* is widespread in the environment (Fox *et al.* 2009) and can resist and even proliferate under hostile environmental conditions thanks to its elaborate physiological adaptation mechanisms (Khelef *et al.* 2006). Several mechanisms can contribute to the persistence of *L. monocytogenes*. The formation of biofilm, for example, is known to increase *L. monocytogenes* tolerance towards disinfectants (Chavant, Gaillard-Martinie and Hébraud 2004). The integration of prophage DNA into the *L. monocytogenes* genome (Verghese *et al.* 2011), the proposed benzylthonium chloride resistance mechanism (Fox, Leonard and Jordan 2011b) or the contribution of the stress inducible sigma factor σ^B (Utratna *et al.* 2012) have also been described as mechanisms contributing to *L. monocytogenes* rapid adaptation to the food processing facility environment and to food.

In Ireland, *L. monocytogenes* occurrence has been reported on dairy farms and in cheesemaking facility environments (Fox *et al.* 2009, 2011a), where 19% and 13.1% of samples were positive, respectively. Its occurrence in the environment may pose a risk of *L. monocytogenes* transfer from the environment to food (D'Amico and Donnelly 2008), even though the routes of contamination are not always clearly identified. While product testing is important, processing environment testing is a more effective way to identify potential routes of contamination, to assess hygiene and to prevent contamination events.

The aim of the present study was to assess the presence and control of persistent *L. monocytogenes* in a food processing facility facing recurrent occurrence of *L. monocytogenes* in the processing environment.

Materials and Methods

Environmental sample collection

Samples from an Irish food processing facility were collected in May, June, August, October, November 2012 and February 2013. Samples were taken in the changing room, the processing area, the ripening rooms, and the washing and packing rooms, at the same spots from one collecting time to the other, as much as possible. Samples were collected from non food contact surfaces (NFCS), which included floors, walls, ceilings, drains and wheels of mobile equipment, and food contact surfaces (FCS), which included utensils, tables, etc. Swab samples were collected using sponge-stick

swabs pre-moistened with neutralising broth (3M, St Paul, Minnesota, USA). Liquid samples were collected using 100-ml sterile containers. All samples were collected at the same time during production and before cleaning and disinfection. Gloves and appropriate protective clothing were worn during sampling. Samples were individually packaged to prevent cross-contamination, placed in a cool box with ice packs and transported directly to the laboratory where they were analysed within 3 hours, including transport.

Microbiological analyses and confirmation of colonies by polymerase chain reaction (PCR)

The two-step enrichment method ISO 11290-1 was used for detection of *L. monocytogenes* (Anonymous 1996), with the exception that only ALOA agar was used to plate the enriched cultures. After each enrichment step, 20 μl of enriched culture was spread on an ALOA agar plate (Oxoid, Hampshire, UK) and incubated for 48 h at 37 °C. When possible, at least two presumptive-positive *L. monocytogenes* colonies (blue-green with a surrounding halo) were isolated per positive plate and frozen at -20 °C after purification, pending further analysis.

All purified isolates were confirmed as *L. monocytogenes* using Real Time-PCR (Rodríguez-Lázaro *et al.* 2004), as described by O'Brien *et al.* (2009).

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) of all *L. monocytogenes* isolates was carried out using the International Standard PulseNet protocol (PulseNet USA 2009). Two restriction enzymes, *Asc*I and *Apa*I, were used and isolate-similarity dendrograms were obtained using Bionumerics version 5.10 software (Applied Maths, Belgium), as previously described (Fox *et al.* 2012).

Results

Occurrence of L. monocytogenes in the processing environment

Of the 205 samples collected from May 2012 to February 2013, 33% were positive for the presence of *L. monocytogenes*. From May to October 2012, the number of positive samples ranged from 30 to 55% (Table 1). Non-food contact surface samples were the most contaminated sample type found and these included floors, drains and wheels of trolleys. However, 10% and 11% of positive FCS samples were found in June and October 2012, respectively. The contaminated FCS were a grinder (June 2012) and a table (October 2012). Due to the contamination level of the processing area, a drastic revision of cleaning and disinfection procedures and of workflows was advised and implemented in October 2012. This included more rigorous cleaning procedures (with additional staff), including use of peracetic acid as a disinfectant. A decrease in the number of positive samples after the implementation of these corrective actions was observed in November 2012. The number of positive NFCS samples was reduced to 5% (Table 1). The effect of improved hygiene practices was confirmed in February 2013 when only 7% of samples were positive for *L. monocytogenes*.

Typing of L. monocytogenes isolates and spatial distribution

From the positive samples, 162 isolates were confirmed as *L. monocytogenes* and characterised using PFGE, combining the patterns from both enzymes *Apa*I and *Asc*I. The eight pulsotypes, T1 to T8, found in the processing environment are shown in Figure 1. Half of the types (T2, T3, T5 and T7) were only found on a single sampling occasion whereas types T1, T4, T6 and T8 were found on at

Table 1. Proportions of samples taken at a food processing facility that were positive for *Listeria monocytogenes* on different sampling occasions (see Figure 2 for details of the sampling sites)

Date	Non-food contact surface			Food-contact surface			Total positive (%)
	Total	No. positive	Positive (%)	Total	No. positive	Positive (%)	
May 12	18	11	61	2	0	0	55
Jun. 12	51	27	53	10	1	10	46
Aug. 12	10	3	30	0	0	-	30
Oct. 12	39	21	54	9	1	11	46
Nov. 12	19	1	5	1	0	0	5
Feb. 13	38	3	8	8	0	0	7
Total	175	66	38	30	2	7	33

least two different occasions. Type T6 was found on each sampling occasion and 115 isolates displayed this pattern, i.e. 71% of the isolates collected (Figure 1). In November 2012 and February 2013, after the improved cleaning strategies were put into practice, type T6 strain was the only strain found in the processing environment. Type T6 was found on NFCS and FCS, whereas all the other types were only isolated from NFCS.

From May to October 2012, the different types were dispersed all over the processing facility (Figure 2). This was especially true for the prevalent type T6 which was widely distributed in the processing area, the wash room, a store-room and the packing room. The type T6 strain was isolated from drains, floors, wheels of trolleys or other mobile equipment, tables, and boots in the changing room. After revised intensive cleaning, type T6 was found on the floor of a store-room, and in the wash room in November 2012 and February 2013 (Figure 2).

Discussion

The results of this study show that sampling of the processing environment can create awareness of the presence of *L. monocytogenes* and that this contamination can be reduced, reducing the risk of cross contamination to food. This facility

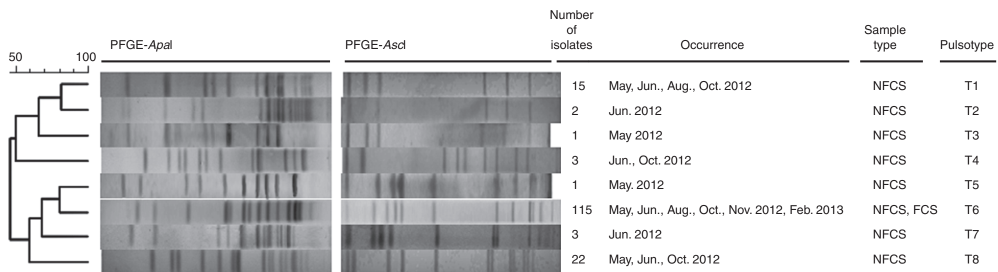

*Figure 1. Dendrogram of PFGE profiles, combining Apa*I *and Asc*I *enzymes, from isolates in non-food contact surfaces (NFCS) and food contact surfaces (FCS) in a food processing plant.*

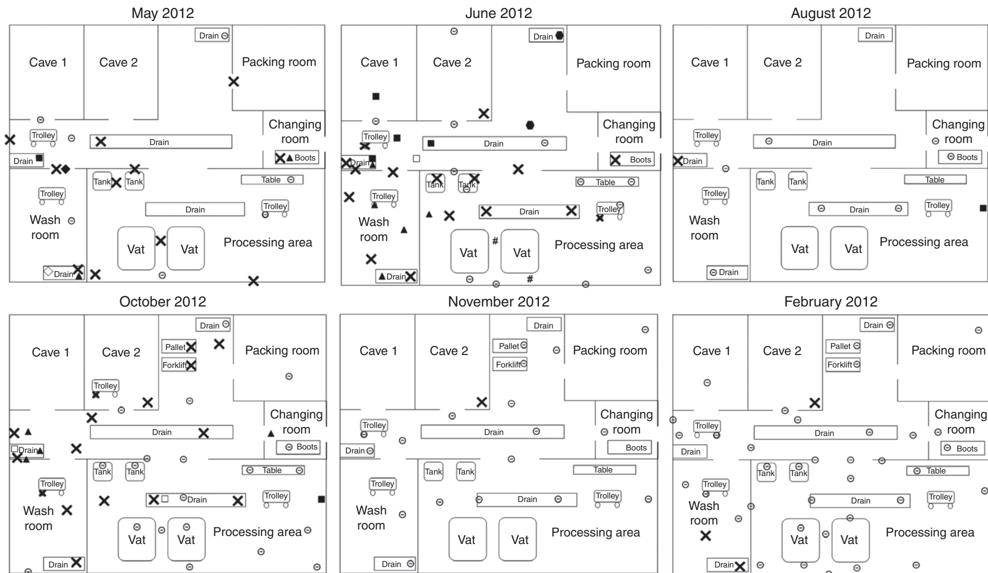

Figure 2. Representation of the location of the pulsotypes found on non-food contact surfaces in the food processing facility from May 2012 to February 2013. The pulsotypes are symbolized as follows: T1 (■), T2 (⬣), T3 (◇), T4 (□), T5 (◆), T6 (×), T7 (#), T8 (▲); no L. monocytogenes found (⊖).

was chosen for intensive sampling because a previous study had shown the occurrence of a strain of *L. monocytogenes* that persisted for 5 years (unpublished data). From May to October 2012, *L. monocytogenes* was detected in 30% to 55% of the samples collected. Similar to the work of Barancelli *et al*. (2011), NFCS were contaminated more often than FCS. The recurrent presence of *L. monocytogenes* in drains may pose the risk of the airborne spreading of *Listeria* by an inadvertent water spray during cleaning (Berrang and Frank 2012). This could be the cause of the transfer of drain microflora to FCS, other NFCS and processing equipment.

The isolates could be divided into three categories, i) sporadic contamination that occurred once (T2, T3, T5 and T7), ii) short-term contamination (T1, T3 and T8), and iii) a persistent strain (T6). Thus, there were two distinct mechanisms of contamination with *L. monocytogenes* at this processing facility. Firstly, there was a persistent strain, type T6, which occurred at all sampling occasions. Secondly, there was the occurrence of sporadic contaminants that appeared on only one or a few occasions and could not be isolated subsequently. The standard HACCP protocol of cleaning practices and workflows of the processing environment were sufficient to address the sporadic contamination, but were inadequate to resolve the short-term or persistent contamination. Advice was given to the food business operator regarding increased cleaning and disinfection practices (including use of peracetic acid as a disinfectant), attention to details in cleaning, and improved workflows in order to limit and prevent the dissemination of *L. monocytogenes* in

the processing environment. Following the implementation of these recommendations, the occurrence of *L. monocytogenes* dramatically dropped in November 2012 and February 2013. As the conditions that influence the occurrence of *L. monocytogenes* in a processing facility (for example temperature, humidity) remain constant, seasonal variation is unlikely to be a factor in the observed reduction in occurrence. The improved cleaning regimen eliminated the short-term contaminating strains and reduced the occurrence of the persistent isolate. Additional measures would be required to eliminate the persistent isolate.

Carpentier and Cerf (2011) described persistence as a loosely defined concept whereby strains of *L. monocytogenes* with indistinguishable PFGE patterns could be isolated over periods ranging from a few months to years. The basis of such persistence has not been fully defined. Carpentier and Cerf (2011) suggest that it is a random event based on a strains ability to grow faster that the rate at which it is inactivated. However, Fox *et al.* (2011b) have shown a potential genetic basis for persistence, where under detergent stress there was increased expression of genes related to detergent resistance. The control of persistent strains is extremely important in order to minimise the risk of contamination of the food product and the dissemination of *L. monocytogenes* in the processing environment. Many examples of *L. monocytogenes* persistence in food processing facilities exist such as in meat, shrimps, ready-to-eat meals, Gorgonzola cheese and fish processing environments (Peccio *et al.* 2003; Gudmundsdottir *et al.* 2006; Keto-Timonen *et al.* 2007; Lomonaco *et al.* 2009; Chen *et al.* 2010). In the current work, the presence of *L. monocytogenes* strains on boots in the changing area before the footbath and on wheels of equipment indicated possible methods of dissemination throughout the processing facility. Mobile equipment, boots and footbaths with inappropriate sanitizer concentration have been identified as potential vectors of dissemination of *L. monocytogenes* in food processing facilities (Reij, Den Aantrekker and ILSI Europe Risk Analysis in Microbiology Task Force 2004; Ho, Lappi and Wiedmann 2007; Schoder *et al.* 2011). Pathogens may also access the food processing environment via staff members, leaks and openings in buildings like doors and windows or even pests (Reij *et al.* 2004). In the present study, the implementation of corrective actions resulted in the elimination of all types from the processing environment with the exception of type T6 which was the predominant persistent type as it was found on each sampling occasion and represented the majority of isolates.

Conclusions

The results of this study show that an environment sampling plan can be an effective contribution to assessing hygiene at a food processing facility, and to preventing future contamination events. It demonstrates that knowledge gained from sampling can lead to appropriate corrective action being introduced (such as changed workflows and use of peracetic acid) to reduce contamination and limit *L. monocytogenes* occurrence in food processing facilities and improve food safety, although additional measures may be required to eliminate very persistent strains.

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