International Journal of Food Microbiology 289 (2019) 64–71

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Listeria monocytogenes colonization in a newly established dairy processing facility

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ARTICLE INFO

Keywords:
Listeria monocytogenes
Cheese
Colonization
Persistence

ABSTRACT

The presence and colonization of *Listeria monocytogenes* were investigated in a newly established dairy processing plant during a one-year period. A total of 250 non-food contact surfaces, 163 food contact surfaces, 46 personnel and 77 food samples were analyzed in two different buildings according to the cheese production chain. Initial steps, including salting, are performed in building I (old facility), while the final steps, including ripening, cutting and packaging, are performed in building II (new facility). Overall, 218 samples were collected from building I and 318 from building II. *L. monocytogenes* isolates were subtyped by PFGE and MLST, and a questionnaire about quality measures was completed. The overall prevalence of *L. monocytogenes* was 8.40%, and while the presence of the pathogen was observed just during the first sampling in building I, *L. monocytogenes* was found in building II at the third sampling event. The salting area in building I had the highest proportion of positive samples with the highest diversity of PFGE types. Moreover, *L. monocytogenes* PFGE type 3 (sequence type -ST- 204) was first detected in building II in the third visit, and spread through this building until the end of the study. The answers to the questionnaire implied that lack of hygienic barriers in specific parts of the facilities and uncontrolled personnel flow were the critical factors for the spread of *L. monocytogenes* within and between buildings. Knowledge of the patterns of *L. monocytogenes* colonization can help a more rational design of new cheesemaking facilities, and improve the food safety within current facilities.

1. Introduction

Listeria monocytogenes is a ubiquitous bacterium that can be isolated from a wide variety of environmental sources, including food-processing environments and a large variety of foods where it can grow over a pH range of 4.39–9.40, even at refrigeration temperatures (Gandhi and Chikindas, 2007; Sauders and Wiedmann, 2007; Swaminathan and Gerner-Smidt, 2007). In 2016, 2536 human cases were reported in 28 EU member states, causing by far the highest number of food-borne diseases-related deaths (EFSA, 2017). Food safety regulations in many countries such as the USA, have tended to adopt a zero tolerance policy for *L. monocytogenes* in ready-to-eat (RTE) food products, as human listeriosis outbreaks have been most often associated with RTE products that are consumed without prior cooking (Painter and Slutsker, 2007; Swaminathan and Gerner-Smidt, 2007). Cheese and other dairy products are within this type of food category. Unlike many other bacterial foodborne pathogens, *L. monocytogenes* can grow in milk at refrigeration temperatures (Kozak et al., 2018; Thamnopoulos et al., 2018) and reach potentially infectious levels in high-moisture and surface-ripened cheeses (Bernini et al., 2013; Cogan, 2011). The survival and growth of *L. monocytogenes* in dairy environments depends on the manufacturing, ripening and storage conditions (Almeida et al., 2013; Pintado et al., 2005). Similarly, the strain-to-strain variability of survival in different storage conditions is associated to the different *L. monocytogenes* genetic lineages (De Jesus and Whiting, 2003; Mataragas et al., 2008).

Although *L. monocytogenes* can decrease in different types of cheeses during ripening and storage (Valero et al., 2014; Wemmenhove et al., 2013), the risk of cross-contamination during processing is still high due to the possible presence of this organism in the dairy environment

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https://doi.org/10.1016/j.ijfoodmicro.2018.09.003
Received 30 April 2018; Received in revised form 30 August 2018; Accepted 3 September 2018
Available online 05 September 2018
0168-1605/

(Muhterem-Uyar et al., 2015; Rückerl et al., 2014; Spanu et al., 2015). Failures of hygiene practices or the incorrect design of equipment or facilities may facilitate *L. monocytogenes* presence and persistence in cheese making facilities (Almeida et al., 2013; Carpentier and Cerf, 2011; Fox et al., 2011; Ibba et al., 2013). Upon colonization of facilities, *L. monocytogenes* can spread easily via contaminated contact materials, inappropriate personnel movements and food workflows, which can constitute an intermediate step in transmission from their original habitat (in biofilms, water and organic soil residues) to surfaces in contact with foods (Muhterem-Uyar et al., 2015; Stessl et al., 2014). Thus, tracing the presence and persistence of *L. monocytogenes* in food commodities is a major issue in food safety.

The aim of this study was to describe the process of colonization by *L. monocytogenes* of a newly opened (6 months), *Listeria*-free dairy processing plant for first samplings by the investigation, during a single year, of the presence and persistence of *L. monocytogenes* and the characterization of its possible routes of contamination.

2. Materials and methods

2.1. Sampling strategy

The presence and persistence of *L. monocytogenes* was investigated in a recently inaugurated dairy processing facility in Castilla y Leon, Spain during a one-year period, in ten different visits from November 2012 to November 2013 every one month and a half. Cheese is produced in two different buildings, 13 km apart (Fig. 1). Building I, which started production in 1984, comprises the first production steps such as 1) milk reception, procession and curdling; 2) salting; and 3) palletizing (crates with unripened cheeses are stacked on a pallet to be transported to the ripening station). Building II, which started production in May 2012, includes the final production steps such as 4) ripening; 5) slicing and modified atmosphere packaging; and 6) cheese grating (Fig. 1). Building II also received cheeses from two other company's plants, located in other regions in Spain.

The selection and number of samples and sites followed a previous described sampling strategy, with a scope of collecting 50 samples per sampling event (Muhterem-Uyar et al., 2015). The sample sites were the same in all the sampling events along the study, taken during the working shift and before cleaning and disinfection. Environmental samples (food contact surfaces –FCS- and non-food contact surfaces -NFCS-), pasteurized cheese, and product-associated samples like brine were tested during the processing. A total of 536 samples were taken in both buildings (218 in the building I and 318 in the building II); comprising 250 NFCS, 163 FCS, 46 personnel and 77 food samples. An average of 53.6 samples per sampling day was collected. Table 1 summarizes all the samplings that were carried out according to the building, production step, sample type and visit. Both the FCS and the NFCS were investigated by swabbing at least 900 cm^2 areas with sterile sponges moistened with 10 mL of buffered peptone water (3 M, St. Paul, MN, USA). All samples were maintained at 4 °C during transportation to the laboratory and were analyzed for the presence of *L. monocytogenes* within 24 h.

2.2. Detection of Listeria monocytogenes

The presence of *L. monocytogenes* in environmental swabs, liquids and solid food samples was investigated as described previously (Muhterem-Uyar et al., 2015). One confirmed *L. monocytogenes* colony by real-time PCR (Rodríguez-Lázaro et al., 2004), was used for further genetic characterization.

2.3. Questionnaire

A questionnaire about personnel behaviour, plant infrastructure and hygienic and cleaning measures was completed by the Quality Manager from each building, to provide a better knowledge of contamination routes (Table 2).

2.4. Genetic characterization

L. monocytogenes PCR-serogrouping was performed using a multiplex PCR as previously described (Doumith et al., 2004). All isolates were genetically characterized by pulsed-field gel electrophoresis (PFGE) using the restriction enzymes *Asc*I and *Apa*I following the standardized PulseNet protocols as previously described (Rodríguez-Lázaro et al., 2015). In the framework of this study, a *L. monocytogenes* strain was defined as persistent when an identical PFGE type was detected at least 6 months apart from the first time. *L. monocytogenes* isolates were further characterized by multilocus sequence typing (MLST) as previously described (Rodríguez-Lázaro et al., 2015).

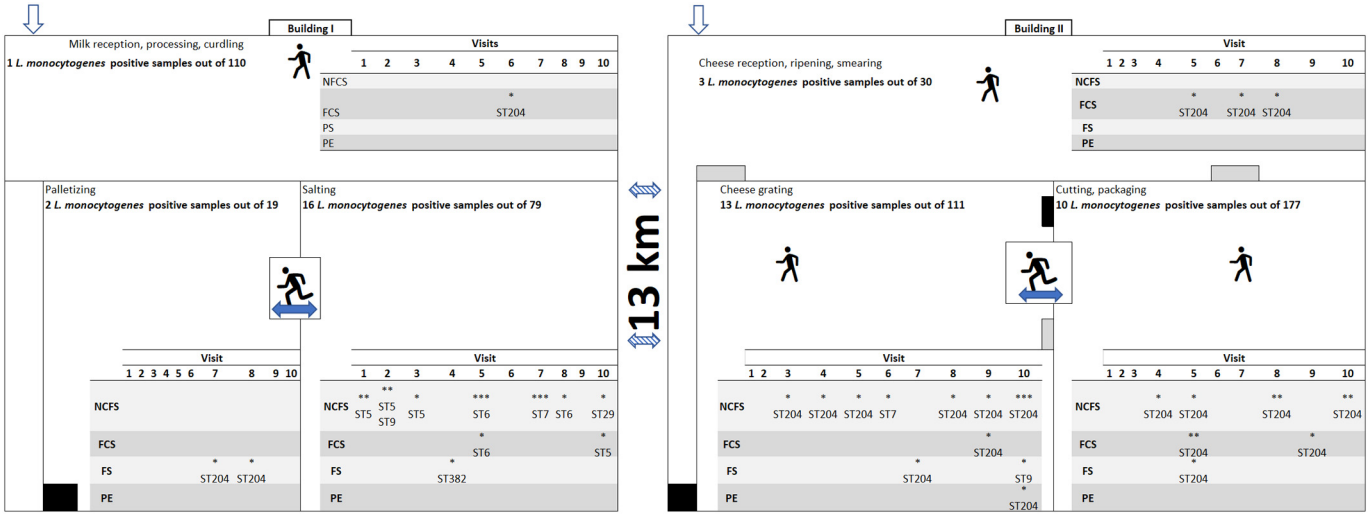

Fig. 1. Schematic representation of the two buildings (located 13 km apart) and the production process/flow of the dairy plant analyzed in this study. Different types of entrances are marked by gray (hygienic) or black (non-hygienic) rectangles. Personnel for which movement is restricted are indicated in black, while personnel moving within different areas are indicated inside a square and with an arrow indicated the direction. An empty arrow indicates the entrance of product in each building. NFCS (Non-food Contact Surfaces); FCS (Food Contact Surfaces); FS (Food Samples); PE (Personnel). * number of positive samples for each visit and each sample type. ST, multi-locus sequence type.

Table 1
Prevalence of *Listeria monocytogenes* in a Spanish dairy processing facility.

				Visit 1[a]	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	n[b] of positive sample/n of total samples	% of positive samples per sample type	% of positive samples per processing area
Building I	Milk reception, processing, curdling	NFCS[c]	Drain	**	**	**	**	**	**	**	**	**	**	0/60	0.0	0.9
			Floor	**	**	**	**	**	**	**	**	**	**			
			Walls	*	*	*	*	*	*	*	*	*	*			
			Food soil	*	*	*	*	*	*	*	*	*	*			
		FCS	Moulds	*	*	*	*	*	*	*	*	*	*	1/30	3.3	
			Conveyor belt	**	**	**	**	**	*+	**	**	**	**			
		FS	Milk	*										0/11	0.0	
			Cheese curd	*	*	*	*	*	*	*	*	*	*			
		PE	Gloves	*	*		*	*	*	*	*	*	*	0/9	0.0	
	Salting	NFCS	Drain	+	*	*	*	+	*	+	*	*	*	13/40	32.5	20.3
			Floor	*	+	*	*	+	*	+	*	*	+			
			Walls	*	*	*	*	*	*	*	*	*	*			
			Food soil	+	+	+	*	+	*	+	+	*	*			
		FCS	Conveyor belt	*	*	*	*	+	*	*	*	*	+	2/19	10.5	
			Salting racks	*	*	*	*	*	*	*	*	*	*			
		FS	Salted cheese	*	*	*	+	*	*	*	*	*	*	1/20	5.0	
			Brine	*	*	*	*	*	*	*	*	*	*			
		PE		Not tested												
	Palletizing	NFCS	Floor	*	*	*	*	*	*	*	*	*	*	0/10		6.9
		FCS	Ripening crate	*	*	*	*	*	*	*	*	*	*	2/19	10.5	
			Lid		*	*	*	*	*	+	+	*	*			
	Total (%)			9.1	9.1	4.8	4.5	18.2	4.5	18.2	9.1	0.0	9.1	19/218		8.7
Building II	Cheese reception, ripening, smearing	NFCS	Floor	*	*	*	*	*	*	*	*	*	*	0/20	0.0	10.0
			Walls	*	*	*	*	*	*	*	*	*	*			
		FCS	Conveyor belt	*	*	*	*	+	*	+	+	*	*	3/10	30.0	
		FS		Not tested												
		PE		Not tested												
	Cutting, packaging	NFCS	Floor	**	**	**	*+	+**	***	***	+**	***	**+	6/61	9.8	5.6
			Walls	**	**	**	**	**	***	***	***	***	***			
			Food soil	*	*	*	*	*	*	*	+	*	+			
		FCS	Packaging bench	**	**	**	**	+*	***	***	***	*+*	***	3/65	4.6	
			Conveyor belt	**	**	**	**	+*	***	***	***	***	***			
			Knife	*	*	*	*	*	**	**	**	**	**			
		FS	Packaged cheese	**	***	**	**	*+	***	***	***	***	***	1/26	3.8	
		PE	Gloves	**	**	**	**	**	***	***	***	***	***	0/25	0.0	
	Cheese grating	NFCS	Floor	**	***	*+*	***	*+*	**+	***	***	**+	*++	9/59	15.3	11.9
			Wall	**	**	**	**	**	**	**	**	**	**			
			Food soil	**	**	**	*+	**	**	**	*+	**	*+			
		FCS	Conveyor belt	*	*	*	*	*	*	*	*	*	*	1/20	5.0	
			Table	*	*	*	*	*	*	*	*	*	*			
		FS	Packaged grated cheese	*	*	*	*	*	*	+	*	*	+	2/20	10.0	
			Packaged sliced cheese	*	*	*	*	*	*	*	*	*	*			
		PE	Gloves	*	*	*	*	**	**	*	*	*	+	1/12	8.3	
	Total (%)			0.0	0.0	3.4	6.9	19.4	2.7	5.6	11.1	8.3	19.4	26/318		8.2

[a] More than one symbols means more than one sampling location; * means negative sample; + means positive sample.
[b] Number.
[c] NFCS (Non-food Contact Surfaces); FCS (Food Contact Surfaces); FS (Food Samples); PE (Personnel).

Table 2
Quality Manager responses to the food safety related questions.

	Building I	Building II	Evaluation of the risk of cross-contamination with *L. monocytogenes*		
			High	Medium	Low
Personnel					
Personnel movements are permitted from clean areas (e.g. end of production, packaging) to the dirty areas (e.g. reception of raw material)	Yes	Yes	✓		
Does personnel from one building move to the other during the shift?	No	No	✓		
Personnel receive training sessions	Yes	Yes	✓		
Personnel change their gloves frequently or after touching something non-food	Yes	Yes	✓		
Plant infrastructure					
Physical separation exists between reception area and product preparation, processing and packaging areas	Yes	Yes	✓		
Floors are well drained	No	Yes	✓		
Drain has removable grates	Yes	Yes		✓	
Hygienic and cleaning measures					
There is a washing area for trucks at the building entrance	No	No		✓	
Presence of hygienic entrance at the entrance of processing area	Yes	Yes	✓		
Presence of hygienic entrance between clean and dirty areas	Yes	No	✓		
The brine is prepared fresh every day	No	–			✓
The brine is pasteurized every day	No	–		✓	
The brine is filtered every day	Yes	–		✓	
Sal concentration in brine is adjusted very day	Yes	–		✓	
The brine tank is well maintained without corrosion and easy to clean	No	–	✓		
Ripening crates and lids are cleaned and disinfected before being reused	Yes	Yes	✓		
Crates and lids have no any physical damage that can difficult the cleaning and disinfection	No	No		✓	
Different crates and lids for each building	No	No	✓		
The plant have equipment and environment washing and cleaning SOPs describing the frequency and products to use	Yes	Yes	✓		
Use of alkaline sanitizers	Yes	Yes		✓	
Use of quaternary ammonium related disinfectants	Yes	Yes		✓	
Sanitizers and disinfectants are often changed to avoid the establishment of resistant bacteria	No	No	✓		
A mid-shift cleaning is performed	No	No	✓		
The processing and packaging area have facilities for hand washing and drying	Yes	Yes	✓		
By-products are stored in a different room to packaged products	Yes	Yes		✓	
The waste is removed from the processing area during the shift	No	No	✓		
The effectiveness of cleaning and disinfection is periodically checked	Yes	Yes	✓		
The effectiveness of cleaning and disinfection is periodically checked for *Listeria monocytogenes*	Yes	Yes	✓		

3. Results

3.1. Prevalence

Forty five of 536 (8.40%) samples were positive for *L. monocytogenes* (Table 1). The overall presence of *L. monocytogenes* in each building was similar. Nineteen out of 218 samples were positive in building I (8.72%) and 26 out of 318 in building II (8.18%) with NFCS samples as the most contaminated ones. However, the prevalence of *L. monocytogenes* in building I (old one) ranged from 0% (visit 9) to 18.2% (visits 5 and 7), while *L. monocytogenes* was not detected in any sample during the first two visits in the building II (new one) (Fig. 1). Interestingly, whereas in building I 84.2% of *L. monocytogenes* positive isolates were found in the salting area, contamination in building II was observed first in the grating cheese area and subsequently in all other areas. The later visits showed an increasing number of positive samples, with the grating cheese area being the most contaminated one (Table 1).

Regarding food samples, in building I only one positive sample out of 20 samples was recovered (i.e. cheese after the salting area), while 3 out of 45 samples were detected in building II (i.e. portion of soft cheese and 2 packages of grated cheese) (Table 1).

3.2. Questionnaire

Table 2 summarizes the responses to the questionnaire. In both buildings, personnel received training sessions and are scrupulous about changing their gloves after touching non-food contact surfaces. However, in both buildings, high risk actions such as non-controlled personnel movement, are permitted. Personnel can move depending on the production requirements, potentially causing cross-contamination between areas (Fig. 1). Although there is a physical separation between reception, production and packaging areas in both buildings, in building II there are no hygienic entrances between these areas (Fig. 1). In the case of building I there is a hygienic entrance between dirty and clean areas, but there are no hygienic barriers between salting and palletizing (Fig. 1). Associations between both buildings were also investigated because they are located 13 km apart; i.e. trucks moving from one to the other building do not have any place for cleaning and disinfection and there is not hygienic entrance between clean and dirty areas in building II. Another potential risk factor is that building II has two other Spanish plants as suppliers. Furthermore, in both buildings sanitizers and disinfectants were not changed during the study.

3.3. Genetic characterization

Four PCR-serotype groups (1/2a-3a; 1/2c-3c; 1/2b-3b-7 and 4b-4d-4e) were detected. The most prevalent one was 1/2a-3a (72.73%) followed by 1/2b-3b-7 (11.36%), 4b-4d-4e (11.36%) and 1/2c-3c (4.55%). The remaining isolate showed an unusual profile that was a mixture of the serogroups 1/2a-3a and 4b-4d-4e (Table 3).

PFGE and MLST typing yielded 7 PFGE types/7 STs, with the most prevalent types being PFGE type 3/ST204 (n = 27); PFGE type 1/ST5 and 5/ST6 (n = 5 each), and PFGE type 6/ST7 (n = 4). PFGE types found in 1 (types 4/ST382 and 7/ST29) or 2 (type 2/ST9) isolates were

Table 3
L. monocytogenes isolated from a Spanish dairy processing facility.

Visit #[a]	Processing area	Building I						Building II					
		Sample type[b]	Sampling details	#[a] of isolates	PCR-serogroup	PFGE type	ST[c]	Sample type	Sampling details	#[a] of isolates	PCR-serogroup	PFGE type	ST[c]
Visit 1	Salting	NFCS	Drain, food soil	2	1/2b-3b-7	1	5						
Visit 2	Salting	NFCS	Drain, food soil	1	1/2b-3b-7	1, 1S[d]	5						
		NFCS	Floor	1	1/2c-3c	2	9						
Visit 3	Salting	NFCS	Drain, food soil	1	1/2b-3b-7	1	5						
	Cheese grating							NFCS	Floor	1	1/2a-3a	3	204
Visit 4	Salting	FS	Cheese after salting	1	1/2a-3a + 4b-4d-4e	4	382						
	Cutting, packaging							NFCS	Floor	1	1/2a-3a	3	204
	Cheese grating							NFCS	Food soil	1	1/2a-3a	3	204
Visit 5	Salting	NFCS, FCS	Drain, floor, food soil, conveyor belt	4	4b-4d-4e	5	6	NFCS					
	Cheese reception, ripening, smearing							FCS	Conveyor belt	1	1/2a-3a	3	204
	Cutting, packaging							NFCS, FCS, FS	Floor, packaging bench, conveyor belt, portion of cheese	4	1/2a-3a	3	204
	Cheese grating							NFCS	Floor	1	1/2a-3a	3	204
Visit 6	Milk reception, processing, curdling	FCS	Conveyor belt	1	1/2a-3a	3	204						
	Cheese grating							NFCS	Floor	1	1/2a-3a	6	7
Visit 7	Salting	NFCS	Drain, floor, food soil	3	1/2a-3a	6	7						
	Palletizing	FCS	Lid	1	1/2a-3a	3	204						
	Cheese reception, ripening, smearing							FCS	Conveyor belt	1	1/2a-3a	3	204
	Cheese grating							FS	Grated cheese	1	1/2a-3a	3	204
Visit 8	Salting	NFCS	Food soil	1	4b-4d-4e	5	6						
	Palletizing	FCS	Lid	1	1/2a-3a	3	204						
	Cheese reception, ripening, smearing							FCS	Conveyor belt	1	1/2a-3a	3	204
	Cutting, packaging							NFCS	Floor, food soil	2	1/2a-3a	3	204
	Cheese grating							NFCS	Food soil	1	1/2a-3a	3	204
Visit 9	Cutting							FCS	Packaging bench	1	1/2a-3a	3	204
	Grating							NFCS, FCS	Floor, table	2	1/2a-3a	3	204
Visit 10	Salting	NFCS	Floor	1	1/2a-3a	7	29						
		FCS	Conveyor belt	1	1/2b-3b-7	1	5						
	Cutting, packaging							NFCS	Floor, food soil	2	1/2a-3a	3	204
	Cheese grating							NFCS, FCS, PE	Floor, food soil, gloves	4	1/2a-3a	3	204
								FS	Grated cheese	1	1/2c-3c	2	9

[a] # number.
[b] NFCS (Non-food Contact Surfaces); FCS (Food Contact Surfaces); FS (Food Samples); PE (Personnel).
[c] ST, multi-locus sequence type.
[d] 1S, PFGE-subtype 1.

considered sporadic (Supplementary Fig. 1). Differences between both buildings can be observed according with the distribution of the PFGE types (Table 3). The salting area in building I, the area with the highest proportion of *L. monocytogenes* positive samples, showed a high diversity of PFGE types and none of the PFGE types predominated over the others. PFGE type 2, 4, 6 and 7 appeared only once, while PFGE type 1 predominates in occurrence and persistence in the salting area, as it appeared during the first three visits and visit 10. In building II on the other hand, PFGE type 3 predominated over type 2 and 6, each appearing only once (Table 3). PFGE type 3 was isolated by the first time in the third visit in the grated cheese area, and it was subsequently observed in other areas and isolated from all the sample types. PFGE type 3 was also isolated in building I in a conveyor belt in the milk reception, processing, curdling area and twice in the lids covering the ripening crates that are transported to the building II for the ripening process.

4. Discussion

This study demonstrates, for the first time, the colonization of a new dairy facility with *L. monocytogenes*. The low prevalence of *L. monocytogenes* found in this study (8.40%) has also been observed in Ireland in 18 dairy facilities where the prevalence ranged from 0 to 14.3% (Leong et al., 2014). In a European survey of dairy plants, prevalence values of 7.22% and 26% were detected in Greece and Austria, respectively (Muhterem-Uyar et al., 2015). However, other studies on cheese processing facilities have reported higher *L. monocytogenes* prevalence; from 33.3% (Ibba et al., 2013) to 50% (De Cesare et al., 2007). The variations observed in different studies could be explained by the different cheeses analyzed, both in terms of processing and the milk used (i.e. different animal sources). In this study, *L. monocytogenes* was detected along the post-ripening process (smearing, cutting and packaging) and in the environment of grated cheese production indicating a potential failure in the application of the hygienic measures that could represent a potential risk of delivery of final products contaminated with this pathogen; *L. monocytogenes* was found in a relatively high percentage of final products tested in building II (i.e. 6.5%) (Table 1). Similarly, Gaulin et al. (2012) described that pasteurized milk cheese was the vehicle of *L. monocytogenes* contamination in an outbreak that occurred in 2008 in Quebec, Canada. The investigation also found that the same strain (LM P93) was isolated from the environment of one cheese processing plant and from the environment of retailers selling that type of cheese (Gaulin et al., 2012; Sauders and D'Amico, 2016).

L. monocytogenes was first detected on the third visit in building II, thus the contamination of the new building appeared just 9 months after the production activity started. In a similar study performed in a newly constructed commercial chicken processing plant, *L. monocytogenes* contamination was observed one month after the processing started, and the microorganism was isolated in a drain during and after processing, prior to clean up (Berrang et al., 2010). Our findings show that the first *L. monocytogenes* isolate collected from the new building (grated cheese area) belongs to main PFGE type 3 (ST204) and persisted during the whole study's timeframe. Moreover, isolates belonging to this PFGE type spread to other areas in the same building. Similarly, some studies reported that some *L. monocytogenes* PFGE types were able to persist over one year in different environmental sites of cheese facilities and even appearing in the final product (Almeida et al., 2013; Kabuki et al., 2004; Stessl et al., 2014; Wagner et al., 2006). Colonization by strains belonging to PFGE type 3 could be likely attributed to the absence of effective hygienic measures between different areas in building II and the lack of restrictions on the movement of personnel across the plant. Lomonaco et al. (2009) revealed the importance of operators in spreading contamination over a processing plant, as they detected in locker rooms, toilets and hallways. Similarly, in this study the same prevalent PFGE type was detected in building II four months after the sampling plan finished, in toilets and hallways connecting both outside and inside areas (data not shown). The persistence of ST204 has previously been described in dairy environments in the Czech Republic (Stessl et al., 2014) and strains belonging to this ST have been isolated from food and food environments (Allnutt et al., 2016; Haase et al., 2014; Rückerl et al., 2014). Although we were not able to trace back the origin of contamination in building II, lids could be a potential source for contamination in this building, in the area where cheese are ripened as it has been proved that they were contaminated after cleaning and disinfection with the same PFGE type 3. The area where lids are cleaned in building II was also sampled 4 months later, and the same PFGE type was obtained (data not shown).

Interestingly, the contamination in building I was characterized by high diversity of PFGE types/STs in the salting area, 4 considered as non-persistent and 2 as persistent. This finding could be explained by the lack of effective hygienic measures, the non-controlled personnel movements and the fact that the floors are not well drained as shown by questionnaire. Indeed, *L. monocytogenes* can adhere to surfaces and form biofilms so that the microorganism is able to adapt and resist sanitation (Chavant et al., 2004; Ibba et al., 2013; Pan et al., 2006), and therefore, hindering the elimination of this pathogen by sanitation procedures (Rovira et al., 2014). Furthermore, in both buildings sanitizers and disinfectants are not often changed, thus *L. monocytogenes* strains can be injured sublethally leading to a better adapted subpopulation (Møretrø et al., 2017). Most of the STs detected in this study have been involved in different outbreaks, highlighting the importance of this kind of studies for a better design of specific hygienic measures to improve the food safety. ST9 has been associated with meat processing plants and listeriosis cases (Henri et al., 2016; Martín et al., 2014; Wang et al., 2012) and ST7 was linked with an outbreak of listeriosis associated with cantaloupe in the US during 2011 (Lomonaco et al., 2013). In addition, both STs have been also isolated from food products (Parisi et al., 2010; Rückerl et al., 2014; Wang et al., 2012). ST382 caused three US outbreaks related to stone fruit, caramel apples, and leafy green salad and represents a novel epidemic clone IX (Chen et al., 2016, 2017a). ST6 is reported to be hypervirulent and often involved in listeriosis with severe outcome (Kremer et al., 2017; Severi et al., 2017; Smith et al., 2016). ST5 is globally distributed and has been also linked to the US cantaloupe and ice-cream outbreaks and is highly prevalent in cheese processing environments (Chen et al., 2017b; Lomonaco et al., 2013; Maury et al., 2016; Muhterem-Uyar et al., 2018). ST204 has been also defined as persistent in food environments in Czech Republic (2007–2008) as well as in Australia where it was also identified from one clinical isolate (1992–2015) (Jennison et al., 2017; Stessl et al., 2014). Finally, ST29 has been associated with outbreaks in EU and USA and clinical samples more than with non-clinical related samples (Den Bakker et al., 2010).

In conclusion, the fact that *L. monocytogenes* could colonize and persist in a cheese processing plant increases the risk of listeriosis associated with this type of RTE product. Moreover, the presence of a high diversity of strains indicates diverse sources of contamination, and highlights the lack of effective hygienic measures and failure in the good manufacturing practices. This study highlights the value of a evidence-based design of sampling schemes over a prolonged period of time, to draw a profile of potential *Listeria* contamination and to determine sources of contamination. Moreover, according to the results obtained, a thorough revision of some Prerequisite Programs such as sanitation and maintenance, raw material control and good manufacturing practices including personnel formation, should be performed to improve food safety in these facilities.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.09.003.

Acknowledgements

This study was supported by the European Union (EU) funded

Integrated Project PROMISE (project number 265877; 7th Framework Program). The authors wish to acknowledge cooperation of the food business owner and quality management board who participated in this work. Additionally, we would like to thank the Institute Pasteur for providing the MLST database of *L. monocytogenes* in the Genotyping of Pathogens and Public Health Platform.

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