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Genetic relatedness among *Listeria monocytogenes* isolated in foods and food production chain in southern Rio Grande do Sul, Brazil

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ABSTRACT

Listeria monocytogenes is a foodborne bacterial pathogen of great concern to food industry. This is mainly due to its capacity to grow at low temperatures, its wide distribution in the environment and ability to adhere to various surfaces that come into contact with food. The aim of this study was to investigate the clonal relationship among *L. monocytogenes* isolates. Our purpose was to better understand the diversity of this pathogen in foods and food production chains in southern Rio Grande do Sul (Brazil). Forty four *L. monocytogenes* strains were characterized by serotyping and PFGE. Six different serotypes were found in the food and food environment (1/2a, 1/2b, 1/2c, 3a, 4b, 4e) and combination of macrorestriction patterns using *Asc*I and *Apa*I, yielded 29 different pulsotypes. Strains with identical restriction patterns were isolated from foods of different sources and environments at different times. The presence of persistent strains of *L. monocytogenes* emphasizes the importance of cross-contamination in these food processing environments. It is likely that this occurs mainly due to ineffective cleaning and sanitization procedures, which allow for the survival and adaptation of these strains in the food processing environment, thereby causing persistence and contamination of final products.

1. Introduction

Listeria monocytogenes, the causative agent of listeriosis, is an important foodborne pathogen of serious public health concern. This pathogen is capable of causing a severe invasive illness in humans with a high fatality rate between 20% and 30% (De Valk et al., 2005; Mead et al., 1999). Clinical manifestations of listeriosis include meningitis, encephalitis, and septicemia, primarily in the elderly or in immunocompromised patients (Dussurget, Pizarro-Cerda, & Cossart, 2004; Farber & Peterkin, 1991). Nevertheless, the infection is most severe in pregnant women and neonates due to its ability to cross the placenta and infect the fetus, causing congenital defects, stillbirth and abortion (Vazquez-Boland et al., 2001). Occasionally, healthy adults may just suffer febrile gastroenteritis after ingesting large numbers of *L. monocytogenes* cells (ILSI, 2005).

Although the bacterium is considered as ubiquitous in nature and usually can be isolated from many different environmental sources (Dauphin, Regimbeau, & Malle, 2001; Swaminathan & Gerner-Smidt, 2007), the main transmission route of *L. monocytogenes* for humans is through consumption of contaminated food (Ramaswamy et al., 2007; Schuchat, Swaminathan, & Broome, 1991). Owing to the ability of *L. monocytogenes* to grow at 4 °C and to contaminate the food processing environment, the most common products implicated in disease transmission include ready-to-eat food such as meat, dairy, seafood, and fresh vegetables produce (Autio et al., 2002; Azevedo et al., 2005; Fugett, Schoonmaker-Bopp, Dumas, Corby, & Wiedmann, 2007).

Discriminative molecular subtyping methods have been used for the characterization of *L. monocytogenes* in order to identify

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sources of contamination and routes of spread through the food system (Kerouanton et al., 1998; Wiedmann, 2002). Pulsed field gel electrophoresis (PFGE), has successfully been used to differentiate isolates of *L. monocytogenes* associated with foods and food processing environments, allowing the identification of persistent contamination (Autio, Keto-Timonen, Lunden, Björkroth, & Korkeala, 2003; Cruz et al., 2008; Thévenot et al., 2006). It provides sensitive discrimination of isolates, has a high reproducibility, can be standardized and is considered the 'gold standard' subtyping method for *L. monocytogenes* (Graves & Swaminathan, 2001; Hunter et al., 2005). Besides, the use of subtyping methods also provides an opportunity to comprehend the genetic population, epidemiology and ecology of *L. monocytogenes*, including differences in their ability to cause human foodborne disease (Wiedmann, 2002).

In this study, we used PFGE to characterize *L. monocytogenes* strains isolated in different sources, including food (sheep carcasses, handmade Minas cheese – a typical Brazilian cheese – chicken carcasses, fresh mixed sausage), food processing plants and commercial poultry farms. Our aim was to investigate the clonal relationship among the *L. monocytogenes* strains isolated in distinct sources and at different times, to better understand the diversity of this pathogen in food and food production chains in southern Rio Grande do Sul (Brazil). To our knowledge, this is the first report of a molecular diversity of *L. monocytogenes* considering PFGE patterns from a set of strains, representative from different serotypes and sources to be published in Brazil.

2. Materials and methods

2.1. Bacterial strains

A total of 44 *L. monocytogenes* strains from culture collection of the Laboratório de Microbiologia de Alimentos (DCTA/FAEM/UFPel) were analyzed. Strains were isolated from four different food sources (sheep carcasses, handmade Minas cheese, chicken carcasses, fresh mixed sausage) and samples of two food industries (processing line of fresh mixed sausage – Plant A, and chicken slaughterhouse – Plant B). The plants had no relationship with each other and were located in different geographical areas of the region investigated. The sampling protocol was performed for the same sampling points after an interval of at least 30–45 days. On the plant A, samples were collected from November 2002 to April 2003, while for Plant B, the samples were taken during the period of November 2005 to May 2006. The entire set of strains, origins and sampling day are described in Table 1. In addition, we also used a strain isolated from chicken cloacal swab found in a commercial chicken house, as well as a strain from food handlers. At this point, it is interesting to note that the strain isolated from chicken cloacal swab belonged to a group of poultry, which were slaughtered and processed industrially on Plant B. In addition, the strain isolated from the hands of a food handler belonged to an employee of Plant A. All strains were isolated during the years of 2001–2006, in southern of Rio Grande do Sul, Brazil. The strain *Salmonella enterica* serotype Braenderup isolate H 9812, established as a molecular size standard

Table 1
Genotypic and serological profile of 44 strains of *L. monocytogenes* isolated in food and food production chain obtained from the industry and grocery store in southern Rio Grande do Sul, Brazil.

Strains**	Source	Isolation year (sampling day)	Serotype	Digestion profile by endonuclease (DPE)		Combined profiles (pulsotype)	Group
				*Asc*I	*Apa*I		
27/39	Trolley[A]/Food handler[A]	2002 (1)/2003 (4)	1/2c	1	G	P1	I
58	Fresh mixed sausage[A]	2003 (2)	1/2c	1	C	P2	I
32	Sausage stuffer[A]	2003 (5)	1/2c	1	B	P3	I
33/34/60/62	Table[A]/Meat grinder[A]/Fresh mixed sausage[A]/Sheep carcass	2003 (5)/2002 (1)/ 2003 (5)/2001	1/2c	2	E	P4	III
40	Fresh mixed sausage[A]	2003 (1)	1/2c	3	D	P5	II
37	Box[A]	2002 (1)	1/2c	4	F	P6	II
12	Sheep carcass	2001	3a	5	I	P7	III
29	Sausage tying machine[A]	2002 (2)	1/2c	6	A	P8	V
9/10/11	Refrigerated chicken carcass[C]/Refrigerated chicken carcass[C]/Chicken cloacal swab[E]	2006/2006/2006	4e	7	H	P9	III
1/6	Sheep carcass/ Refrigerated chicken carcass[C]	2000/2006	1/2a	8	J	P10	I
5	Refrigerated chicken carcass[C]	2006	1/2a	9	L	P11	I
2/ 3/ 4	Handmade Minas cheese[C]/Handmade Minas cheese[C]/ Handmade Minas cheese[C]	2006/2006/2006	1/2a	10	K	P12	IVb
16	Handmade Minas cheese[C]	2006	1/2b	11	N	P13	Iva
26	Refrigerated chicken carcass[C]	2006	1/2b	12	N	P14	Iva
18	Fresh mixed sausage[A]	2002 (1)	1/2b	13	P	P15	Iva
22/44/50/51	Chicken carcass[B]/ Chicken carcass in pre-chiller[B]/Poultry Conveyor before slaughter[B]/Poultry Conveyor after slaughter[B]	2006 (4)/2006 (3)/2006 (4)/2006 (4)	1/2b	14	N	P16	Iva
23	Refrigerated chicken carcass[C]	2006	1/2b	14	M	P17	Iva
42	Poultry pallet[B]	2006 (2)	1/2b	14	P	P18	Iva
43/45	Floor[B]/Chicken carcass in chiller[B]	2006 (3)/2006 (3)	1/2b	14	Q	P19	IVa
20/41	Fresh mixed sausage[A]/ Fresh mixed sausage[A]	2003 (5)/2002 (1)	1/2b	15	O	P20	IVd
52	Drain[B]	2006 (3)	1/2b	15	M	P21	IVd
19	Sheep carcass	2001	1/2b	16	P	P22	Iva
14/17	Handmade Minas cheese[C]/Handmade Minas cheese[C]	2006/2006	1/2b	17	N	P23	IVb
73	Fresh mixed sausage[A]	2002 (1)	4b	18	U	P24	III
7	Industrial brush[B]	2005 (1)	4b	18	S	P25	**
70	Handmade Minas cheese[C]	2006	4b	19	R	P26	Ivc
71	Fresh mixed sausage (raw material)[B]	2002 (1)	4b	19	T	P27	Ivc
8	Table[B]	2005 (1)	4b	20	S	P28	**
66	Handmade Minas cheese[C]	2006	4b	20	R	P29	IVb

**: Identification number of strains; **: Profile excluded from dendrogram; [A]: Plant A; [B]: Plant B; [C]: Products purchased from local grocery store; [D]: Chicken from commercial chicken house; Sampling day: numbers 1, 2, 3, 4, 5 represent the day of each sampling with an interval of at least 30–45 days.

universal for PFGE, was used in order to serve as a reference for comparative analysis among the pulsotypes.

2.2. Serotyping

Strains of *L. monocytogenes* were characterized by serotyping by the Laboratório de Zoonoses Bacterianas, Departamento de Bacteriologia, Instituto Osvaldo Cruz (FIOCRUZ).

2.3. Pulsed Field Gel Electrophoresis (PFGE)

The standardized CDC PulseNet protocol (Graves & Swaminathan, 2001) with slightly modifications was used to perform the *L. monocytogenes* analysis by PFGE. Briefly, *L. monocytogenes* strains were grown overnight in TSB-YE medium at 37 °C for 16–18 h. Bacterial cells were then pelleted by centrifugation at 13,000 rpm for 5 min and resuspended in TE buffer. Bacterial suspensions (240 μl) were added with 60 μl of 10 mg mL^{-1} lysozyme solution and the mixture were incubated at 37 °C for 10 min. Equal volumes (300 μl) of SSP solution [1.2% Agarose Pulsed Field (Bio-rad, USA); 1% sodium dodecyl sulfate (Invitrogen), 0.2 mg mL^{-1} proteinase K (LGC Bio)] were added to the bacterial suspension and the mixture transferred to PFGE molds (Bio-Rad, USA). The agarose blocks (plugs) were lysed in lysis buffer [50 mM Tris-HCL, 50 mM EDTA, 1% sodium lauryl sarcosine, 0.15 mg mL^{-1} Proteinase K] for 2 h at 54 °C in a water bath. After proteolysis, the lysis solution was removed and plugs were washed. Plugs were digested with *Asc*I (25 U μL^{-1}) e *Apa*I (160 U μL^{-1}) (New England BioLabs) in buffer solutions according to the manufacturer's instructions. DNA fragments were electrophoretically separated through 1% agarose gel (Pulsed Field Certified Agarose, Bio-Rad) in 0.5× TBE buffer (Bio-Rad Laboratories) at 6 V cm^{-1}, 14 °C in CHEF DR II apparatus (Bio-Rad). The pulse time was ramped from 4 to 40 s for 23 h. *S. enterica* serotype Braenderup (H 9812) was used for fragment size determination, after digestion with restriction enzyme *Xba*I (Fermentas, Glen Burnie). After electrophoresis, the gels were stained with ethidium bromide and photographed. The resulting pulsed field electrophoretic macrorestriction patterns were compared using Bionumerics software (version 5.10, Applied Maths, Sint-Martens-Latem, Belgium). The similarity between PFGE patterns was expressed as a Dice coefficient (position tolerance 1.0%). The clustering and construction of dendrograms were performed by the unweighted pair group method using arithmetic averages (UPGMA).

3. Results and discussion

The isolates analyzed in this study came from four different food categories and samples from two different processing plants, collected over a period of six years (2001–2006). In addition, we also used one strain isolated from chicken cloacal swab kept in commercial chicken house, as well as a strain of a food handler who worked at Plant A (Table 1). Among the 29 food strains, four of them were isolated from sheep carcasses, eight from handmade Minas cheese, nine strains were obtained from chicken carcasses and eight from fresh mixed sausage. The 13 isolates from food processing plants were selected from different environments. Thus, six strains were isolated from Plant A (processing line of fresh mixed sausage) and the other seven strains were selected from Plant B (chicken slaughterhouse).

All *L. monocytogenes* strains were serotyped and six different serotypes were identified among the 44 isolates evaluated (Table 1). Most of the strains were identified as serotype 1/2b representing 38.6% ($n = 17$). The next most common was serotype 1/2c (25.1%, $n = 11$), followed by 1/2a (13.6%, $n = 6$), 4b (13.6%, $n = 6$), 4e (6.8%, $n = 3$) and 3a (2.3%, $n = 1$). In particular, serotype 1/2b was associated with strains isolated from chicken slaughterhouse (Plant B) and chicken carcasses, whereas serotype 1/2c was isolated in food and environmental samples from processing line of fresh mixed sausage (Plant A). Our data showed higher percentages (65.9%) of strains belonging to serotypes 1/2a, 1/2b and 4b in foods and food processing environment in this region, which is relevant since many studies have reported that the majority of cases and outbreaks of listeriosis is caused by strains of these serotypes (Farber & Peterkin, 1991; Miettinen et al., 1999; Vazquez-Boland et al., 2001). Our isolation data of serotype 1/2c showed that this serotype was frequently found in food and environment (Table 1). Similar information has been observed in some studies, reporting that this serotype is often found in food, however, it is rarely implicated in cases of human listeriosis (Kathariou, 2002). It is believed that the fact of serotype 1/2c usually is not involved in outbreaks of listeriosis, contrasts with the frequency of isolation of this serotype in food (Vitas & Garcia-Jalon 2004; Von Laer et al., 2009). Another significant observation of this study was that strains 4b, which is the serotype of *L. monocytogenes* most frequently associated with human listeriosis, was isolated from food and environmental samples. It is known that the isolates belonging to serotype 4b have proven pathogenic capacity even when not prevalent in foods (Borucki & Call, 2003; Torres, Sierra, Poutou, Carrascal, & Mercado, 2005). However, according to Bruhn, Vogel, and Gram (2005), strains belonging to this serotype are more sensitive to selective enrichment media used for isolation of *L. monocytogenes* than other serotypes, which could underestimate its presence in foods.

Serological and molecular techniques have been applied in several countries to trace back *L. monocytogenes* implicated in outbreaks (Autio et al., 2002; De Valk et al., 2005; Miettinen et al., 1999; Swaminathan & Gerner-Smidt, 2007). In this situation, serotyping still has limited discriminatory value, and techniques like PFGE provide enhanced discrimination for outbreak investigations and surveillance purposes. Therefore, the genetic fingerprint of the *L. monocytogenes* strains isolated in food and food production chain from southern Rio Grande do Sul (Brazil), were determined using PFGE protocol proposed for Graves and Swaminathan (2001). The strains were divided into 20 and 21 distinct profiles by *Asc*I ($D = 0.94$) and *Apa*I ($D = 0.95$) enzymes, respectively, and also named 1 to 20 and A to U, schematically represented in Table 1. They were considered to have the same profile when the numbers and position of the bands were indistinguishable, as well as, distinct when composed of one or more bands of difference (Rivoal et al., 2010; Sperry, Kathariou, Edwards, & Wolf, 2008).

Analyses of PFGE data for the 44 strains yielded a total of 29 different pulsotypes, increasing the discriminatory power up to 0.98. This highest D-value and the greatest number of distinguishable pulsotypes were obtained when the results of analysis of *Asc*I and *Apa*I were combined. Table 1 shows the *L. monocytogenes* serotypes and distribution of PFGE profiles according to sources over the years. Among the 29 pulsotypes, 20 PFGE patterns were unique, being composed of only one strain, isolated from different sources. The other 9 pulsotypes, grouped the remaining 24 strains: 2 were composed of four strains (P4 and P16), 2 grouped three strains (P9 and P12) and 5 consisted of only two strains (P1, P10, P19, P20 and P23). Our data also showed that there was a huge correlation between pulsotypes and serotypes, since 100% of isolates with identical PFGE patterns belonged to the same serotype.

In this study, strains of *L. monocytogenes* isolated from different sources at different times, when cleaved with both restriction endonucleases *Asc*I and *Apa*I, shared identical restriction patterns. This characteristic was observed between the strains 1 and 6 (pulsotype 10), which were isolated from a sheep carcass in the year 2001 and a refrigerated chicken carcass purchased from the

local grocery store in Pelotas in 2006 (Table 1), respectively. The same was observed with strains 33, 34, 60 and 62 (pulsotype 4): the strain 62 was isolated from a sheep carcass in 2001, while strains 33, 34 and 60 (isolated from Plant A) were obtained from a meat processing table (2003), a meat mincer (2002) and from fresh mixed sausage (2003), respectively (Table 1).

The presence of pulsotypes P4 and P10 in different years indicate that some *L. monocytogenes* subtypes may be ubiquitous in this region, adapting and persisting in different foods and environments, as was observed in other studies (Autio et al., 2002; Fugett et al., 2007; Lawrence & Gilmour, 1995; Thévenot et al., 2006). Beside this, it was establish that the same *L. monocytogenes* clones were found in different food sources or products, such as sheep carcass, chicken carcass and fresh mixed sausage (Table 1). This confirms previous reports, which indicate that similar isolates can be found in different product types from different geographical areas and periods (Autio et al., 2002; Filiousis, Johansson, Frey, & Perreten, 2009; Gianfranceschi, D'Ottavio, Gattuso, Bella, & Aurelli, 2009). Leite et al. (2006) evaluated *L. monocytogenes* in seven farms that produce sheep cheese in Portugal, these researchers found that the isolates from different industries, shared PFGE patterns identical to the same serotype. Moreover, they concluded that some pulsotypes were common in different farms, which are separated from each other, and that did not share flocks or equipment, suggesting the wide distribution of these genotypes in the environment.

According to our results, identical PFGE profiles in food and environmental isolates were identified among the strains that shared the pulsotypes P1, P4, P16 and P19 (Table 1). Strains 27 and 39 (P1) were both isolated in Plant A, from a stainless steel trolley used to transport meat and a food handler, respectively. The trolley used to transport the raw material (meat) may have played an important role in the spread of persistent clones within that industry (Plant A), since strains with similar pulsotypes were found in different periods and sampling points (Table 1). This situation was observed when these two strains 27 and 39 were cleaved with only a single restriction enzyme (*Asc*I) and they have been clustered in the same genotypic profile, with two other strains 32 and 58, which were isolated in the stuffer and the final product (fresh mixed sausage), with a percentage of similarity of 64% and 94% respectively (Fig. 1). On the other hand, the handlers can also contribute in a significant way to the spread of pathogens to foods through cross-contamination (Cocolin et al., 2009; Lomonaco et al., 2009). This was corroborated in our study, since strain 27 with the same genetic profile to the strain isolated in the handler, was isolated from a different place (trolley), showing a percentage of similarity of 100% (Fig. 1). It is interesting to note that the Plant A is a industry with a small staff population, therefore, these employees did not present a fixed function during the production process, circulating around the area of processing and performing different activities in a single day of work, as reported by Silva et al. (2004). According to Destro, Leitao, and Farber (1996) and Autio et al. (1999), the importance of employees in food contamination, may be higher when a single employee performs different functions along the processing line. The importance of food handlers on the contamination by *L. monocytogenes* was also highlighted by Cruz et al. (2008) who assessed a processing plant for smoked salmon in Brazil and observed that the bacterium was present in 34% of samples from food handlers. When strains isolated in this industry were characterized by PFGE, it was observed that strains in the

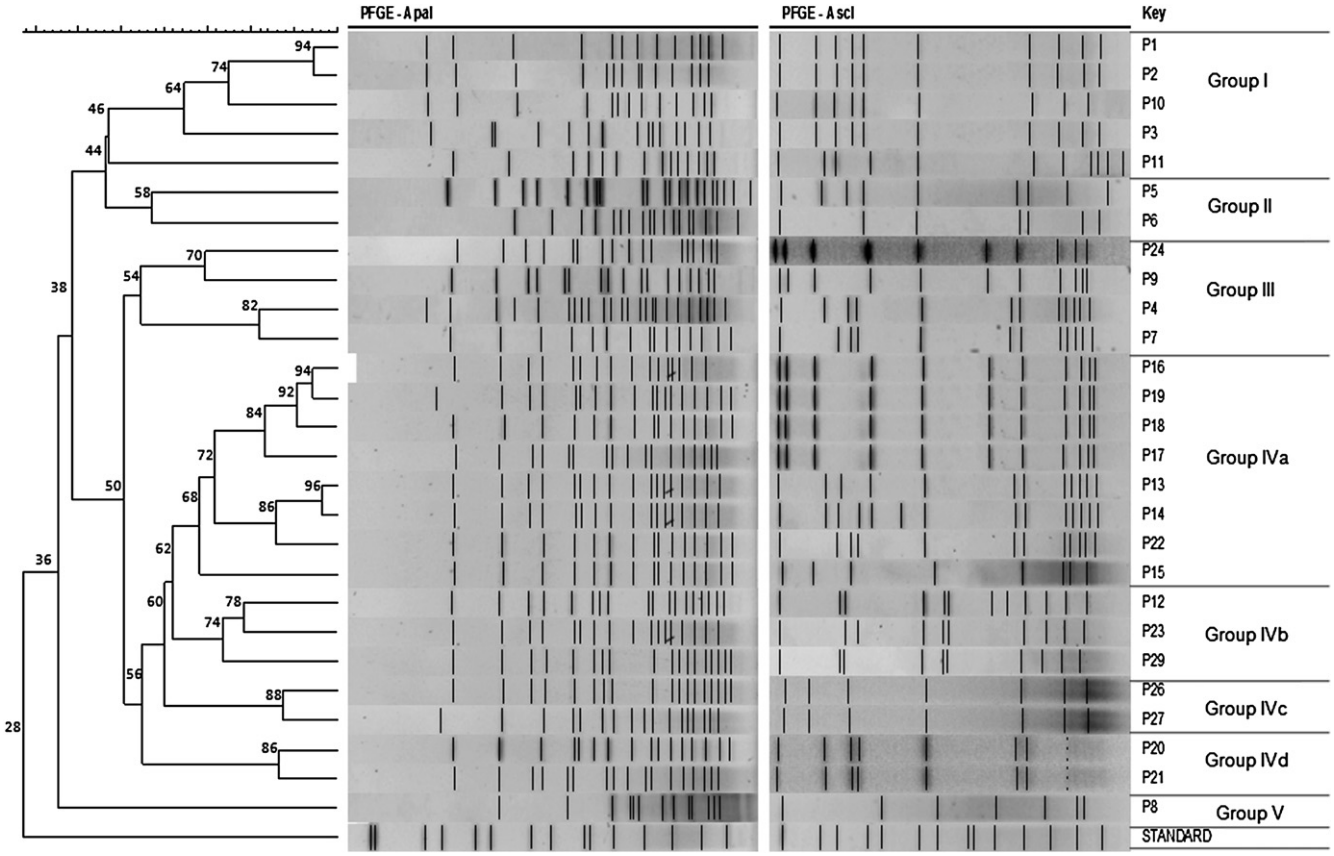

Fig. 1. Dendrogram representing the genetic relationship among 44 strains of *L. monocytogenes* analyzed by PFGE using the restriction enzymes *Apa*I and *Asc*I. Molecular Weight Marker: *S. enterica* serotype H 9812 Braenderup digested with *Xba*I.

handlers had the same genotypic profile of strains isolated in raw material and final product. These researchers suggested that this may be due to the fact that in this factory, the handlers played multiple functions and could work at the same day, with both, the raw material and the final product, similar to that observed in Plant A, evaluated in our study.

Strains belonging to pulsotypes P16 and P19 (serotype 1/2b) were all isolated in Plant B, being found in chicken carcasses and in the processing environment (Table 1). Interestingly, the comparison between food and environmental isolates showed that profiles of food isolates were corresponding to environmental isolates, suggesting that they may have been derived from the same source of contamination. As was observed with P19, it is interesting to note that the contamination in the floor, can possibly bring the pathogen to anywhere in the factory through the use of equipment and utensils, as well as by food handlers. Moreover, it is worth mentioning that the samples collected in this poultry slaughterhouse over a period of six months, had strains with identical genotypic profile, which demonstrates its persistence in this environment. The presence of persistent strains of *L. monocytogenes* in the environment of the Plant B, along with the fact of the collection had been made before start of slaughter activities, as was observed for P16 since strains with the same profile were isolated with an interval of approximately 45 days, suggests the possibility of contamination of poultry carcasses from the processing environment. This fact has been described by other researchers, who report that the contamination of the final product usually occurs during processing and is result of the presence of persistent strains on the environment (Autio et al., 2002; Heir et al., 2004; Miettinen et al. 1999; Thévenot et al., 2006).

In the present study, *L. monocytogenes* strains were considered to be persistent when the same pulsotype profile occurred in samples collected from the same food industry or even from the same equipment after an interval of at least 30 days. Furthermore, according to Thévenot et al. (2006), the persistence of *L. monocytogenes* in food processing environments occurs through the interaction of two main factors. Firstly due to measures of cleaning and disinfecting inefficient, since for the cleaning to be effective must reach the site of contamination in sufficient quantities and duration. In addition, another factor that may contribute to persistence is the nature of the strains, in view of the fact that some are better adapted to industrial environments.

Our data also showed that strains 9 and 10 (serotype 4e) isolated from refrigerated chicken obtained in the local supermarket of Pelotas and strain 11 (serotype 4e) isolated from a cloacal swab sample from chicken, showed the same PFGE profile, thus compounding the pulsotype P9 (Table 1). This result is very interesting because it demonstrates the importance of raw material in the introduction of strains in the industry environment, and subsequent contamination of the final product. Only these three strains belonged to serotype 4e and all were genetically indistinguishable. It is important to note that, even though samples of different brands marketed in the southern region had been collected, these strains were isolated from the final product of Plant B at the same time that strain 11 was isolated from cloacal swabs from poultry slaughtered by the same industry.

The 15 strains of *L. monocytogenes* isolates in Plant A, were divided into 11 pulsotypes. Seven (P2, P4, P5, P15, P20, P24 and P27) were found in food, however, only P27 was found in the raw material. It is interesting to note that the final product (fresh mixed sausage) had six different pulsotypes, five of which were unique pulsotypes because they were not found in any other sample. Among the strains isolated in the processing environment of Plant A, we identified five pulsotypes (P1, P3, P4, P6 and P8), where P4 was the only one that brought together both strains of *L. monocytogenes* found in environment and food (Table 1). Furthermore, six pulsotypes were detected in 10 strains of *L. monocytogenes* isolated in Plant B. Among these, five (P18, P19, P21, P25 and P28) were isolated only in the processing environment, while pulsotype P16 was isolated in food and environment (Table 1). Interestingly, only one pulsotype was found in the final product (chicken carcass), although six different pulsotypes were identified from the processing environment from Plant B. This means that some strains of *L. monocytogenes*, have better survivability than others due to its ability to withstand the various stages of industrial processing.

The 4 strains of *L. monocytogenes* isolated from sheep carcasses were grouped into four pulsotypes (P4, P7, P10 and P22), two of which were exclusive, P7 and P22. It is important to note that these two strains with unique pulsotypes, strain 12 (serotype 3a) and strain 19 (serotype 1/2b), were isolated from the same sheep carcass, suggesting that different sources may be contaminating the carcasses in the slaughterhouse or that different strains occupied the same niche. It is notable that in the slaughterhouse where the sheep were slaughtered, none of the samples were collected from the environment. However, when the sheep were sampled prior to slaughter no *L. monocytogenes* was isolated from their feces (Antoniollo, Bandeira, Jantzen, Duval, & Silva, 2003), indicating that the contamination occurred during the skinning process.

Six strains of *L. monocytogenes* isolated from refrigerated chicken carcasses purchased in a grocery store, were divided into five pulsotypes (P9, P10, P11, P14 and P17), with pulsotypes P11, P14 and P17 being exclusive. In addition, eight strains isolated from handmade Minas cheese were also divided into five pulsotypes (P12, P13, P23, P26 and P29), only found in this source (Table 1).

PFGE typically provides high sensitivity for the identification of differences in molecular subtypes. Eight clusters were identified among 44 foods and environment strains from different geographic areas in southern Rio Grande do Sul, Brazil. The dendrogram was generated by the combination of the results of *Asc*I and *Apa*I, and PFGE pulsotypes were divided into five main groups, with group IV being subdivided in groups IVa, IVb, IVc and IVd (Fig. 1). Nevertheless, due to problems of DNA degradation during digestion, it was not possible to perform an adequate visualization of the bands from profile S, and the pulsotypes P25 and P29 could not be used to construct the dendrogram (Table 1). Five clusters (II, IVa, IVc, IVd and V) harbored strains of the same serotype (Table 1). The cluster IVa has been the predominant group, gathering eight pulsotypes and 12 of 44 strains of *L. monocytogenes* analyzed. However, the remaining 3 clusters were formed by strains with different serotypes. The cluster I was composed of strains of serotypes 1/2a ($N = 3$) and 1/2c ($N = 4$); in addition the Cluster III contained strains 1/2c ($N = 4$), 3a ($N = 1$), 4b ($N = 1$) and 4e ($N = 3$), as well as the Cluster IVb which consisted of serotypes 1/2a ($N = 3$), 1/2b ($N = 2$) and 4b ($N = 1$).

Based on the observations of dendrogram, PFGE pulsotypes were divided into eight distinct clusters: The strains of cluster IVb (P12, P23 and P29) were isolated only in samples of handmade Minas cheese, sold in local grocery store of Pelotas in 2006. Strains belonging to clusters II and V were isolated only in the Plant A, between the years 2002–2003. Cluster II was composed of pulsotypes P5 and P6, which contained a single, strain each, with the similarity level of 58%. The cluster V was highly divergent from all other isolates, with a level of similarity of only 36%, indicating that even strains collected from a limited geographic area could show a very high variability in the PFGE profile.

IVc and IVd clusters contained strains of the same serotype but from different sources. The cluster IVc, contained two strains of serotype 4b, isolated from meat used as raw material for fresh mixed sausage (Plant A) and another isolated from handmade

Minas cheese, isolated in 2002 and 2006, respectively. Group IVd consisted of strains of serotype 1/2b, being isolated from two fresh mixed sausages (Plant A) and one isolated from a drain (Plant B), in studies conducted in 2002–2003 and 2006, in this order. The similarity level among pulsotypes that formed the genomic groups IVc (P26 and P27) and IVd (P20 and P21) were 88% and 86%, respectively.

Cluster IVa, in addition to grouping the largest number of strains (12), all of serotype 1/2b, was most frequently detected among strains isolated from Plant B. Among the seven strains detected in this Plant during the year 2006, four strains were isolated from the food processing environment and three strains were isolated from chicken carcasses. Furthermore, belonging to the group IVa another strain isolated from handmade Minas cheese, two strains isolated from refrigerated chicken (both obtained from the local supermarket in 2006), one strain isolated from sheep carcass in 2001 and one strain of fresh mixed sausage isolated in the processing Plant A in 2002. Among the pulsotypes clustered in genomic group IVa, the profiles of P13 and P14, which include strains of the same serotype (1/2b), though from different origins, showed a similarity of 96%. Likewise, the pulsotypes P16 and P19, which also included strains of serotype 1/2b, but all isolated from Plant B, showed a similarity level of 94%. The similarity level of the other pulsotypes compared to the profiles previously reported (P16 and P19) was below 90%, with the exception of the profile P15, which showed a similarity of 68%, compared to all other pulsotypes which were included in this cluster.

Clusters I and III grouped strains of different origins and serotypes. Group I harbored 7 strains with two different serotypes, while group III was formed by 9 strains, including four different serotypes. Among the seven strains included in cluster I, four belonged to serotype 1/2c and were isolated from the Plant A during the years 2002–2003, from the trolley, the handler, the packaging equipment and the final product (fresh mixed sausage). The remaining three strains, serotypes 1/2a, were isolated from a sheep carcass in 2001 and from two refrigerated chickens obtained in a local supermarket in 2006. Among the five pulsotypes of genomic group I, P1 and P2 showed a similarity level of 94%, while the similarity level of other profiles of this group as compared to previous genotypic profiles was below 74%.

Lastly, cluster III grouped nine strains: one strain belonging to serotype 4b was isolated in 2002 in a sample of fresh mixed sausage; 2 strains of serotype 4e, which were isolated in 2006 from refrigerated chicken obtained in a local grocery store and one strain of serotype 4e which was also isolated in 2006 from a chicken cloacal swab sample. Besides these, three strains of serotype 1/2c were isolated from the Plant A in 2002–2003, from the working tables, mincer and fresh mixed sausage; one strain of serotype 1/2c was isolated from a sheep carcass in 2001 and one strain of serotype 3a was isolated from a sheep carcass in 2001. The similarity level between the profiles presented in P4 and P7 (group III) was 82%, while the other pulsotypes of this group showed similarities below 70% compared to the P4 and P7.

These results indicate that strains of *L. monocytogenes* with identical restriction patterns were isolated from foods of different origins and in environments at different times, in southern Rio Grande do Sul, Brazil. One strain isolated in the final product (fresh mixed sausage) from a food processing industry in 2003, showed the same genotypic profile as that of a strain isolated from sheep carcass two years before (2001). Similar situation occurred with strains isolated from sheep carcass and refrigerated chicken carcass, which showed the same genotypic profile, despite having been isolated five years apart. Moreover, some strains of *L. monocytogenes* were periodically isolated from the same type of product or processing environment, suggesting a possible persistence of these strains at these sources. According to Autio et al. (2002), the recovery of *L. monocytogenes* strains, which share identical restriction patterns in different foods and over different years, indicates that the strains are not geographically or temporally specific. Besides, certain strains of *L. monocytogenes* may be more widely distributed in nature and therefore are more easily introduced and reintroduced in processing plants via raw materials, with some strains causing persistent contamination. López et al. (2008) reported that persistent strains have specific characteristics, such as better adherence to food contact surfaces and increased resistance to disinfectants, allowing them to survive and adapt in the processing environment, causing persistent contamination of products.

Furthermore, some profiles were found only in a particular food, as in the case of some pulsotypes which were isolated only in handmade Minas cheese. However, this result should be evaluated carefully, considering that it may be a coincidence, although this may indicate that these pulsotypes of *L. monocytogenes* are associated with the cheese or the raw material, or with its processing environment or sale. Nevertheless to prove this hypothesis, further studies are needed to reveal if these strains are more adapted to that source.

In addition, the current study also showed that the raw material could be an important way to introduce *L. monocytogenes* in the environment of chicken slaughterhouse (Plant B), since the same serotype (4e) and pulsotype (P9) found in *L. monocytogenes* isolated from a cloacal swab sample from chicken prior to slaughter, was also isolated in a refrigerated chicken carcass obtained in a local grocery store, produced by the same industry.

In conclusion, our results provide an important insight into the potential presence of *L. monocytogenes* in different foods and food production chains in southern Rio Grande do Sul, Brazil. *L. monocytogenes* strains with identical restriction patterns were isolated from different foods and environments at different times. Moreover, the presence of persistent strains in these food processing environments emphasizes the significance of cross-contamination in the food production chain. The presence of these persistent strains could be mostly due to ineffectiveness of cleaning and sanitization procedures, which allow survival and adaptation of *L. monocytogenes* strains in the food processing environment, resulting in persistence and contamination of final products.

Authors contributions

Conceived and designed the experiments: KSM, GBM, AEL, MRIC, WPS. Performed the experiments: KSM, GBM. Analyzed the data: KSM, GBM, AEL, WPS. Contributed reagents/materials/analysis tools: DBM, MRIC, WPS. Wrote the paper: KSM, WPS.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.foodcont.2012.04.014.

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