Food Microbiology 84 (2019) 103234

Contents lists available at ScienceDirect

Food Microbiology

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

Distribution, adhesion, virulence and antibiotic resistance of persistent *Listeria monocytogenes* in a pig slaughterhouse in Brazil

Mallu Jagnow Sereno[a,*], Cibeli Viana[b], Kadigia Pegoraro[a], Danilo Augusto Lopes da Silva[b], Ricardo Seiti Yamatogi[b], Luís Augusto Nero[b], Luciano dos Santos Bersot[a]

[a] Universidade Federal do Paraná - Setor Palotina, Departamento de Ciências Veterinárias, Rua Pioneiro, 2153, Jardim Dallas, 85950-000, Palotina, PR, Brazil
[b] Universidade Federal de Viçosa, Departamento de Veterinária, Avenida PH Rolfs, s/n, Campus Universitário, 36570-900, Viçosa, MG, Brazil

ARTICLE INFO

Keywords:
Biofilm
Conveyor belt
Cutting room
Disk diffusion
Minimum inhibitory concentration
PFGE

ABSTRACT

Listeria monocytogenes is a relevant pathogen usually associated with meat and ready-to-eat products. This study aimed to assess the distribution, adhesion, virulence and antibiotic resistance of *L. monocytogenes* in a pork production chain. Environment, carcass and food samples (n = 894) were obtained from different steps of a pork production chain over a 6-month period (10 samplings), including from farms and the slaughterhouse (reception, slaughtering, processing, storage and end products). *L. monocytogenes* was detected in samples from the reception (lairage floor, 1/10), slaughtering (drains, 2/20) and cutting room stages (conveyor belts in the final packing stage – 11/20, knife – 1/40, and cutting boards – 1/20). Positive results for conveyor belts were recorded in seven consecutive samplings. *L. monocytogenes* isolates (n = 87) were characterized as belonging to serogroup IVb and presented positive PCR results for *inlA*, *inlB*, *inlC*, *inlJ*, *hlyA*, *plcA*, *actA* and *iap*. Isolates were selected according to the original samples (n = 31) and subjected to Pulsed Field Gel Electrophoresis (PFGE), demonstrating their high clonal identity (98.4–100%). According to PFGE results and their original samples, isolates were selected (n = 16) and subjected to phenotypic assay to assess their adhesion potential and tested for resistance against 15 antibiotics; all tested isolates presented weak adhesion potential and were resistant to ampicillin. The present study demonstrated the persistence of *L. monocytogenes* in the pork processing facility, indicating the potential risk for cross-contamination with a potential virulent and resistant clone.

1. Introduction

Listeria monocytogenes is the causative agent of listeriosis, a foodborne disease that is highly associated with ready-to-eat (RTE) products and meats (Camargo et al., 2017). Listeriosis is particularly risky for elderly, immunocompromised individuals, pregnant women and newborns, whereas in healthy people it is characterized by gastrointestinal disorders. Despite the low morbidity, listeriosis is a concern in public health due to its severity, leading to hospitalization in 90% of confirmed cases, and mortality in 20–30% of cases (EFSA, 2015; Garner and Kathariou, 2016; Lamont et al., 2011; Swaminathan and Gerner-Smidt, 2007).

L. monocytogenes strains isolated from food and human cases of listeriosis belong mainly to serotypes 1/2a, 1/2b, 1/2c and 4b (Borucki and Call, 2003; Orsi et al., 2011; Swaminathan and Gerner-Smidt, 2007). Strains of these serotypes contain the main virulence genes related to listeriosis, located in the Pathogenicity Island of *Listeria* 1: *prfA, plcA, hly, mpl, actA* and *plcB* (De las Heras et al., 2011; Mertins et al., 2007; Vázquez-Boland et al., 2001). These genes, associated with internalin genes (*inlA, inlB, inlC* and *inlJ*), confer the ability of *L. monocytogenes* to internalize, survive and disseminate in host cells (Camargo et al., 2016; De las Heras et al., 2011; Liu et al., 2007; Pizarro-Cerda et al., 2012).

Antibiotics are the usual therapy used to fight listeriosis, especially its invasive form (Swaminathan and Gerner-Smidt, 2007). β-lactams (penicillin and ampicillin), with or without gentamicin, are the main antibiotics considered for listeriosis treatments; vancomycin and trimethoprim/sulfamethoxazole can be used as alternative therapy for penicillin-allergic patients (Pagliano et al., 2017; Temple and Nahata, 2000). *L. monocytogenes* strains are considered relatively sensitive to antibiotics, but resistance is increasing as antimicrobials are being inadequately used in animal production and human medicine (EFSA, 2008; Granier et al., 2011; Kovacevic et al., 2013). So, the effectiveness of listeriosis therapy is directly linked to the reliable surveillance of antibiotic resistance in *L. monocytogenes* strains obtained from food and clinical cases (Gómez et al., 2014).

* Corresponding author. Department of Veterinary Science, Federal University of Paraná, Palotina, 2153 – Dallas, CEP: 85950-000, Paraná, Brazil.
E-mail address: mallusereno@gmail.com (M.J. Sereno).

https://doi.org/10.1016/j.fm.2019.05.018
Received 5 February 2019; Received in revised form 28 May 2019; Accepted 29 May 2019
Available online 30 May 2019
0740-0020/

Although swine are not considered to be the usual carriers of *L. monocytogenes*, pork and pork products (mainly RTE) are highly susceptible to contamination by this pathogen during processing, due to cross-contamination in industrial facilities via equipment and utensils (Bonaventura et al., 2008; Bonsaglia et al., 2014; Møretrø and Langsrud, 2004). *L. monocytogenes* is known for its ability to survive in harsh conditions at such sites, forming biofilms and being a constant source of contamination (Barros et al., 2007; Carpentier and Cerf, 2011). In addition, *L. monocytogenes* is resistant to different concentrations of NaCl and nitrates, it can multiple at low temperatures and it survives under modified atmospheres, leading to its potential presence in pork products, as described in other studies (Camargo et al., 2017; Gandhi and Chikindas, 2007; Zeng et al., 2014).

Given the importance of controlling contamination by *L. monocytogenes* in food processing facilities, this study aimed to identify the presence, diversity, virulence potential, and antibiotic resistance of this foodborne pathogen in a pork processing chain.

2. Material and methods

2.1. Sampling

Ten different finishing pig farms and a swine slaughterhouse inspected by the Brazilian Ministry of Agriculture (MAPA, Brasília, DF, Brazil) were selected for the present study for sampling at different steps of pig production, slaughtering and pork processing. Sampling was conducted from September 2016 to February 2017, at approximately 15-day intervals.

A total of 894 samples were collected. On pig farms, samples of water (n = 10), feed (n = 10) and barn floor (feces) (n = 10) were collected on the day prior to transport to the slaughterhouse. In the slaughterhouse, samples were obtained in different sectors and environments: 1) in the pig reception area, the lairage floor (feces) was sampled (n = 10); 2) in the slaughtering environment, samples were collected from walls (n = 20), drains (n = 20), evisceration tables (n = 20), steel gloves from employees (n = 18), and knives (n = 20); 3) during slaughtering, 100 pigs were sampled in four slaughtering steps: before bleeding (n = 100), after buckling (n = 100), after evisceration (n = 100) and after the final washing (n = 100); 4) in the cold chamber, samples were collected from the walls (n = 20) and floor (n = 20); 5) in the cutting room, samples were collected from the walls (n = 20), drains (n = 20), cutting table (n = 20), deboning table/conveyor belts (n = 40), conveyor belts for final packing (n = 20), steel gloves from employees (n = 40), knives (n = 40), cutting boards (n = 20), plastic boxes (n = 20) and pork cuts (n = 76). Samples of carcasses, meat cuts and surfaces were obtained by swabbing four 100 cm^2 areas with sterile sponges (3M Microbiology, St. Paul, MN, USA) pre-moistened with 10 mL NaCl 0.85% (w/v); glove and knife samples comprised a pool of four units, also collected using previously moistened sterile sponges; the drain samples were obtained by swabbing the inner area of approximately 400 cm^2, also with sterile sponges; feces from barn and lairage floors were sampled by using the footprint technique (Botteldoorn et al., 2003). All samples were placed in sterile bags under refrigeration until analysis.

2.2. Listeria spp. detection and identification

Detection of*L. monocytogenes* was performed based on ISO 11.290–1 (ISO, 1996, 2004). For each carcass sample, two of the swabbed sponges were transferred to a sterile bag containing 180 mL of buffered peptone saline (BPS, with peptone at 0.01%, w/v, and NaCl at 0.85%, w/v) and homogenized at 230 rpm for 1 min (Stomacher 400®, Seward, Worthing, UK). Then, aliquots of 40 mL were centrifuged at 2,000 × *g* for 15 min, the supernatant discarded, and the pellet suspended in 10 mL of *Listeria* Enrichment Broth (LEB, Oxoid, Basingstoke, UK), then incubated at 30 °C for 24 h. For water and feed samples, aliquots of 25 mL or g were transferred to 225 mL of LEB (Oxoid), homogenized and incubated at 30 °C for 24 h. For other samples, one of the swabbed sponges was transferred to a sterile bag containing 90 mL of LEB (Oxoid), homogenized and incubated at 30 °C for 24 h. Aliquots of the obtained cultures in LEB (0.1 mL) were transferred to Fraser broth (Oxoid), incubated at 35 °C for 48 h, and then streaked onto plates containing chromogenic *Listeria* agar (Oxoid) and Oxford *Listeria* agar (Oxoid), and incubated at 35 °C for up to 48 h. Colonies that presented characteristic morphologies of *Listeria* spp. were subjected to phenotypic tests for species identification (catalase, fermentation of dextrose, xylose, rhamnose and mannitol, β-hemolysis, motility and Gram staining).

Isolates identified as *L. monocytogenes* (n = 87) and *L. innocua* (n = 16) by phenotypic tests were subjected to a multiplex PCR protocol for serotyping, targeting *prs*, *lmo1118*, *lmo0737*, *ORF2819* and *ORF2110* (Doumith et al., 2004a). Isolates were grown in trypticase soya broth (TSB, Oxoid) at 30 °C for 18 h, and then the DNA was extracted using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) and lysozyme (at 10 mg/mL, Promega). Primer sequences, PCR conditions and sizes of the products are detailed in Table 1. PCR products were subjected to electrophoresis with agarose 2% (w/v) containing GelRed (Biotium Inc., Hayward, CA, USA) and visualized in a transilluminator (L-PIX-HE, Loccus, Cotia, SP, Brazil).

2.3. L. monocytogenes and L. Innocua characterization

2.3.1. Pulsed Field Gel Electrophoresis (PFGE)

Isolates of *L. monocytogenes* (n = 31) and *L. innocua* (n = 6) were selected based on the origin of the sample and subjected to PFGE as described by Graves and Swaminathan (2001), following PulseNet (Center for Diseases Control and Prevention, CDC, Atlanta, GE, USA) recommendations. Briefly, isolates were cultured overnight at 30 °C in TSB (Oxoid), subjected to lysis and DNA extraction using lysozyme (20 mg/mL, at 55 °C for 10 min) and proteinase K (20 mg/mL, at 55 °C for 10 min), and digested using the restriction enzymes *AscI* (40 U/sample) and *ApaI* (50 U/sample), New England Biolabs Inc., Ipswich, MA, USA). Plugs were prepared and subjected to electrophoresis using CHEF-DR II (BioRad, Philadelphia, NY, EUA), with the following running parameters: 6 V/cm, initial switch time of 4 s, final switch time of 63.8 s and length of 20 h. The obtained gels were stained in a GelRed bath (Biotium) and the bands were visualized in a transilluminator (Loccus). The images were analyzed using the software BioNumerics 6.6.4 (Applied Maths NV, Sint-Martens-Latem, Belgium) and the pulsotypes were compared using the unweighted pair group method with arithmetic mean (UPGMA) and dice coefficient of 5%. A standard digestion of *Salmonella* Braenderup ATCC BAA-664 with *XbaI* was used for gel normalization.

2.3.2. Adhesion potential

Based on PFGE and sample origin, 16 *L. monocytogenes* isolates were selected and subjected to a phenotypic assay in order to assess their adhesion potential (Stepanović et al., 2000; Lee et al., 2017). Briefly, each strain was cultured overnight at 30 °C in TSB (Oxoid), diluted to achieve a turbidity similar to tube 0.5 (McFarland scale, corresponding to approximately 10^8 CFU/mL) and transferred (200 μL) in triplicate to wells of a 96-well flat-bottom polystyrene microplate (NEST Scientific, Rahway, NJ, USA). Plates were incubated at 35 °C for 48 h and washed three times with sterile PBS (250 μL per well) to remove the sessile bacterial cells. Bacterial cells adhering to the wells were fixed with 200 μL of methanol (99%, v/v) for 15 min and allowed to dry at room temperature. Then, fixed bacterial cells were stained with crystal violet (200 μL per well, 1%, w/v) and washed three times with distilled water, after which the plates were left at room temperature for drying. Finally, glacial acetic acid (33%, v/v) was added to the wells and optical reading (λ = 595 nm) was performed in a microplate reader (Anthos 2010, Biochrom Ltd., Cambridge, UK). For each prepared microtiter

Table 1
Primers, annealing and product sizes of PCRs used to characterize *Listeria* spp. isolates obtained from different sites in a pork production chain located in Brazil.

Reaction	PCR	Target gene	Function	Primers	Annealing[a]	Product size (bp)	Reference
1	multiplex	*prs*	identification	F: GCTGAAGAGATTGCGAAAGAAG R: CAAAGAAACCTTGGATTTGCGG	53	370	Doumith et al. (2004b)
		lmo0737	serotyping	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC		691	
		lmo1118	serotyping	F: AGGGGTCTTAAATCCTGGAA R: CGGCTTGTTCGGCATACTTA		906	
		ORF2819	serotyping	F: AGCAAAATGCCAAAACTCGT R: CATCACTAAAGCCTCCCATTG		471	
		ORF2110	serotyping	F: AGTGGACAATTGATTGGTGAA R: ATCCATCCCTTACTTTGGAC		597	
2	multiplex	*inlA*	invasion	F: ACGAGTAACGGGACAAATGC R: CCCGACAGTGGTGCTAGATT	55	800	Liu et al. (2007)
		inlC	invasion	F: AATTCCCACAGGACACAACC R: CGGGAATGCAATTTTTCACTA		517	
		inlJ	invasion	F: TGTAACCCCGCTTACACAGTT R: AGCGGCTTGGCAGTCTAATA		238	
3	simplex	*inlB*	invasion	F: TGGGAGAGTAACCCAACCAC R: GTTGACCTTCGATGGTTGCT	55	884	Liu et al. (2007)
4	simplex	*plcA*	vacuole lysis	F: CTGCTTGAGCGTTCATGTCTCATCCCCC R: ATGGGTTTCACTCTCCTTCTAC	60	1,484	Notermans et al. (1991)
5	multiplex	*hlyA*	intracellular parasitism	F: GCAGTTGCAAGCGCTTGGAGTGAA R: GCAACGTATCCTCCAGAGTGATCG	60	456	Paziak-Domanska et al., 1999
		actA	intracellular motility	F: CGCCGCGGAAATTAAAAAAAGA R: ACGAAGGAACCGGGCTGCTAG		839	Suarez and Vazquez-Boland (2001)
		Iap	invasion (p60)	F: ACAAGCTGCACCTGTTGCAG R: TGACAGCGTGTGTAGTAGCA		131	Furrer et al. (1991)

[a] PCR conditions: reaction 1: 25 µL, 94 °C for 3 min, 35 cycles at 94 °C for 0.40 min, 53 °C for 1.15 min, 72 °C for 1.15 min, 72 °C for 7 min; reaction 2: 25 µL, 94 °C for 2 min, 30 cycles at 94 °C for 20 s, 55 °C for 20 s, 72 °C for 50 s, 72 °C for 2 min; reaction 3: 25 µL, 94 °C for 2 min, 30 cycles at 94 °C for 20 s, 55 °C for 20 s, 72 °C for 50 s, 72 °C for 2 min; reaction 4: 25 µL, 95 °C for 2 min, 35 cycles at 95 °C for 15 s, 60 °C for 30 s, 72 °C for 1.5 min, 72 °C for 10 min; reaction 5: 25 µL, 95 °C for 2 min, 35 cycles at 95 °C for 15 s, 60 °C for 30 s, 72 °C for 1.5 min, 72 °C for 10 min.

plate, three wells were inoculated with TSB (Oxoid) only as the negative control. This assay was conducted in three independent repetitions.

Based on the optical readings, the isolates were classified according Stepanović et al. (2000) by considering the mean optical densities (OD), compared to the three standard deviations above the mean OD of the negative control (ODc):

Non-adherent: OD < ODc
Weakly adherent: ODc < OD < 2.ODc
Moderately adherent: 2.ODc < OD < 4.ODc
Strongly adherent: OD > 4.ODc

2.3.3. Virulence-related genes

L. monocytogenes isolates (n = 87) were subjected to PCR targeting the virulence-related genes *inlA, inlB, inlC, inlJ, plcA, hlyA, actA*, and *iap* (Liu et al., 2007; Rawool et al., 2007). Primer sequences, PCR conditions and product sizes are described in Table 1. PCR products were stained, subjected to electrophoresis and visualized as described above.

2.3.4. Antibiotic resistance

The selected *L. monocytogenes* isolates (n = 16) were also subjected to the disk-diffusion method (CLSI, 2017) to identify their resistance to a panel of 12 antibiotics from nine classes: 1) aminoglycosides: gentamicin (10 µg), kanamycin (30 µg) and tobramycin (10 µg), 2) ansamycins: rifampicin (5 µg), 3) carbapenem: meropenem (10 µg), 4) phenicols: chloramphenicol (30 µg), 5) lincosamides: clindamycin (2 µg), 6) fluoroquinolones: ciprofloxacin (5 µg), 7) tetracyclines: minocycline (30 µg) and tetracycline (30 µg), 8) glycopeptides: vancomycin (30 µg), 9) macrolides: erythromycin (15 µg). *Staphylococcus aureus* ATCC 25923 was used as a control and interpretation was based on CLSI recommendations for *Enterococcus* sp. (vancomycin) and *Staphylococcus* sp., the isolates being classified as presenting resistance, intermediate resistance or susceptibility to the tested antibiotic.

The selected isolates were also subjected to the broth microdilution assay for antibiotic susceptibility (CLSI, 2012) to determine the minimum inhibitory concentration (MIC) for ampicillin, penicillin and for the combination of sulfamethoxazole and trimethoprim, as determined for *L. monocytogenes* (CLSI, 2006). *S. aureus* ATCC 29213 was used as a control and the results were interpreted according to previously established breakpoint values, classifying the isolates as sensitive or resistant (CLSI, 2017).

3. Results

Listeria spp. was detected in 18 samples, as detailed in Table 2. Only some of the environmental samples (lairage, slaughtering and cutting room) were positive for *Listeria* spp., 16 for *L. monocytogenes* (mainly the conveyor belt for final packing) and two for *L. innocua*. None of the samples from pig farms (water, feed and feces from barn floors), carcasses or end products were positive for *Listeria* spp. (Table 2). Table 3 shows the distribution of positive results for *L. monocytogenes* over the

Table 2
Frequency of positive results for *Listeria* at different sites in a pig slaughterhouse in Brazil.

Slaughterhouse site	Sample*	n	*L. monocytogenes*	*L. innocua*
Lairage	Floor	10	1	1
Slaughtering	Drain	20	2	0
Cutting room	Drain	20	0	1
	Conveyor belt (final packing)	20	11	0
	Knife	40	1	0
	Cutting board	20	1	0

*samples from pig barns (n = 30), slaughtering (wall, n = 20, evisceration table, n = 20, steel glove, n = 18, knife, n = 20), carcasses (before bleeding, n = 100, after buckling, n = 100, after evisceration, n = 100, after final washing, n = 100), cold chamber (wall, n = 20, floor, n = 20), cutting room (wall, n = 20, cutting table, n = 20, deboning table/conveyor belt, n = 40, steel glove, n = 40, plastic box, n = 20) and end cuts (n = 76) were negative for Listeria spp. based on the adopted methodology.

Table 3
Distribution of positive results (×) for *Listeria monocytogenes* in samples obtained from different sites through the study period in a pig slaughterhouse located in Brazil.

Slaughterhouse site	Sample	Sampling period									
		2016 Sep	2016 Oct	2016 Nov (1)	2016 Nov (2)	2016 Nov (3)	2016 Dec	2017 Jan (1)	2017 Jan (2)	2017 Jan (3)	2017 Feb
Lairage	Floor							×			
Slaughtering	Drain				×			×			
Cutting room	Conveyor belt (final packing)	×	×	×	×	×	×	×			
	Knife								×		
	Cutting board					×					

sampling period. The cutting room was the local where the pathogen isolation occurred more times. When considering the results obtained in this place, utensil and equipment samples presented the pathogen. Among the utensils, it was possible to find the agent in one knife that was being used to make cuts at the moment of collection, and one cutting board sanitized prior to use by handlers. About the equipment, all eleven positive samples were obtained from seven distinct collections, from September 2016 to January 2017, from the same conveyor belt that carried the meat cuts to final packaging.

A total of 103 isolates were isolated from *Listeria* spp. positive samples, being identified by phenotypic tests as *L. monocytogenes* (n = 87) and *L. innocua* (n = 16). All *L. monocytogenes* isolates were identified as belonging to serogroup IVb (amplification of PCR products for *prs*, *ORF2110* and *ORF2819*). These isolates presented two genetic profiles by PFGE that shared 98.4% of similarity, while all *L. innocua* isolates presented a single genetic profile (Fig. 1). The selected *L. monocytogenes* isolates (n = 16) were characterized as weakly adherent, based on the classification indicated by Stepanović et al. (2000). All *L. monocytogenes* isolates (n = 87) presented amplification products for all virulence-related genes assessed in this study (*inlA, inlB, inlC, inlJ, plcA, hlyA, actA* and *iap*).

The frequencies of *L. monocytogenes* isolates that presented resistance to the tested antibiotics are presented in Table 4. In general, it was found resistance to clindamycin, kanamycin, tetracycline and erythromycin in the disk-diffusion test. In the MIC test, the isolates showed 100% (n = 16) resistance to ampicillin (breakpoint > 2 μg/ml), 18,8% (n = 03) to penicillin (breakpoint > 2 μg/ml), and 6,2% (n = 01) to the combination of sulfamethoxazole and trimethoprim (breakpoint ≥ 4/76 μg/ml), following the CLSI interpretation. Two isolates presented resistance to three or more classes, therefore, classified as multiresistant (Table 5). One of the multiresistant isolate was obtained from the lairage floor and showed resistance to four different classes of antibiotics (kanamycin, clindamycin, erythromycin, tetracycline and ampicillin) and the another multiresistant isolate was collected from the conveyor belt final packing and was resistance to clindamycin, ampicillin, and to the combination of sulfamethoxazole and trimethoprim.

Table 4
Results for antibiotic resistance of *L. monocytogenes* isolates (n = 16) obtained from different stages of pork processing.

Assay	Antibiotic	Result		
		Resistance	Intermediate resistance	Susceptible
Disk-diffusion	Gentamicin	0	0	16
	Kanamycin	1	0	15
	Tobramycin	0	0	16
	Rifampicin	0	0	16
	Meropenem	0	0	16
	Chloramphenicol	0	0	16
	Clindamycin	5	11	0
	Ciprofloxacin	0	0	16
	Minocycline	0	0	16
	Tetracycline	1	0	15
	Vancomycin	0	0	16
	Erythromycin	1	1	14
MIC	Ampicillin	16	0	0
	Penicillin	3	0	13
	Sulfamethoxazole & trimethoprim	1	0	15

Table 5
Antibiotic resistance profiles of selected isolates of *L. monocytogenes* (n = 16) and their origin from a pork processing facility.

Resistance profile[a]	n	Origin
AMP	09	conveyor belt (final packing); drain; knife
AMP-PEN	02	conveyor belt (final packing)
AMP-CLI	02	conveyor belt (final packing); cutting board
AMP-CLI-PEN	01	conveyor belt (final packing)
AMP-CLI-SUT	01	conveyor belt (final packing)
AMP-CLI-KAN-ERY-TET	01	lairage

[a] AMP: ampicillin, PEN: penicillin, CLI: clindamycin, SUT: Sulfamethoxazole & trimethoprim, KAN: kanamycin, ERY: erythromycin, TET: tetracycline.

4. Discussion

L. monocytogenes was not recorded in the samples obtained from pig farms and a low contamination rate was found in the lairage at the slaughterhouse (Table 2). In general, infected animals are asymptomatic carriers that shed the bacterium in their feces, and to evaluate the sanitary status of the sampled lots, it was decided to collect feces by using the footprint technique. Since we did not find positive samples, this proves that pigs are not important in the spread of *L. monocytogenes* to the slaughter environment, although their relevance in disseminating

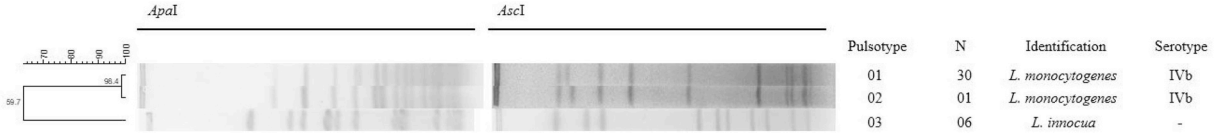

Fig. 1. PFGE patterns after the macrorestriction (*Apa*I and *Asc*I enzymes) and serotypes (Doumith et al., 2004a) of 37 *Listeria* sp. isolates obtained from samples from a swine slaughterhouse during ten collections carried out between September 2016 and February 2017. Similarities between the identified PFGE pulsotypes were compared using the unweighted pair group method with arithmetic mean (UPGMA) and dice coefficient of 5%.

other microorganisms to this environment is known (Boscher et al., 2012). Also, none of the sampled carcasses was positive for *Listeria* spp.; Prencipe et al. (2012) reported a low frequency of *L. monocytogenes*-positive carcasses in Italy (23/774). Due to the widespread distribution and survival of *L. monocytogenes* in the slaughtering and processing environments, carcasses can be contaminated at any stage of slaughtering: however, the level of contamination tends to increase during meat processing procedures. Higher percentages of *L. monocytogenes* are found at the end of processing stages than in carcasses immediately after the slaughtering stage, which can be explained by the amount of handling during processing, which involves the use of utensils and equipment in direct contact with the meat (Gamboa-Marin et al., 2012; Prencipe et al., 2012).

The results (Table 2) confirmed the environmental characteristic of *L. monocytogenes*, with higher frequencies of positive results in the cutting room when compared to other slaughter sites. This ability to survive and persist in different sites and conditions makes it possible to isolate *L. monocytogenes* in industrial environments, as observed in many other studies (Autio et al., 2000; Berzins et al., 2010; Chasseignaux et al., 2002; Larivière-Gauthier et al., 2014; Meloni et al., 2013; Neira et al., 2015; Ortiz et al., 2010; Peccio et al., 2003; Sala et al., 2016; Thévenot et al., 2005). However, despite being present at higher frequencies in the cutting room environment, none of the samples of pork cuts was positive for *L. monocytogenes*.

It is known that some *L. monocytogenes* strains may reside in facilities, utensils and equipment, being considered persistent in the industrial environment (Carpentier and Cerf, 2011; Garner and Kathariou, 2016). In the current study, *L. monocytogenes* was repeatedly isolated from conveyor belts (final packing) from September 2016 to January 2017 (Table 3), and the obtained isolates presented identical and/or highly similar genetic profiles (Fig. 1), confirming the persistence of this strain in the facility. The presence of *L. monocytogenes* in the final stages of processing demonstrates the epidemiological role of these stages in spreading the pathogen (Carpentier and Cerf, 2011). Despite not being detected in the pork cuts (Table 2), the persistence of *L. monocytogenes* in the final stages of processing poses a potential risk for cross-contamination to end products, like RTE, that are destined for retail and consumption.

Considering the persistence behavior of *L. monocytogenes*, reports of these isolates sharing high similarity in their genetic profiles are common. Meloni et al. (2013) in Italy, and López et al. (2008) in Spain, found only six and nine different pulsotypes, respectively, among isolates obtained from swine slaughterhouses. Camargo et al. (2014) found the same genetic profile in 100% of the *L. monocytogenes* isolates obtained from bovine carcasses in two slaughterhouses located in Brazil. This low diversity could be related to the pathogen's ability to persist in the same ecological niche for months or years, becoming endemic and specific to a slaughterhouse and processing plants (Carpentier and Cerf, 2011; Ferreira et al., 2014; Meloni et al., 2013).

The abilities of *L. monocytogenes* to adhere and produce biofilm are considered key factors for the pathogen's persistence in food-processing facilities (Bonaventura et al., 2008; Bonsaglia et al., 2014; Møretrø and Langsrud, 2004). Once established, *L. monocytogenes* biofilms act as permanent sources of contamination and dispersal in the environment, leading to cross-contamination (Markkula et al., 2005). Also, adhered strains tend to be more resistant to cleaning and disinfection than suspended bacteria (Simões et al., 2010; Van der Veen and Abee, 2011). In this sense, even surfaces without direct contact with food, like floors, drains and walls, can be considered as important sites for *L. monocytogenes* maintenance, as observed in this study (Table 2). Biofilms formed in these sites can easily detach and spread *L. monocytogenes* cells to end products and other sites, mainly due to aerosols formed during hygienic procedures (Berrang et al., 2010; Zhao et al., 2006). It is important to highlight that despite being classified as weakly adherent, the data indicate the persistence of the identified *L. monocytogenes* strains in the processing facility, specifically on the conveyor belts of the cutting room (Tables 2 and 3, Fig. 1).

Other factors may contribute to the persistence of bacteria in a food-processing facility, such as tolerance to "environmental stress", usually caused by temperature fluctuations, lack of nutrients or the action of disinfectants, and specific characteristics of the utensils, equipment, and facilities, like the material, corners and grooves that facilitate the adhesion and housing of bacterial cells (Camargo et al., 2014; Carpentier and Cerf, 2011; López et al., 2008). The conveyor belts in which the *L. monocytogenes* strains were persistent presented some of these conditions, as they were subjected to constant contact with sharp bones from pig carcasses, forming grooves that allow the adherence of organic material and consequent matrix for bacterial growth of adhered cells.

The identified serogroup IV included the serotype 4b, which has been associated with more than 50% of listeriosis cases worldwide (Borucki and Call, 2003; Doumith et al., 2005), indicating the pathogenic potential of its isolates. This potential was confirmed by the presence of all virulence-related genes targeted in this study (*inlA, inlB, inlC, inlJ, hlyA, plcA, actA* and *iap*), these being responsible for the main pathogenic pathways of *L. monocytogenes* (Camargo et al., 2016; De las Heras et al., 2011; Liu et al., 2007; Pizarro-Cerda et al., 2012). The presence of these genes in *L. monocytogenes* isolates obtained from food-processing facilities and foods has already been described in a number of studies, demonstrating the relevance of these genes for the characterization of the virulence potential of this foodborne pathogen (Camargo et al., 2015; Haubert et al., 2015; Jamali et al., 2013; Lomonaco et al., 2012; Silva et al., 2016).

Despite presenting a high virulence potential, the antibiotic resistance of the selected isolates can be considered as low (Table 4), as observed in other studies (Camargo et al., 2014; Haubert et al., 2015; Korsak et al., 2012; Lotfollahi et al., 2017; Ruiz-Bolivar et al., 2011). All tested isolates presented resistance to ampicillin, and almost all isolates presented intermediate resistance to clindamycin (Table 4). Only one isolate presented simultaneous resistance to five antibiotics, and it was obtained from the lairage floor; the majority of tested isolates from conveyor belts presented resistance only to ampicillin (Table 5). Studies on the resistance among isolates of *L. monocytogenes* against these antibiotics are becoming more numerous, with high variation in resistance rates mainly for the more usual drugs adopted for listeriosis treatment: ampicillin, penicillin and sulfamethoxazole associated with trimethoprim (Du et al., 2017; Harakeh et al., 2009; Haubert et al., 2015; Jamali et al., 2013; Lotfollahi et al., 2017; Olaniran et al., 2015). Despite the high susceptibility to antibiotics of the tested *L. monocytogenes* isolates, the presence of resistance to ampicillin, penicillin and sulfamethoxazole-trimethoprim is an important concern for public health (Charpentier and Courvalin, 1999; CLSI, 2006; Hansen et al., 2005; Poros-Gluchowska and Markiewicz, 2003).

The increase in antibiotic resistance in *L. monocytogenes* emphasizes the need for constant surveillance of this characteristic in food chains, including pork. The widespread use of antibiotics in different stages of food production can easily lead to the development and spread of resistance in bacteria normally present in the animal production environment and processing chain (Charpentier and Courvalin, 1999; Kovacevic et al., 2013; Lungu et al., 2011). Resistance can be transferred through mobile genetic elements, such as plasmids, which are considered the main mechanism by which *L. monocytogenes* acquires resistance (Bertrand et al., 2005; Bertsch et al., 2013, 2014; Charpentier and Courvalin, 1999; Haubert et al., 2015; Sharma et al., 2017). This may explain the differences in antibiotic resistance profiles observed for the tested isolates, even those sharing highly similar genetic profiles (Tables 4 and 5, Fig. 1).

In summary, although *L. monocytogenes* was identified at low frequencies along the pork production chain, the continuous presence of the same clonal strain on a conveyor belt in the slaughterhouse cutting room indicates its persistence in the studied facility and poses a risk for cross-contamination to end products. This *L. monocytogenes* clone

persisted despite its weak adhesion ability. Given its identified serogroup (IVb), virulence potential and antibiotic resistance (mainly to ampicillin), this persistent *L. monocytogenes* clone could pose a potential risk for public health.

Acknowledgments

The authors thank CAPES (Finance Code 001), FAPEMIG and CNPq.

References

Autio, T., Sateri, T., Fredriksson-Ahomaa, M., Rahkio, M., Lunden, J., Korkeala, H., 2000. *Listeria monocytogenes* contamination pattern in pig slaughterhouses. J. Food Prot. 63, 1438–1442. http://doi.org/10.4315/0362-028X-63.10.1438.

Barros, M.A., Nero, L.A., Silva, L.C., D'Ovidio, L., Monteiro, F.A., Tamanini, R., Fagnani, R., Hofer, E., Beloti, V., 2007. *Listeria monocytogenes*: Occurrence in beef and identification of the main contamination points in processing plants. Meat Sci. 76, 591–596. https://doi.org/10.1016/j.meatsci.2007.01.016.

Berrang, M.E., Meinersmann, R.J., Frank, J.F., Ladely, S.R., 2010. Colonization of a newly constructed commercial chicken further processing plant with Listeria monocytogenes. J. Food Prot. 73, 286–291. https://doi.org/10.4315/0362-028X-73.2.286.

Bertrand, S., Huys, G., Yde, M., D'Haene, K., Tardy, F., Vrints, M., Swings, J., Collard, J.M., 2005. Detection and characterization of tet(M) in tetracycline-resistant *Listeria* strains from human and food-processing origins in Belgium and France. J. Med. Microbiol. 54, 1151–1156. http://doi.org/10.1099/jmm.0.46142-0.

Bertsch, D., Muelli, M., Weller, M., Uruty, A., Lacroix, C., Meile, L., 2014. Antimicrobial susceptibility and antibiotic resistance gene transfer analysis of foodborne, clinical, and environmental *Listeria* spp. isolates including *Listeria monocytogenes*. MicrobiologyOpen 3, 118–127. http://doi.org/10.1002/mbo3.155.

Bertsch, D., Rau, J., Eugster, M.R., Haug, M.C., Lawson, P.A., Lacroix, C., Meile, L., 2013. *Listeria fleischmannii* sp. nov., isolated from cheese. Int. J. Syst. Evol. Microbiol. 63, 526–532. http://doi.org/10.1099/ijs.0.036947-0.

Berzins, A., Hellstrom, S., Silins, I., Korkeala, H., 2010. Contamination patterns of *Listeria monocytogenes* in cold-smoked pork processing. J. Food Prot. 73, 2103–2109. http://doi.org/10.4315/0362-028X-73.11.2103.

Bonaventura, G., Piccolomini, R., Paludi, D., D'Orio, V., Vergara, A., Conter, M., 2008. Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: Relationship with motility and cell surface hydrophobicity. J. Appl. Microbiol. 104, 1552–1561. https://doi.org/10.1111/j.1365-2672.2007.03688.x.

Bonsaglia, E.C.R., Silva, N.C.C., Júnior, A.F., Júnior, J.A., Tsunemi, M.H., Rall, V.L.M., 2014. Production of biofilm by *Listeria monocytogenes* in different materials and temperatures. Food Control 35, 386–391. https://doi.org/10.1016/j.foodcont.2013.07.023.

Borucki, M.K., Call, D.R., 2003. *Listeria monocytogenes* serotype identification by PCR. J. Clin. Microbiol. 41, 5537–5540. https://doi.org/10.1128/JCM.41.12.5537-5540.2003.

Boscher, E., Houard, E., Denis, M., 2012. Prevalence and distribution of *Listeria monocytogenes* serotypes and pulsotypes in sows and fattening pigs in farrow to finish farms (France, 2008). J. Food Prot. 75, 889–895. http://doi.org/10.4315/0362-028X.JFP-11-340.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003. *Salmonella* on pig carcasses: Positive pigs and cross contamination in the slaughterhouse. J. Appl. Microbiol. 95, 891–903. https://doi.org/10.1046/j.1365-2672.2003.02042.x.

Camargo, A.C., Lafisca, A., Cossi, M.V.C., Lanna, F.G.P.A., Dias, M.R., Pinto, P.S.A., Nero, L.A., 2014. Low occurrence of *Listeria monocytogenes* on bovine hides and carcasses in minas gerais state, Brazil: Molecular characterization and antimicrobial resistance. J. Food Prot. 77, 1148–1152. http://doi.org/10.4315/0362-028X.JFP-13-434.

Camargo, A.C., Dias, M.R., Cossi, M.V.C., Lanna, F.G.P.A., Cavicchioli, V.Q., Valim, D.C., Pinto, P.S.A., Hofer, E., Nero, L.A., 2015. Serotypes and pulsotypes diversity of *Listeria monocytogenes* in a beef-processing environment. Foodb. Pathog. Dis. 12, 323–326. http://doi.org/10.1089/fpd.2014.1875.

Camargo, A.C., Woodward, J.J., Nero, L.A., 2016. The continuous challenge of characterizing the foodborne pathogen *Listeria monocytogenes*. Foodb. Pathog. Dis. 13, 405–416. http://doi.org/10.1089/fpd.2015.2115.

Camargo, A.C., Woodward, J.J., Call, D.R., Nero, L.A., 2017. Listeria monocytogenes in food-processing facilities, food contamination, and human listeriosis: The brazilian scenario. Foodb. Pathog. Dis. 14, 623–636. http://doi.org/10.1089/fpd.2016.2274.

Carpentier, B., Cerf, O., 2011. Review - persistence of *Listeria monocytogenes* in food, industry equipment and Premises. Int. J. Food Microbiol. 145, 1–8. https://doi.org/10.1016/j.ijfoodmicro.2011.01.005.

Charpentier, E., Courvalin, P., 1999. Minireview: Antibiotic resistance in *Listeria* spp. Antimicrob. Agents Chemoter 43, 2103–2108. http://aac.asm.org/content/43/9/2103.

Chasseignaux, E., Géraukt, P., Toquin, M.T., Salvat, G., Colin, P., Ermel, G., 2002. Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw pork meat processing plants. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 210, 271–275. https://doi.org/10.1111/j.1574-6968.2002.tb11192.x.

CLSI – Clinical and Laboratory Standards Institute, 2006. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. Clinical and Laboratory Standards Institute CLSI document M45-A.

CLSI – Clinical and Laboratory Standards Institute, 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, ninth ed. Clinical and Laboratory Standards Institute CLSI document M07-A9.

CLSI – Clinical and Laboratory Standards Institute, 2017. Performance Standards for Antimicrobial Susceptibility Testing, 27th ed. Clinical and Laboratory Standards Institute CLSI supplement M100.

De las Heras, A., Cain, R.J., Bielecka, M.K., Vázquez-Boland, J., 2011. Regulation of *Listeria* virulence: PrfA master and commander. Curr. Opin. Microbiol. 14, 118–127. http://doi.org/10.1016/j.mib.2011.01.005.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004a. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J. Clin. Microbiol. 42, 3819–3822. http://doi.org/10.1128/JCM.42.8.3819–3822.2004.

Doumith, M., Cazalet, C., Simoes, N., Frangeul, L., Jacquet, C., Kunst, F., Martin, P., Cossart, P., Glaser, P., Buchrieser, C., 2004b. New aspects regarding evolution and virulence of *Listeria monocytogenes* revealed by comparative genomics and DNA arrays. Infect. Immun. 72, 1072–1083. http://doi.org/10.1128/IAI.72.2.1072-1083.2004.

Doumith, M., Jacquet, C., Gerner-Smidt, P., Graves, L.M., Loncarevic, S., Mathisen, T., Morvan, A., Salcedo, C., Torpdahl, M., Vazquez, J.A., Martin, P., 2005. Multicenter validation of a multiplex PCR assay for differentiating the major *Listeria monocytogenes* serovars 1/2a, 1/2b, 1/2c, and 4b: Toward an international standard. J. Food Prot. 68, 2648–2650.

Du, X.J., Zhang, X., Wang, X., Su, Y.I., Li, P., Wang, S., 2017. Isolation and characterization of *Listeria monocytogenes* in Chinese food obtained from the central area of China. Food Control 74, 9–16. https://doi.org/10.1016/j.foodcont.2016.11.024.

EFSA – European Food Safety Authority, 2008. Scientific opinion of the panel on biological hazards on a request from the health and consumer protection, directorate general, European commission on microbiological risk assessment in feedingstuffs for food producing animals. EFSA J. 1–84.

EFSA – European Food Safety Authority, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J. 13, 165.

Ferreira, V., Wiedmann, M., Teixeira, P., Stasiewicz, M.J., 2014. Listeria monocytogenes persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. J. Food Prot. 77, 150–170. http://doi.org/10.4315/0362-028X.JFP-13-150.

Furrer, B., Candrian, U., Hoefelein, C.H., Luethy, J., 1991. Detection and identification of *Listeria monocytogenes* in cooked sausage products and in milk by *in vitro* amplification of haemolysin gene fragments. J. Appl. Microbiol. 70, 372–379. https://doi.org/10.1111/j.1365-2672.1991.tb02951.x.

Gamboa-Marin, A., Buitrago, S., Perez-Perez, K., Mercado, M., Poutou-Pinales, R., Carrascal-Camacho, A., 2012. Prevalence of *Listeria monocytogenes* in pork-meat and other processed products from the Colombian swine industry. Rev. MVZ Córdoba 17, 2827–2833.

Gandhi, M., Chikindas, M.L., 2007. *Listeria*: A foodborne pathogen that knows how to survive. Int. J. Food Microbiol. 113, 1–15. http://doi.org/10.1016/j.ijfoodmicro.2006.07.008.

Garner, D., Kathariou, S., 2016. Fresh produce-associated listeriosis outbreaks, sources of 427 concern, teachable moments, and insights. J. Food Prot. 79 (2), 337–344. http://doi.org/10.4315/0362-028X.JFP-15-387.

Gómez, D., Azón, E., Marco, N., Carramiñana, J.J., Rota, C., Ariño, A., Yangüela, J., 2014. Antimicrobial resistance of *Listeria monocytogenes* and *Listeria innocua* from meat products and meat-processing environment. Food Microbiol. 42, 61–65. https://doi.org/10.1016/j.fm.2014.02.017.

Granier, S.A., Moubareck, C., Colaneri, C., Lemire, A., Roussel, S., Dao, T.T., Courvalin, P., Brisabois, A., 2011. Antimicrobial resistance of *Listeria monocytogenes* isolates from food and the environment in France over a 10-year period. Appl. Environ. Microbiol. 77, 2788–2790. http://doi.org/10.1128/AEM.01381-10.

Graves, L.M., Swaminathan, B., 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int. J. Food Microbiol. 65, 55–62. https://doi.org/10.1016/S0168-1605(00)00501-8.

Hansen, J.M., Gerner-Smidt, P., Bruun, B., 2005. Antibiotic susceptibility of *Listeria monocytogenes* in Denmark 1958–2001. APMIS 113, 31–36. https://doi.org/10.1111/j.1600-0463.2005.apm1130105.x.

Harakeh, S., Saleh, I., Zouhairi, O., Baydoun, E., Barbour, E., Alwan, N., 2009. Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products. Sci. Total Environ. 407, 4022–4027. http://doi.org/10.1016/j.scitotenv.2009.04.010.

Haubert, L., Mendonça, M., Lopes, G.V., De Itapema, M.R.C., Da Silva, W.P., 2015. *Listeria monocytogenes* isolates from food and food environment harbouring tetM and ermB resistance genes. J. Appl. Microbiol. 62, 23–29. http://doi.org/10.1111/lam.12516.

ISO – International Organization for Standardization, 1996. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of *Listeria Monocytogenes* - Part 1: Detection Method. ISO 11290-1.

ISO – International Organization for Standardization, 2004. Modification of the Isolation Media and the Haemolysis Test, and Inclusion of Precision Data –. ISO 11290-1/Amd 1.

Jamali, H., Radmehr, B., Thong, K.L., 2013. Prevalence, characterisation, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk tanks. Food Control 34, 121–125. https://doi.org/10.1016/j.foodcont.2013.04.023.

Korsak, D., Borek, A., Daniluk, S., Grabowska, A., Pappelbaum, K., 2012. Antimicrobial susceptibilities of *Listeria monocytogenes* strains isolated from food and food processing environment in Poland. Int. J. Food Microbiol. 158, 203–208. http://doi.org/10.1016/j.ijfoodmicro.2012.07.016.

Kovacevic, J., Sagert, J., Wozniak, A., Gilmour, M.W., Allen, K.J., 2013. Antimicrobial

resistance and coselection phenomenon in *Listeria* spp. recovered from food and food production environments. Food Microbiol. 34, 319–327. http://doi.org/10.1016/j.fm.2013.01.002.

Lamont, R.F., Sobel, J., Mazaki-Tovi, S., Kusanovic, J.P., Vaisbuch, E., Kim, S.K., Uldbjerg, N., Romero, R., 2011. Listeriosis in human pregnancy: A systematic review. J. Perinat. Med. 39, 227–236. http://doi.org/10.1515/JPM.2011.035.

Larivière-Gauthier, G., Letellier, A., Kérouanton, A., Bekal, S., Quessy, S., Fournaise, S., Fravalo, P., 2014. Analysis of *Listeria monocytogenes* strain distribution in a pork slaughter and cutting plant in the province of Quebec. J. Food Prot. 77, 2121–2128. http://doi.org/10.4315/0362-028X.JFP-14-192.

Lee, S.H.I., Barancelli, G.V., De Camargo, T.M., Corassin, C.H., Rosim, R.E., Da Cruz, A.G., Cappato, L.P., De Oliveira, C.A.F., 2017. Biofilm producing ability of *Listeria monocytogenes* isolates from Brazilian cheese processing plants. Food Res. Int. 91, 88–91. https://doi.org/10.1016/j.foodres.2016.11.039.

Liu, D.Y., Lawrence, M.L., Austin, F.W., Ainsworth, A.J., 2007. A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. J. Microbiol. Methods 71, 133–140. http://doi.org/10.1016/j.mimet.2007.08.007.

Lomonaco, S., Patti, R., Knabel, S.J., Civera, T., 2012. Detection of virulence associated genes and epidemic clone markers in *Listeria monocytogenes* isolates from PDO Gorgonzola cheese. Int. J. Food Microbiol. 160, 76–79. https://doi.org/10.1016/j.ijfoodmicro.2012.09.011.

López, V., Villatoro, D., Ortiz, S., López, P., Navas, J., Davila, J.C., Martmez-Suarez, J.V., 2008. Molecular tracking of *Listeria monocytogenes* in na Iberian pig abattoir and processing plant. Meat Sci. 78, 30–134. https://doi.org/10.1016/j.meatsci.2007.05.002.

Lotfollahi, L., Chaharbalesh, A., Rezaee, M.A., Hasani, A., 2017. Prevalence, antimicrobial susceptibility and multiplex PCR-serotyping of *Listeria monocytogenes* isolated from humans, foods and livestock in Iran. Microb. Pathog. 107, 425–429. https://doi.org/10.1016/j.micpath.2017.04.029.

Lungu, B., O'Bryan, C.A., Muthaiyan, A., Milillo, S.R., Johnson, M.G., Crandall, P.G., Ricke, S.C., 2011. *Listeria monocytogenes*: Antibiotic resistance in food production. Foodb. Pathog. Dis. 8, 569–578. https://doi.org/10.1089/fpd.2010.0718.

Markkula, A., Autio, T., Lundén, J., Korkeala, H., 2005. Raw and processed fish show identical *Listeria monocytogenes* genotypes with pulsed-feld gel electrophoresis. J. Food Prot. 68, 1228–1231.

Meloni, D., Piras, F., Mureddu, A., Fois, F., Consolati, S.G., Lamon, S., Mazzette, R., 2013. *Listeria monocytogenes* in five Sardinian swine slaughterhouses: Prevalence, serotype, and genotype characterization. J. Food Prot. 76, 1863–1867. http://doi.org/10.4315/0362-028X.JFP-12-505.

Mertins, S., Joseph, B., Goetz, M., Ecke, R., Seidel, G., Sprehe, M., Hillen, W., Goebel, W., Müller-Altrock, S., 2007. Interference of components of phosphoenolpyruvate system with the central virulence gene regulator *PrfA* of *Listeria monocytogenes*. J. Barcetriology 189, 473–490. http://doi.org/10.1128/JB.00972-06.

Møretrø, T., Langsrud, S., 2004. *Listeria monocytogenes*: Biofilm formation and persistence in food-processing environments. Biofilms 1, 107–121. https://doi.org/10.1017/S1479050504001322.

Neira, K., Cherifi, T., Fournaise, S., Surprenant, C., Letellier, A., Fravalo, P., 2015. Residual Contamination Detection and Serovar Distribution of *Listeria Monocytogenes*. pp. 63–68 Safepork.

Notermans, S.H., Dufrenne, J., Leimeister-Wachter, M., Domann, E., Chakraborty, T., 1991. Phosphatidylinositol-specific phospholipase C activity as a marker to distinguish between pathogenic and nonpathogenic *Listeria* species. Appl. Environ. Microbiol. 57, 2666–2670.

Olaniran, A.O., Nzimande, S.B.T., Mkize, N.G., 2015. Antimicrobial resistance and virulence signatures of *Listeria* and *Aeromonas* species recovered from treated wastewater effluent and receiving surface water in Durban, South Africa. BMC Microbiol. 15, 234. http://doi.org/10.1186/s12866-015-0570-x.

Orsi, R.H., Den Bakker, H.C., Wiedmann, M., 2011. *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. Int. J. Med. Microbiol. 301, 79–96. http://doi.org/10.1016/j.ijmm.2010.05.002.

Ortiz, S., López, V., Villatoro, D., López, P., Dávila, C., Martínez-Suárez, J.V., 2010. A 3-Year Surveillance of the genetic diversity and persistence of *Listeria monocytogenes* in an Iberian slaughterhouse and processing plant. Foodb. Pathog. Dis. 7, 1177–1184. https://doi.org/10.1089/fpd.2010.0535.

Pagliano, P., Arslan, F., Ascione, T., 2017. Epidemiology and treatment of the commonest form of listeriosis: Meningitis and bacteraemia. Infezioni Med. Le 3, 210–216.

Paziak-Domanska, B., Bogulawska, E., Wiekowska-Szakiel, M., Kotlowski, R., Rozalska, B., Chmiela, M., Kur, J., Dabrowski, W., Rudnicka, W., 1999. Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 171, 209–214. https://doi.org/10.1111/j.1574-6968.1999.tb13434.x.

Peccio, A., Autio, T., Korkeala, H., Rosmini, R., Trevisani, M., 2003. *Listeria monocytogenes* occurrence and characterization in meat-producing plants. Lett. Appl. Microbiol. 37, 234–238. http://doi.org/10.1046/j.1472-765X.2003.01384.x.

Pizarro-Cerdá, J., Kuhbacher, A., Cossart, P., 2012. Entry of *Listeria monocytogenes* in mammalian epithelial cells: An updated view. Cold Spring Harb. Perspect. Med. 2, 1–17. http://doi.org/10.1101/cshperspect.a010009.

Poroś-Głuchowska, J., Markiewicz, Z., 2003. Antimicrobial resistance of *Listeria monocytogenes*. Acta Microbiol. Pol. 52, 113–129.

Prencipe, V.A., Rizzi, V., Acciari, V., Iannetti, L., Giovannini, A., Serraino, A., Calderone, D., Rossi, A., Morelli, D., Marino, L., Migliorati, G., Caporale, V., 2012. *Listeria monocytogenes* prevalence, contamination levels and strains characterization throughout the Parma ham processing chain. Food Control 25, 150–158. http://doi.org/10.1016/j.foodcont.2011.10.018.

Rawool, D.B., Malik, S.V.S., Barbuddhe, S.B., Shakuntala, I., Aurora, R.A., 2007. A multiplex PCR for detection of virulence associated genes in *Listeria monocytogenes*. Int. J. Food Safety 9, 56–62.

Ruiz-Bolivar, Z., Neuque-Rico, M.C., Poutou-Pinãles, R.A., Carrascal-Camacho, A.K., Mattar, S., 2011. Antimicrobial susceptibility of *Listeria monocytogenes* food isolates from different cities in Colombia. Foodb. Pathog. Dis. 8, 913–919. http://doi.org/10.1089/fpd.2010.0813.

Sala, C., Morar, A., Tîrziu, E., Nichita, I., Imre, M., Inre, K., 2016. Environmental occurrence and antibiotic susceptibility profile of *Listeria monocytogenes* at a slaughterhouse raw processing plant in Romania. J. Food Prot. 79, 1794–1797. http://doi.org/10.4315/0362-028X.JFP-16-052.

Sharma, S., Sharma, V., Dahiya, D.K., Khan, A., Mathur, M., Sharma, A., 2017. Prevalence, virulence potential, and antibiotic susceptibility profile of *Listeria monocytogenes* isolated from bovine raw milk samples obtained from Rajasthan, India. Foodb. Pathog. Dis. 14, 132–140. http://doi.org/10.1089/fpd.2016.2118.

Silva, D.A.L., Camargo, A.C., Todorov, S.D., Nero, L.A., 2016. *Listeria* spp. contamination in a butcher shop environment and *Listeria monocytogenes* adhesion ability and sensitivity to food contact surface sanitizers. J. Food Saf. 1–8. http://doi.org/10.1111/jfs.12313.

Simões, M., Simões, L.C., Vieira, M.J., 2010. A review of current and emergent biofilm control strategies. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 43, 573–583. https://doi.org/10.1016/j.lwt.2009.12.008.

Stepanović, S., Vukovic, D., Dakic, I., Savic, B., Svabic-Vlahovic, M., 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods 40, 175–179. https://doi.org/10.1016/S0167-7012(00)00122-6.

Suarez, M., Vazquez-Boland, J.A., 2001. The Bacterial Actin Nucleater Protein act*A* Is Involved in Epithelial Cell Invasion by *Listeria Monocytogenes*. [Accession No. AF103807]. www.ncbi.nlm.nih.gov/pubmed.

Swaminathan, B., Gerner-Smidt, P., 2007. The epidemiology of human listeriosis. Microb. Infect. 9, 1236–1243. http://doi.org/10.1016/j.micinf.2007.05.011.

Temple, M.E., Nahata, M.C., 2000. Treatment of listeriosis. Ann. Pharmacother. 34, 656–661.

Thévenot, D., Delignette-Muller, M.L., Christieans, S., Vernozy-Rozand, C., 2005. Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. Int. J. Food Microbiol. 102, 85–94. http://doi.org/10.1016/j.ijfoodmicro.2004.12.008.

Van der Veen, S., Abee, T., 2011. Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and paracetic acid. Int. J. Food Microbiol. 144, 421–431. http://doi.org/10.1016/j.ijfoodmicro.2010.10.029.

Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., Gonzalez-Zorn, B., Wehland, J., Kreft, J., 2001. *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. 14, 584–640. http://doi.org/10.1128/CMR.14.3.584-640.2001.

Zeng, W., Vorst, K., Brown, W., Marks, B.P., Jeong, S., Perez-Rodriguez, F., Ryser, E.T., 2014. Growth of *Escherichia coli* 0157:H7 and *Listeria monocytogenes* in packaged fresh-cut Romaine mix at fluctuating temperatures during commercial transport, retail storage, and display. J. Food Prot. 77, 197–206.

Zhao, T., Podtburg, T.C., Zhao, P., Schmidt, B.E., Baker, D.A., Cords, B., Doyle, M.P., 2006. Control of *Listeria* spp. by competitive-exclusion bacteria in floor drains of a poultry processing plant. Appl. Environ. Microbiol. 72, 3314–3320. https://doi.org/10.1128/AEM.72.5.3314–3320.2006.