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Distribution, virulence, genotypic characteristics and antibiotic resistance of Listeria monocytogenes isolated over one-year monitoring from two pig slaughterhouses and processing plants and their fresh hams

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1	Distribution, virulence, genotypic characteristics and antibiotic resistance of Listeria monocytogenes isolated over one-year monitoring from
2	two pig slaughterhouses and processing plants and their fresh hams
3	
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15	Abstract
16	Listeria monocytogenes contamination in raw pork and ready to eat foods is an important food safety concern, also for the increasing detection of

17 antimicrobial-resistant isolates. Data on *L. monocytogenes* occurrence, persistence, distribution and genetic characterization in two different plants,

18	namely in continuum from slaughtered pigs, environment and unfinished products (fresh hams) were observed by one-year monitoring and were
19	integrated with their antimicrobial resistance patterns. A total of 98 samples out of the overall 1,131 (8.7%) were positive for L. monocytogenes,
20	respectively 2.6% and 13.2% in plants A and B: only three serotypes were identified, 1/2c (50%), 1/2b (36.7%) and 1/2a (13.27%), and strains were
21	classified in 35 pulsotypes and 16 clusters by PFGE; a unique P-type was highlighted according to the detection of virulence genes. The contamination
22	flow of <i>L. monocytogenes</i> has a low occurrence in slaughterhouse (Plant A=1.1%, Plant B: 3.1%; p>0.05) and increased throughout the processing
23	chain with trimming area as the most contaminated (Plant A: 25%, Plant B: 57%; (p<0.05)), both in the environment and in unfinished products (80%)
24	in hams before trimming in plant B). The dominant role of environmental contamination in post-slaughter processing is confirmed to be a significant
25	cause of meat contamination by L. monocytogenes. Very high levels of resistance were observed for clindamycin (57%) and high resistance levels
26	(>20-50%) to ciprofloxacin, oxacillin, levofloxacin and daptomycin, confirming the L. monocytogenes resistance trend to a wide range of antimicrobial
27	agents. A total of 11 L. monocytogenes isolates were multidrug resistant and 7 out of them were isolated from slaughtered pigs. An interesting
28	significant (p<0.05) statistical correlation has been found between resistance to some antimicrobial agents and lineage/serotypes. Microbiological
29	sampling of food and environments after sanitization are commonly used as verification procedure for the absence of L. monocytogenes in food plants
30	and to give assurance of food safety, but strains characterization is necessary for industries to target specific control measures, like the enforcement
31	of the hygiene program and of the control of operator activities, at least for permanent strains. The only presence of L. monocytogenes could not be
32	considered as the conclusive assessment of a potential risk for public health, also in terms of emerging and emerged antimicrobial resistances.

# 34 Keywords

35 Foodborne pathogen, pig industry, pork product, molecular epidemiology, antimicrobial resistance

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#### 41 1. Introduction

Pork is the most frequently consumed meat in the European Union (Devine, 2003). In Italy, dry cured hams, in particular those designed by the 42 43 European Union as PDO (Protected Designation Origin) like Parma and San Daniele, represent a significant source of income for the pork industry, being exported worldwide, even if raw meat and other pork products are produced with carcass parts other than thighs. Listeria monocytogenes 44 45 contamination in raw pork is an important food safety concern (Li et al., 2018), and the management of microbiological hazards transmitted to humans by pork consumption is of major health and economic significance (De Cesare et al., 2018). L. monocytogenes is a ubiquitous foodborne pathogen 46 which causes listeriosis mainly among the so called YOPIs (young, old, pregnant, immunocompromised) sensitive groups. Although still relatively 47 48 rare, in Europe human listeriosis is one of the most serious food-borne diseases under surveillance causing hospitalization, high morbidity and high mortality (EFSA, 2019); treatment with antibiotics is usually needed for the control of the infection caused by this bacterium. 49

*L. monocytogenes* grows and survives in different types of habitats, even in adverse conditions, such as dry environment, high salt concentrations (10%, wt/vol), at refrigeration temperatures and a wide pH range (4.7-9.2). This ability to persist and multiply in the food environment, even by biofilm formation, makes the genus *Listeria* hard to control in food processing plants (Conficoni et al., 2016). In the pig industry, the contamination

routes of *L. monocytogenes* are well reported and its incidence in pig carcasses and slaughter plant equipment is usually low (Meloni et al., 2013; Ortiz et al., 2010), whereas a relatively common contamination of raw pork products and ready to eat (RTE) food with *L. monocytogenes* is observed (Camargo et al., 2017; EFSA, 2018). Persistence of *L. monocytogenes* in food processing environments is still considered the major source of RTE food contamination and this persistence appears to be the result both of improper hygiene conditions and of the high adaptive capacity of this bacterium (EFSA, 2018).

For these reasons *L. monocytogenes* poses a significant risk to the food industry, particularly producers of RTE foods, and meat products continue to be one of the three most RTE food categories typically associated with human listeriosis (EFSA, 2018). In addition, further concern is due to the increasing detection of antimicrobial-resistant *L. monocytogenes* isolates, mainly for antibiotics commonly used for the treatment of listeriosis, namely a combination of penicillin or ampicillin with aminoglycosides (gentamicin) and trimethoprim-sulfamethoxazole (Sosnowski et al., 2019). Future outbreaks may be more difficult to manage because of the emergence of antimicrobial resistance among *L. monocytogenes* strains isolated from food products (Olaimat et al., 2018).

In literature, there are several studies worldwide on *L. monocytogenes* presence, persistence, distribution and genetic characterization in pig slaughterhouses and processing plants, as well as in fresh hams and pork meat products. It is well recognized that, on the one hand, raw pork meat has rarely been implicated in foodborne illness, and perhaps it could be considered a potential source of domestic cross contamination of other food (Thevenot et al., 2006), and that, on the other, the level of *L. monocytogenes* contamination increases along the pork supply chain (López et al., 2008). When contaminated surfaces are involved in food contamination, the risk of the cross-contamination of pork by *L. monocytogenes* could be assessed by predictive models able to predict sporadic event affecting the number of contaminated food samples and the influence of food processing factors

and the indirect mechanisms involved in cross-contamination (Jiang et al., 2018; Møller et al., 2016; Possas et al., 2017). Being the role of the after-70 71 slaughter phases, such as cooling and/or cutting, crucial in the contamination of fresh hams (Camargo et al., 2017; Larivière-Gauthier et al., 2014; Thevenot et al., 2006), the reduction of L. monocytogenes prevalence in the processing plant environment and, as a consequence, the decrease of the 72 initial L. monocytogenes load in intermediate fresh hams may lead to a significant reduction in the likelihood of L. monocytogenes contamination 73 downstream the meat production chain. 74 75 Moreover, resistance of *L. monocytogenes* to many antimicrobial agents has emerged and evolved during the past few decades (Olaimat et al., 2018). 76 Resistance is increasingly observed from humans, food, plants and the environment: in humans, L. monocytogenes isolates showed resistance for tetracycline and ciprofloxacin; in food, high prevalence of oxacillin and clindamycin resistance was described from meat and fish production chains 77 and significant percentages of resistance against ampicillin, penicillin G, and tetracycline were reported in L. monocytogenes strains isolated from 78 meat, fish, and dairy production chains (Caruso et al., 2019). However, among this increasing number of studies on the emergence of antibiotic 79 resistance in L. monocytogenes from food products (Camargo et al., 2015; Caruso et al., 2019; Chen et al., 2019; Escolar et al., 2017; Gómez et al., 80 2014; Li et al., 2016; Moreno et al., 2014; Sala et al., 2016; Sereno et al., 2019; Sosnowski et al., 2019), it is noteworthy that the investigated isolates 81 usually belong to strain collections or to different food sources considered all together, with any correlation to a specific scenario and/or phase within 82 the food chains. No correlation of these antimicrobial resistance levels with information about presence, distribution and genomic characterization is 83 84 performed along pork chains, let alone food chains in general. Thus, the aim of this study was to integrate these scattered information by one-year of L. monocytogenes monitoring in two Italian plants in order to: i) describe the occurrence of L. monocytogenes in continuum in two slaughterhouses 85 with annexed cutting and trimming plants, from slaughtered pigs, environment and unfinished products (fresh hams); ii) trace the relevant sources of 86

contamination for fresh hams by PFGE typing method; iii) characterize the *L. monocytogenes* isolates in relation to their serotyping, profiles of
 virulence genes and antimicrobial resistance patterns.

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- 90 2. Material and methods
- 91 **2.1. Sampling in the two plants**

92 Two ham processing facilities, located respectively in the Lombardia and Emilia-Romagna Regions, in Italy, were investigated. Plants A and B 93 comprise two large scale (>400pigs/hour) slaughterhouses authorized to export pork meat products to the Unites States, each annexed to a processing plant, with cutting and trimming areas, that produce fresh hams for the "Parma ham" production and other fresh pork products. Plants A and B were 94 95 monthly sampled from April 2014 to September 2015 with a sampling plan including food and environment samples collected in continuum at slaughtering, cutting, cooling, trimming areas and storage chill room, respectively. One variable day within the week for each sampling was scheduled. 96 The sampled animals belonged to different batches of about 135 heavy pigs (160-180 kg weight) 9-10 months old. The animals received by the 97 slaughterhouses came for most part (88.9% of pigs and 89.4% of batches) from Emilia-Romagna or from nearby northern Italian regions (Piedmont 98 99 and Lombardy), within a radius of about 250 km from the slaughterhouses. Travel duration resulted always under 8 hours, and about 60% of batches with less than 90 min. Overall, plants A and B were sampled 10 and 9 times, respectively. At each sampling time point, the following samples were 100 101 collected from different areas of the processing plant: i) from slaughtering area, cecal content and amygdales (and ileo-cecal lymph nodes only for 102 plant B) were collected after evisceration from 15 pigs randomly selected; environment and facilities, namely splitting saws (2 swabs in pool) and gloves, bib, and surfaces in contact with the carcasses (4 swabs in pool); ii) from both cutting and trimming areas, hams after cutting (HAC) (4 swabs 103

104 in pool) and hams before trimming (HBT) (4 swabs in pool) were randomly sampled; environment and facilities, namely meat contact surface swabs 105 (MCS) (4 swabs in pool for hams conveyor, gloves and knives, respectively) and non-meat contact surface swabs (NMCS) (drain); iii) in cooling room, room walls (4 swabs in pool); iv) in storage chill room, ham post-trimming and before curing (HBC) (20 swabs examined in single) were 106 collected from hams belonging to 10 different batches. Plants A and B used different cleaning and disinfection protocols: i) a preliminary cleaning 107 aimed at achieving "visual cleanliness" of the workplace was performed by both plants by washing down with a low-pressure hose, but using cold 108 water in plant A and hot water (between 45°C and 55°C) in plant B; ii) a cleaning stage was performed with alkaline foaming detergent (at a dilution 109 110 rate of 3% in plant A and 5% in plant B), alternating with acids products at the week-ends only in plant A; in this plant, a specific biofilm-disruptor product was used weekly; iv) intermediate rinsing with hot water (<45°C) at low pressure in plant A and with hot water (between 45°C and 55°C) at 111 high pressure in plant B; v) disinfection performed by rotating products on a daily basis in both plants: chlorine products or equivalents in plant A and 112 citric acid and hydrogen peroxide or quaternary ammonium compound in plant B. In the latter plant, an amphoteric disinfectant based on acetic acid 113 and propanediamine derivatives was used once a fortnight; vi) final rinsing with water at very low pressure in plant A and with cold water at low 114 pressure in plant B. 115 The environmental samples were collected by swabbing 30x30 cm of the surface, during processing. Overall, a total of 1,131 samples were collected, 116

- 117 namely 487 in plant A and 644 in plant B. More details are reported in Table 1. All samples were carried under chilled conditions to the laboratory of
- 118 the Experimental Institute for Zooprophylaxis of Lombardy and Emilia Romagna, and processed within 12 h after collection.

119

120 **2.2. Isolation and identification of** *Listeria monocytogenes* 

121	For the microbiological analysis, the ileo-cecal lymph nodes and amygdales were separated from the fat and surrounding tissues, respectively, and
122	both were processed using 95% alcohol to flame on specimen surface and then disrupted using a hammer to expose the interior before analysis. Fecal
123	and swab samples were homogenized 1/10 with Fraser broth base (Biolife, Milan, Italy) in a Stomacher Lab Blender 400 (PBI, Italy) for 2 min.
124	The detection of L. monocytogenes was performed according to the international standard method ISO 11290-1:1996\Amd 1:2004. Only culture-
125	confirmed samples by biochemical tests (Gram staining, catalase, hemolysis and CAMP tests, carbohydrate utilization test for xylose, rhamnose and
126	mannitol) were deemed positive.
127	One L. monocytogenes isolate for each positive sample was considered and preserved in brain heart infusion broth (Biolife) with glycerol (15%,
128	vol/vol) at -80°C. All the considered isolates underwent their characterization by serotyping and by the presence of putative virulence genes. Bacterial
129	DNA extracted from the selected collection of L. monocytogenes isolates was subjected to multiplex PCR-based serotyping assay targeting genes
130	Imo0737, Imo1118, ORF2819, ORF2110, and prs, as previously described (Doumith et al., 2004), and confirmed with classical agglutination
131	serotyping according to the method described in the Bacteriological Analytical Manual using commercial anti O and H antisera (Denka Seiken, Tokyo,
132	Japan).

## 134 **2.3.** Antibiotics Susceptibility Testing

All the isolates were tested for their antimicrobial resistance using a commercial microbroth dilution method, namely Sensititre<sup>TM</sup> Gram Positive Plate Format (GPN3F plates, Thermo Scientific, USA) containing 18 antimicrobials (dilution range in mg/l) were used: ampicillin (AMP; 0.12–16), ceftriaxone (AXO; 8–64), ciprofloxacin (CIP; 0.5–2), clindamycin (CLI; 0.12–2), daptomycin (DAP; 1–8), erythromycin (ERY; 0.25–4), gatifloxacin 138 (GAT; 1-8), gentamicin (GEN; 2-16), levofloxacin (LEVO; 0.25-8), linezolid (LZD; 0.5-8), oxacillin (OXA; 0.25-8), penicillin (PEN; 0.06-8), 139 quinupristin/dalfopristin (SYN; 0.12–4), rifampin (RIF; 0.5–4), streptomycin (STR; 1000), tetracycline (TET; 2–16), trimethoprim/sulfamethoxazole (SXT; 0.5/9.5–4/76), vancomycin (VAN; 1–128). The antimicrobials were selected among antimicrobials used for treatment of human Listeria 140 infections or for antibiotic therapy that is usually applied against Gram-positive bacteria. The minimal inhibitory concentration (MIC) records were 141 manually read. Antimicrobial resistance of the isolates was determined as first choice according to epidemiological cut-off values for L. 142 143 monocytogenes proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), following by EUCAST clinical breakpoints 144 for L. monocytogenes and Staphylococcus spp., and lastly by Clinical and Laboratory Standards Institute (CLSI) breakpoints for L. monocytogenes 145 and Staphylococcus spp. (CLSI, 2012; CLSI, 2016). Isolates were considered multidrug resistant (MDR) when showing resistance to three or more 146 antimicrobial classes (Schwarz et al., 2010).

147

### 148 2.4. Typing of *Listeria monocytogenes* isolates and detection of virulence genes

Pulsed-field gel electrophoresis (PFGE) was performed according to the Pulse-Net standardized protocol for *L. monocytogenes* (https://www.cdc.gov/pulsenet/pdf/listeria-pfge-protocol-508c.pdf) using *Asc*I and *Apa*I restriction enzymes. *Salmonella enterica* serovar Braenderup strain H9812 restricted with *Xba*I was used as standard according to the above protocol. The PFGE profiles were analyzed using BioNumerics software (version 6.7; Applied Maths, Sint-Martens-Latern, Belgium); similarity between PFGE profiles was determined using the Dice coefficient with 1% value of tolerance limit and optimization. Pulsotypes were obtained combining AscI and ApaI profiles. Dendrograms were obtained by cluster analysis of the pulsotypes, set at a 0.90% similarity level, by the Unweighted Pair Group Method Analysis (UPGMA). Isolates showing a PFGE similarity

155	level $\geq$ 90% were assigned to the same pulsotype. Isolates showing pulsotypes with a similarity level of $>$ 80% were grouped in the same "PFGE"
156	cluster" and were identified by progressive Roman numbers. The definition of the clusters was performed by evaluating the two plants separately.
157	The reliability of cluster analysis was evaluated by calculating the cophenetic correlation coefficient. The discriminatory index (DI) of the PFGE
158	analysis was calculated using the Simpson's diversity index described by Hunter and Gaston (Hunter and Gaston 1988), considering the combined
159	PFGE AscI and ApaI profiles, and using the Comparing Partitions Website (http://www.comparingpartitions.info).
160	For the identification of virulence genes, DNA was amplified by PCR to detect the <i>inlA</i> , <i>inlB</i> , <i>inlC</i> , and <i>inlJ</i> genes (Liu et al., 2007), which code for
161	internalin proteins A, B, C, J, respectively, and of plcA, hlyA, actA, and iap genes (Rawool et al., 2007). According to the presence of these genes,
162	different pathotypes (P-types) were determined.
163	

## 164 **2.5. Statistical analysis**

- 165 Descriptive statistics (absolute frequencies and percentages) have been provided regarding the occurrence and characteristics (serotype and P-type)
- 166 of *L. monocytogenes* isolates. Findings were presented for the two plants by sampling area, sample types and sources. Descriptive statistics (absolute
- 167 frequencies and percentages) have been also provided for their antimicrobial resistance patterns, namely the number of isolates resistant to, at least,
- 168 one AA and MDR isolates. Findings were presented by lineage, origin, sources and clusters. Chi-square test or Fisher's exact test were used to compare
- 169 nominal variables between different sampling area, origin and sources, as well as serotypes and lineages. Significance was set at a P value of <0.05.
- 170 PRISM 5.0 software was used.
- 171

#### 172 **3. Results**

#### 173 **3.1.** Isolation and identification of *Listeria monocytogenes*

A total of 98 samples out of the overall 1,131 (8.7%) were positive for L. monocytogenes by culture examination, 13 (2.6%) and 85 (13.2%) in plants 174 175 A and B, respectively. For both plants a low and a relatively low contamination rates were reported in the slaughterhouse area (1.1% and 3.1% for plants A and B, respectively; p>0.05), whereas the trimming area resulted the most contaminated (25% and 57% for plants A and B, respectively; 176 p < 0.05), reaching a maximum level of occurrence of 80% for HBT samples. For the other areas, not negligible differences in L. monocytogenes 177 178 occurrence between the plants were found: in plant A, L. monocytogenes was isolated in 2.2 % of samples from cutting area, whereas it was not found in cooling areas and in HBC; on the other hand, in plant B L. monocytogenes was isolated in 40%, 10% and 27% of samples collected from cutting 179 area, cooling area and in HBC, respectively. The environment resulted more contaminated than food, with the only exception of the slaughterhouse 180 area of both plants, in which the contamination rate in slaughtered pigs, even if low, resulted higher than in environment. More details in relation to 181 182 the occurrence, distribution of *L. monocytogenes* in the two plants and their statistical comparisons are reported in Table 1. 183 Only three serotypes were identified in the overall 98 L. monocytogenes isolates, namely 1/2c (50%; n=49), 1/2b (36.7%; n=36) and 1/2a (13.27%; n=13). Three lineages were identified in the population of L. monocytogenes: the lineage I includes isolates of serotype 4b/4e, 1/2b, 3b, 4b/4e and 3c, 184 185 the lineage II isolates of serotype 1/2a, 1/2c and 3a, and the lineage III isolates of serotype 4a and 4c. Of the overall 98 strains, 63.2% (62/98) belonged 186 to the lineage II and 36.7% (36/98) to the lineage I. Occurrence, distribution in the two plants and characteristics of the 98 L. monocytogenes isolates 187 in relation to lineage distribution, serotyping, and their source are summarized in Table 1.

188

#### 189 3.2. Antibiotics Susceptibility Testing

The MIC evaluation of the 98 isolates is reported in Table 2. In total, all the isolates were susceptible to 8 out of 18 tested antimicrobials, namely, 190 191 quinupristin/dalfopristin, vancomycin, ampicillin, gentamicin, rifampin, penicillin, streptomycin and gatifloxacin. Ceftriaxone was not considered 192 because L. monocytogenes is intrinsically resistant to cephalosporins (CRAB, 2020). A total of 93 isolates (94.9%) showed resistance to at least one 193 antimicrobial agent and 11 isolates (11.2%) were MDR, and it is worth noting that 7 (64%) of the overall 11 MDR isolates were from slaughtered 194 pigs. The percentage of MDR isolates was significant higher (p < 0.05) in slaughtered pigs in comparison to environment and food, and significant 195 differences were (p < 0.05) in the lineages and serotypes (see Table 3). The most common resistances of L. monocytogenes isolates were to clindamycin, with very high level of resistance (n=56/98 isolates; 57.1%), followed 196 by high resistance levels (>20-50%) to ciprofloxacin (n=42/98 isolates; 42.9%), oxacillin (n=35/98 isolates; 35.7%), levofloxacin (n=34/98 isolates; 197 198 34.7%) and daptomycin (23/98 isolates; 23.5%). Lastly, low resistance levels (>1-10%) were observed for linezolid (n=4/98 isolates; 4%), tetracycline and trimethoprim/sulfamethoxazole (both n=3/98 isolates; 3.1%) and erythromycin (n=2/98 isolates; 2.04%). Tables 3 and 4 detail antimicrobial 199 200 susceptibility findings of L. monocytogenes isolates. 201 Different resistance levels were observed in relation to the lineage and serotype, and in relation to the origin: i) the percentage of resistant L. 202 monocytogenes was significantly higher (p<0.05) in isolates of lineage II for clindamycin, levofloxacin and ciprofloxacin, whereas for daptomycin 203 and oxacillin was significantly higher (p < 0.05) in isolates of lineage I, and no differences were found for the other antimicrobial agents (see Table 3); ii) the percentage of resistant L. monocytogenes was significantly higher (p < 0.05) in isolates of serotype 1/2c for clindamycin, levofloxacin and 204

205 ciprofloxacin, and of serotype 1/2a for daptomycin and oxacillin (see Table 3); iii) although the percentage of MDR isolates was significantly higher

(p<0.05) in slaughtered pigs, no significant differences were found between the percentage of resistant *L. monocytogenes* isolated from different sources (slaughtered pigs, environment and food) for all the antimicrobial agents, with the exception of clindamycin for which the percentage of resistant *L. monocytogenes* was significantly lower (p<0.05) in isolates from food source (see Table 4).

209

#### 210 3.3. Typing of *Listeria monocytogenes* isolates and detection of virulence genes

211 The 98 L. monocytogenes isolates characterized using PFGE with AscI and ApaI were classified in 35 pulsotypes and 16 clusters. The pulsotypes 212 indicated as P16, P01, P04, P13, P15, P26, P03, P12, P18, P08, P14 and P07 were shared between 2 and 29 isolates, whereas the other 23 pulsotypes 213 were identified in single isolates. More details were reported in Figures 1-2. Pulsotypes with >10% of isolates were arbitrarily considered as predominant, namely pulsotypes P16, P01, P04. The most common pulsotype was that indicated as P16, exclusively associated with 29 isolates of 214 215 serotype 1/2b and with environmental sources, followed by pulsotypes P01, P04, P13 and P15. The only pulsotype identified from all the investigated 216 sources, namely slaughtered pigs, food and environment, was pulsotype P04, even if the unique strain isolated from food shows a different serotype, 217 namely 1/2b, versus serotype 1/2c of all the other 8 isolates of this pulsotype. The resistant isolates were spread in all clusters (except in cluster XIV) 218 even if the L. monocytogenes isolates showing resistance to the highest number of antimicrobial agents (>4 antimicrobial agents) were grouped in 219 cluster V, IV and I. For each antimicrobial agent, the percentage of resistant isolates varies between the different clusters, with some clusters gathering 220 the great majority of resistant isolates: in cluster VI were included 32 out of the overall 35 (91%) and 16 of the overall 23 (70%) isolates resistant to oxacillin and daptomycin, respectively. Cluster IV showed 18/34 (53%) and 20/42 (48%) resistant isolates to levofloxacin and ciprofloxacin, as well 221

as 26/56 (46%) of clindamycin resistant isolates were observed in cluster III. MDR isolates belong to 5 clusters (IV, V, VII, IX and XI). See Table 5
 for more details.

224 The DI of PFGE method ranged between 0.883 (C.I. 95%: 0.843-0.923) for plant A and 0.971 (C.I. 95%: 0.906-1.000) for plant B.

All the genes were detected among the investigated 98 isolates *L. monocytogenes* strains, thus a unique P-type was highlighted according to the

226 detection of virulence genes, hindering comparison and evaluation on virulence genotypes, but highlighting their potential pathogenicity.

227

#### 228 4. Discussion

229 The control of L. monocytogenes in meat processing facilities continues to be an important challenge consistent with the continuous, even if 230 intermittent, introduction and/or reintroduction of the microorganism in the processing plants, and with the presence of persistent strains in the same 231 environment and the parallel complexity of proper sanitization practices. The first objective of this study was to describe the occurrence and 232 distribution of L. monocytogenes in different areas of two different pig industries, from slaughterhouses to fresh hams. Our study confirms the contamination flow of L. monocytogenes observed in different pork processing plants with a low occurrence in slaughterhouse that increased 233 234 throughout the processing chain, both in the environment and in unfinished products (Larivière-Gauthier et al., 2014; Ortiz et al., 2010; Prencipe et al., 2012; Thevenot et al., 2006). Indeed, the low level of contamination at slaughterhouse resulted in line with most of the previous studies reporting 235 236 an occurrence ranging from 0 to 16.7% in pork carcasses (Larivière-Gauthier et al., 2014; Ortiz et al., 2010; Prencipe et al., 2012; Thevenot et al., 237 2006) and 0 to 3 % on slaughter equipment (Ortiz et al., 2010) but lower than the study of Meloni and Colleagues (2013) reporting prevalence of 33% 238 in slaughtered pigs. Our findings showed that few batches of pigs entering the slaughterhouse were contaminated with L. monocytogenes (from 3 out

239 of 9 samplings for plant A to 5 out of 10 samplings for plant B, with higher frequencies in amygdales, followed by ileo-cecal lymph nodes and feces) but with a high strain diversity (13 pulsotypes among 17 overall isolates) supporting the observations of Larivière-Gauthier and Colleague (2014) of 240 241 an infrequent entry of positive batches with a great variety of strains. Furthermore, these strains could be classified as not persistent strains because 242 they were never detected on consecutive visits and they have different pulsotypes, with the exception of pulsotype (P04) observed in the only one 243 splitting saw resulted positive, ileo-cecal lymph nodes, knives and gloves in cutting and trimming area, drains in the trimming area as well as HBC, 244 the latter isolated more than one year later (see P04 in cluster IV of dendrogram, Figure 2). P04 resulted the only pulsotype identified from all the 245 investigated sources: this event demonstrated that these isolates were in-house strains of this plant environment, that a contamination of a plant with 246 L. monocytogenes could originate from primary production and that, reciprocally, utensils (splitting saw) could be responsible for cross contamination 247 of the fresh hams, in line with observations of Larivière-Gauthier and Colleagues (2014). Among the debate on the possible origin of contaminations of fresh hams, and as a consequence of the end products, namely if contaminations were incoming from earlier phases of the processing chain or were 248 rather directly originating from the processing environment, our study highlighted the marginality of transfers of L. monocytogenes from primary 249 250 production and, at the same time, the dominant role of environmental contamination. Post-slaughter processing confirmed to be a significant cause of 251 meat contamination, namely in cutting and trimming environment where this contamination is amplified (see Table 1, and cluster III and VI of plant B in Figure 2), even if the initial microbial load as well as the environmental conditions favoring the microbial growth (nutrients from meat and 252 temperature) could have also played an increase in the L. monocytogenes level of contamination in both plants (Camargo et al., 2017; Larivière-253 254 Gauthier et al., 2014; Thevenot et al., 2006).

255 Only five pulsotypes (P01, P04, P12, P13 and P16) persisted in the plant during the 1-year period of this study but they represented the 60% of the 256 overall L. monocytogenes isolates and included all the three predominant pulsotypes (P16, P01, P04). This is a common scenario for food processing plants and a significant risk factor for the application and selection of the appropriate food plant sanitization procedures. The presence of L. 257 258 monocytogenes belonging to these predominant pulsotypes in cutting, trimming and storage chill room areas, and their circulation on plant B through the transmission of indistinguishable or closely related pulsotypes between food and the environment (both MCS and NMCS), probably represent the 259 260 mechanism for L. monocytogenes maintenance on the plant. Indeed, differently from slaughter area and cooling room, significant differences (p<0.05) 261 in the occurrence between plants A and B were observed in the other investigated areas, in which, even with wide fluctuations, the frequencies of L. 262 monocytogenes isolation were higher and affected all the samples types and sources. Moreover, the overall occurrence in fresh hams of both plants are in line with literature and the overall occurrence in fresh hams observed in plant B was similar to the worst findings among 13 Italian 263 slaughterhouses (Prencipe et al., 2012) as well as in an Iberian pig slaughterhouse and processing plant underwent to a 3-year surveillance (Ortiz et 264 265 al., 2010). This difference in contamination between the two plants as well as the varied contamination levels across the processing suggests the 266 existence of limitations to cross-contamination between compartments within the same plant and reflects the operative features of a specific plant, also in terms of sanitization procedures and behavior of workers. Based on the findings observed in cutting and trimming areas in plant B, on one 267 hand, gloves and knives of workers were frequently contaminated and could be considered carriers for HBT and HBC contamination, and on the other, 268 269 MCS (e.g. ham conveyors) as well as NMCS (e.g. drain) are significant niches indicative of the tendency of L. monocytogenes to persist in processing 270 facilities or in general inside the plant. In this context, it is essential the adoption of proper sanitization practices trying to avoid cross-contamination 271 to the products by environmental contamination, a good training of operators on working procedures like movement of materials, and cleaning and

272	disinfection (C&D) procedures. For example, no preventing crossover circuits for equipment and forklift, and the improper use of high-pressure hoses,
273	both observed only in plant B (data not shown), could have respectively represented a possible source of environmental bacterial contamination and
274	provoked the spreading of aerosol particles belonging to drainage water, as observed by Conficoni et al., 2016. These observations could justify the
275	high number of L. monocytogenes strains isolated in HBT and HBC in plant B. Finally, besides within-plant strains similarity, pulsotype P01 (namely
276	represented by isolate MN28 in plant A) was found in both the establishments, in line with two studies reporting the presence of indistinguishable
277	strains in different plants, namely for the contamination pattern of L. monocytogenes in the environment of Cured Ham food chain (Morganti et al.,
278	2016) and in finished pork-meat products of several unrelated factories (Autio et al., 2002).
279	For serotyping, in line with literature, 1/2a, 1/2b and 1/2c L. monocytogenes serotypes were observed in the pig slaughterhouse and processing plants,
280	in slaughtered pigs and from fresh meat (unfinished products). Serotypes 4b, 1/2b, and 1/2a are described as pathogenic and have been shown to be
281	predominant in human listeriosis cases (Orsi et al., 2011): the 4b is responsible for the majority of human listeriosis outbreaks, while sporadic cases
282	of L. monocytogenes gastroenteritis-are typically caused by consumption of food products contaminated with high bacterial loads of serotype 1/2a and
283	1/2b (Halbedel et al., 2019). Differently from previous studies on pork meat industry showing that serotype 1/2a is the dominant serotype and the
284	most prevalent in food (Li et al., 2016; López et al., 2008; Ortiz et al., 2010; Thevenot et al., 2006), our findings agree with other Italian studies
285	(Meloni et al., 2013; Prencipe et al., 2012) in which serotype 1/2c resulted the most dominant in plants, whereas serotype 1/2a seems to be the
286	prevalent, but not exclusive, in pig animals, in which serotypes 1/2b and 1/2c were also isolated. Although the reasons for dominance of the different
287	serotypes are unclear, strains belonging to serotype 1/2a and 1/2c have demonstrated to be more capable of biofilm formation when compared to
288	lineage I (Borucki et al., 2003; Kalmokoff et al., 2001). In particular, serotype 1/2c adhers significantly more to stainless steel, demonstrating highest

289	degree of adsorption (Ortiz et al., 2010). The L. monocytogenes serovars depend upon peptidoglycan-anchored cell wall teichoic acids (WTAs)
290	diversity within the cell wall that defines the O-antigens; WTAs are known to be involved in regulation of cell morphology and division, autolytic
291	activity, ion homeostasis, protection from host defenses and antibiotics, and may mediate host cell invasion and colonization. Recently, the study of
292	Sumrall and Colleagues (2019) demonstrated a switch from serovar 4b to 4d by bacteriophages with a loss of WTA galactosylation, which is involved
293	in cells adhesion; this leads to the loss of biofilm formation capacity of L. monocytogenes cells, consequently allowing their easy elimination during
294	sanitization. No hypothesis could be performed in this study for the dominance of serotype 1/2c, but both the mechanisms of serotype selection as a
295	result of sanitization conditions or change in serotype could be taken into account. In our study, all the serotypes are distributed in all sources with
296	some differences: serotype 1/2a, 1/2b and 1/2c isolates were mostly present in slaughtered pigs (61.5%), food (58.3%) and in the environment (59.2%),
297	respectively. In similar studies, serotype 1/2a isolates were present in the environment and equipment, and in different product categories, whereas
298	serotype 1/2b was mostly presented in environmental sites and raw products (Larivière-Gauthier et al., 2014; López et al., 2008; Ortiz et al., 2010;)
299	or even serotype 1/2c in fresh hams (Prencipe et al., 2012).
300	From source attribution studies, it was evidenced that not all L. monocytogenes strains are equally capable of causing invasive disease: overall, several
301	studies have shown that L. monocytogenes strains belonging to lineage I are on average more virulent and more frequently associated with human
302	clinical cases than lineage II strains (Filipello et al., 2020). Additionally, a recent study showed that a significant proportion of L. monocytogenes
303	isolated from food production environments have reduced virulence (Van Stelten et al., 2016) but our findings otherwise showed that all tested L.
304	monocytogenes strains had all detected virulence genes, and this suggests that L. monocytogenes strains isolated from food and/or environment are
305	potentially pathogenic and consequently may play an important role in epidemics, independently from the sources.

306 In relation to data on antimicrobial resistance in L. monocytogenes, some studies are available in literature but most of them reported data generically 307 from different food products or meat and human; very few studies investigated the pork chain, and only one study considered the continuum from slaughterhouses, environment and fresh hams in the same plants (Sereno et al., 2019). Furthermore, comparisons are arduous due to differences in 308 309 antimicrobial agents and breakpoints used. In most studies, the most common resistances were observed for oxacillin, clindamycin, tetracycline, ampicillin and trimethoprim-sulfamethoxazole and MDR isolates varied between zero to 27% (Camargo et al., 2015; Caruso et al., 2019; Chen et al., 310 311 2019; Escolar et al., 2017; Gómez et al., 2014; Li et al., 2016; Moreno et al., 2014; Sala et al., 2016; Sereno et al., 2019; Sosnowski et al., 2019). In 312 our study very low resistance or full susceptibility were observed for ampicillin (treatment of choice for listeriosis), tetracycline, and trimethoprim-313 sulfamethoxazole (used as second choice therapy or as alternative therapy for penicillin-allergic patients). However, the presence of very high and high level of resistances to clindamycin, ciprofloxacin, oxacillin, levofloxacin and daptomycin, is noteworthy and of important concern for public 314 health, since most of these antibiotics are widely used in hospitals to treat Gram-positive infections. Our findings confirm the L. monocytogenes 315 increasing trend of resistance to a wide range of antimicrobial agents and the fact that the genus Listeria spp. could no longer be reported as susceptible 316 317 to almost all antimicrobials. In addition, attention should be demanded for the risk of increasing multidrug resistance in *Listeria* and the possibility of 318 its transfer to other bacteria (Moreno et al., 2014). 319 Slaughtered pigs were the only source of strains that resulted resistant to all the 9 antimicrobial agents for which at least a resistance was observed

and for which the highest resistance was reported for all these antimicrobials, notwithstanding no significant differences were observed between the different origins (see table 4), whereas resulted the source with significant higher percentage of MDR strains. This finding could be due to the fact that emergence and spread of antimicrobial-resistant *Listeria* spp. has been attributed to the overuse of antibiotics in disease treatments and growth

promotion in domestic livestock (Charpentier et al., 1995; Walsh et al., 2001). The most common antibiotic classes worldly used in global pig 323 324 production are penicillins and tetracyclines. However, the use of Critically Important Antimicrobials for humans is also reported, even at different levels across countries, differently across countries: macrolides were reported at 20% and at 7.4% of total use in France and Austria, fluoroquinolones 325 326 at 2.4% and 5% and third and fourth generation cephalosporins at 2.2% and 11% of total use in Austria and Belgium (Lekagul et al., 2019). In one study performed in Italy on evaluation of antibiotic usage in swine reproduction farms, penicillins, macrolides, tetracyclines, and polymyxins (colistin) 327 328 were the most common antimicrobials used; third-generation cephalosporins, penicillins, colistin and fluoroquinolones had the highest prevalence, 329 while sulphonamides and trimethoprim, tetracyclines had the highest intensity of use (DDDs/animals) (Scoppetta et al., 2017). 330 An interesting statistical correlation (p < 0.05) has been found between resistance and lineage/serotypes. Clindamycin, levofloxacin and ciprofloxacin and, similarly, oxacillin and daptomycin resistances were significantly (p < 0.05) more frequently identified respectively in lineage II and in particular 331 332 in serotype 1/2c, and, in lineage I and in particular in serotype 1/2b. This correlation has been previously reported for oxacillin and lineage I, but in 333 particular with serotype 4b/4e, whereas intermediate clindamycin or ciprofloxacin resistances resulted higher in serotype 4b/4e (Caruso et al., 2019) 334 or 1/2a (Kovacevic et al., 2013; Safdar and Armstrong, 2003), but in several other cases no correlations were found (Safdar and Armstrong, 2003). No observations for these correlations have been proposed yet except that serotypes originating from different countries have different antibiotic 335 336 resistance profiles (Ayaz and Erol, 2010). 337 In conclusion, microbiological sampling of food and environments after sanitization are commonly used as verification procedure for the absence of

L. monocytogenes in food plants and to give assurance of food safety, but the findings of this study underlined that the presence without recognizing

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of the real pattern of contamination and the characteristics of the strains could not be considered as the conclusive assessment of a potential risk for 339

340	public health. Genotyping L. monocytogenes strains, mostly in cases in which a previous identification of the pathogen occurred within the plant or
341	in unfinished products, is necessary for industries to target specific control measures, for example the enforcement of the hygiene program and of the
342	control of operator activities, and may help reducing the risk of cross-contamination at the consumer level. L. monocytogenes microbiological sampling
343	and its conjunction with a specific evaluation of virulence and antimicrobial resistance of the L. monocytogenes strains, at least for permanent strains,
344	are necessary since it should result in a more comprehensive food safety vision and control, also in terms of emerging and emerged antimicrobial
345	resistances.
346	
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349	
350	Declaration of competing interest
351	The authors declare that they have no conflict of interest.
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353	References
354	Devine, R., 200. Meat consumption trends in the world and the European Union. INRA Prod. Anim.16, 325-327.
355	Li, H., Wang, P., Lan, R., Luo, L., Cao, X., Wang, Y., Wang, Y., Li, H., Zhang, L., Ji, S., Ye, C., 2018. Risk factors and level of Listeria monocytogenes
356	contamination of raw pork in retail markets in China. Front. Microbiol. 29, 9:1090. https://doi.org/10.3389/fmicb.2018.01090

- De Cesare, A., Doménech, E., Comin, D., Meluzzi, A., Manfreda, G., 2018. Impact of cooking procedures and storage practices at home on
   consumer exposure to *Listeria monocytogenes* and *Salmonella* due to the consumption of pork meat. Risk Anal. 38(4), 638-652.
   https://doi.org/10.1111/risa.12882
- 360 EFSA (European Food Safety Authority), 2019. Scientific report on the European Union One Health 2018 Zoonoses Report. EFSA J. 17(12), 5926.
   361 https://doi.org/10.2903/j.efsa.2019.5926
- 362 Conficoni, D., Losasso, C., Cortini, E., Di Cesare, A., Cibin, V., Giaccone, V., Corno, G., Ricci, A., 2016. Resistance to biocides in Listeria
- 363 *monocytogenes* collected in meat-processing environments. Front. Microbiol. 19, 7:1627. <u>https://doi.org/10.3389/fmicb.2016.01627</u>
- 364 Meloni, D., Piras, F., Mureddu, A., Fois, F., Consolati, S.G., Lamon, S., Mazzette, R., 2013. Listeria monocytogenes in five sardinian swine
- 365 slaughterhouses: prevalence, serotype, and genotype characterization. J. Food Prot. 76(11), 1863-1867. <u>https://doi.org/10.4315/0362-</u>
   366 028X.JFP-12-505
- 367 Ortiz, S., López, V., Villatoro, D., López, P., Dávila, J.C., Martínez-Suárez, J.N., 2010. A 3-year surveillance of the genetic diversity and
- persistence of *Listeria monocytogenes* in an Iberian pig slaughterhouse and processing plant. Foodborne Pathog. Dis. 7(10), 1177-1784.
   https://doi.org/10.1089/fpd.2010.0535
- 370 Camargo, A.C., Woodward, J.J., Call, D.R., Nero, L.A., 2017. Listeria monocytogenes in food-processing facilities, food contamination, and human
- 371 listeriosis: the brazilian scenario. Foodborne Pathog Dis. 14(11), 623-636. <u>https://doi.org/10.1089/fpd.2016.2274</u>

- 372 EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P.S.,
- 373 Girones, R., Herman, L., Koutsoumanis, K., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E.,
- 374 Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Takkinen, J., Wagner, M., Arcella, D., Da Silva Felicio, M.T., Georgiadis, M.,
- 375 Messens, W., Lindqvist, R., 2018. Scientific Opinion on the Listeria monocytogenes contamination of ready-to-eat foods and the risk for
- 376 human health in the EU. EFSA J. 16(1), 5134. <u>https://doi.org/10.2903/j.efsa.2018.5134</u>
- 377 Sosnowski, M., Lachtara, B., Wieczorek, K., Osek, J., 2019. Antimicrobial resistance and genotypic characteristics of *Listeria monocytogenes*
- isolated from food in Poland. Int. J. Food Microbiol. 16(289), 1-6. <u>https://doi.org/10.1016/j.ijfoodmicro.2018.08.029</u>
- 379 Olaimat, A.N., Al-Holy, M.A., Shahbaz, H.M., Al-Nabulsi, A.A., Abu Ghoush, M.H., Osaili, T.M., Ayyash, M.M., Holley, R.A., 2018. Emergence
- 380 of antibiotic resistance in *Listeria monocytogenes* isolated from food products: a comprehensive review. Compr. Rev. Food Sci. F. 17, 5.
- 381 <u>https://doi.org/10.1111/1541-4337.12387</u>
- 382 Thévenot, D., Dernburg, A., Vernozy-Rozand, C., 2006. An updated review of *Listeria monocytogenes* in the pork meat industry and its products. J.
- 383 Appl. Microbiol. 101, 7-17. <u>https://doi.org/10.1111/j.1365-2672.2006.02962.x</u>
- 384 López, V., Villatoro, D., Ortiz, S., López, P., Navas, J., Dávila, J.C., Martínez-Suárez, J.V. 2008. Molecular tracking of Listeria monocytogenes in an
- 385 Iberian pig abattoir and processing plant. Meat Sci. 78(1-2), 130-4. <u>https://doi.org/10.1016/j.meatsci.2007.05.002</u>
- 386 Jiang, R.H., Wang, X., Wang, W., Liu, Y.T., Du, J.P., Cui, Y., Zhang, C.Y., Dong, Q.L., 2018. Modelling the cross-contamination of Listeria
- 387 *monocytogenes* in pork during bowl chopping. Int J Food Sci Tech. 53, 837-846. <u>https://doi.org/10.1111/ijfs.13660</u>

- 388 Møller, C.O., Sant'Ana, A.S., Hansen, S.K., Nauta, M.J., Silva, L.P., Alvarenga, V.O., Maffei, D., Silva, F.F., Lopes, J.T., Franco, B.D., Aabo, S.,
- 389 Hansen, T.B., 2016. Evaluation of a cross contamination model describing transfer of *Salmonella* spp. and *Listeria monocytogenes* during
- 390 grinding of pork and beef. Int. J. Food Microbiol. 226, 42-52. <u>https://doi.org/10.1016/j.ijfoodmicro.2016.03.016</u>
- Possas, A., Carrasco, E., Garcia-Gimeno, R.M., Valero, A., 2017. Models of microbial cross-contamination dynamics. Curr. Opin. Food Sci. 14, 43 49. http://dx.doi.org/10.1016/j.cofs.2017.01.006
- 393 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(12), 2121-8. <u>https://doi.org/10.4315/0362-</u>
   028X.JFP-14-192
- 396 Caruso, M., Fraccalvieri, R., Pasquali, F., Santagada, G., Latorre, L.M., Difato, L.M., Miccolupo, A., Normanno, G., Parisi, A., 2019. Antimicrobial
- 397 Susceptibility and Multilocus Sequence Typing of *Listeria monocytogenes* isolated over 11 years from food, humans, and the environment in
- 398 Italy. Foodborne Pathog. Dis. 17(4), 284-294. <u>https://doi.org/10.1089/fpd.2019.2723</u>
- 399 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
- 400 and pulsotypes diversity of *Listeria monocytogenes* in a beef-processing environment. Foodb. Pathog. Dis. 12, 323-326.
   401 <u>http://doi.org/10.1089/fpd.2014.1875</u>

- 402 Chen, M., Cheng, J., Zhang, J., Chen, Y., Zeng, H., Xue, L., Lei, T., Pang, R., Wu, S., Wu, H., Zhang, S., Wei, X., Zhang, Y., Ding, Y., Wu, Q., 2019.
- 403 Isolation, potential virulence, and population diversity of *Listeria monocytogenes* from meat and meat products in China. Front. Microbiol.
- 404 10, 946. <u>https://www.frontiersin.org/article/10.3389/fmicb.2019.00946</u>
- 405 Escolar, C., Gómez, D., Del Carmen Rota García, M., Conchello, P., Herrera, A., 2017. Antimicrobial resistance profiles of Listeria monocytogenes
- 406 and *Listeria innocua* isolated from ready-to-eat products of animal origin in Spain. Foodborne Pathog. Dis. 14(6), 357-363.
   407 https://doi.org/10.1089/fpd.2016.2248
- 408 Gómez, D., Azón, E., Marco, N., Carramiñana, J.J., Rota, C., Ariño, A., Yangüela, J., 2014. Antimicrobial resistance of Listeria monocytogenes and
- 409 *Listeria innocua* from meat products and meat-processing environment. Food Microbiol. 42, 61-65. <u>https://doi.org/10.1016/j.fm.2014.02.017</u>
- 410 Li, L., Olsen, R.H., Ye, L., Wang, W., Shi, L., Yan, H., Meng, H., 2016. Characterization of antimicrobial resistance of *Listeria monocytogenes* strains
- 411 isolated from a pork processing plant and its respective meat markets in southern China. Foodborne Pathog. Dis. 13(5), 262-268.
  412 https://doi.org/10.1089/fpd.2015.2087
- 413 Moreno, L.Z., Paixão, R., Gobbi, D.D.S., Raimundo, D.C., Ferreira, T.P., Moreno, A.M., Hofer, E., Reis, C.M.F., Matté, G.R., Matté, M.H., 2014.
- 414 Characterization of antibiotic resistance in *Listeria* spp. isolated from slaughterhouse environments, pork and human infections. J. Infect. Dev.
- 415 Ctries. 8(4), 416-23. <u>https://jidc.org/index.php/journal/article/view/24727506</u>

- Sala, C., Morar, A., Tîrziu, E., Nichita, I., Imre, M., Imre, K., 2016. Environmental Occurrence and Antibiotic susceptibility profile of *Listeria monocytogenes* at a slaughterhouse raw processing plant in Romania. J. Food Protect. 79(10), 1794-1797. <u>https://doi.org/10.4315/0362-</u>
  <u>028X.JFP-16-052</u>
- 419 Sereno, M.J., Viana, C., Pegoraro, K., da Silva, D.A.L., Yamatogi, R.S., Nero, L.A., Bersot, L.D.S., 2019. Distribution, adhesion, virulence and
   420 antibiotic resistance of persistent *Listeria monocytogenes* in a pig slaughterhouse in Brazil. Food Microbiol. 84, 103234.
   421 https://doi.org/10.1016/j.fm.2019.05.018
- 422 ISO, 2004. Microbiology of food and animal feeding stuffs Horizontal method for the detection and enumeration of Listeria monocytogenes —
- 423 Part 1: Detection method (ISO 11290-1:1996/AMD 1:2004). International Organization for Standardization, Geneva, Switzerland.
- 424 Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex
- 425 PCR. J. Clin. Microbiol. 42(8), 3819-22. <u>https://dx.doi.org/10.1128%2FJCM.42.8.3819-3822.2004</u>
- 426 CLSI (Clinical and Laboratory Standards Institute), 2012. Performance Standards for Antimicrobial Susceptibility Testing Twenty Second
- 427 Informational Supplement. vol. 33, no. 3 Clinical and Laboratory Standards Institute, Wayne, PA, USA (Document M100-S30-20).
- 428 CLSI (Clinical and Laboratory Standards Institute), 2016. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently
- 429 Isolated or Fastidious Bacteria. Clinical and Laboratory Standards Institute, Wayne, PA, USA (Document M45).
- 430 Schwarz, S., Silley, P., Simjee, S., Woodford, N., van Duijkeren, E., Johnson, A. P., Gaastra, W., 2010. Editorial: assessing the antimicrobial
- 431 susceptibility of bacteria obtained from animals. J. Antimicrob. Chemother. 65(4):601-604. <u>https://doi.org/10.1093/jac/dkq037</u>

- Hunter, P.R. and Gaston, M.A., 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of
  diversity. J. Clin. Microbiol. 26(11), 2465-6.
- 434 Liu, D., Lawrence, M.L., Austin, F.W., Ainsworth, A.J., 2007. A multiplex PCR for species- and virulence-specific determination of Listeria
- 435 *monocytogenes*. J. Microbiol. Methods 71, 133-140. <u>https://doi.org/10.1016/j.mimet.2007.08.007</u>
- 436 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
- 437 *Listeria monocytogenes*. Int. J. Food Safety 9, 56–62.
- 438 CRAB, 2020. Centro di Referenza Nazionale per l'Antibioticoresistenza. Table 1. URL: <u>http://www.izslt.it/crab/wp-</u>
- 439 <u>content/uploads/sites/8/2018/08/Tabelle-Resistenze-intrinseche-in-batteri-di-interesse-veterinario.pdf</u> (Accessed 08.01. 2020).
- 440 Prencipe, V.A., Rizzi, V., Acciari, V., Iannetti, L., Giovannini, A., Serraino, A., Calderone, D., Rossi, A., Morelli, D., Marino, L., Migliorati, G.,
- 441 Caporale, V., 2012. Listeria monocytogenes prevalence, contamination levels and strains characterization throughout the Parma ham
- 442 processing chain. Food Control, 25(1), 150-158. <u>https://doi.org/10.1016/j.foodcont.2011.10.018</u>
- 443 Morganti, M., Scaltriti, E., Cozzolino, P., Bolzoni, L., Casadei, G., Pierantoni, M., Foni, E., Pongolini, S., 2016. Processing-Dependent and Clonal
- 444 Contamination Patterns of Listeria monocytogenes in the Cured Ham Food Chain Revealed by Genetic Analysis. Appl. Environ. Microbiol.
- 445 82(3), 822-831. <u>https://doi.org/10.1128/AEM.03103-15</u>.
- 446 Autio, T., Lundén, J., Fredriksson-Ahomaa, M., Björkroth, J., Sjöberg, A.M., Korkeala, H., 2002. Similar Listeria monocytogenes pulsotypes detected
- 447 in several foods originating from different sources. Int. J. Food Microbiol. 77(1-2), 83-90. <u>https://doi.org/10.1016/S0168-1605(02)00055-7</u>

- 448 Orsi, R.H., den Bakker, H.C., Wiedmann, M., 2011. Listeria monocytogenes lineages: Genomics, evolution, ecology, and phenotypic characteristics.
- 449 Int. J. Medical Microbiol. 301(2), 79-96. <u>https://doi.org/10.1016/j.ijmm.2010.05.002</u>
- 450 Halbedel, S., Prager, R., Banerji, S., Kleta, S., Trost, E., Nishanth, G., Alles, G., Hölzel, C., Schlesiger, F., Pietzka, A., Schlüter, D., Flieger, A., 2019.
- 451 A Listeria monocytogenes ST2 clone lacking chitinase ChiB from an outbreak of non-invasive gastroenteritis. Emerg. Microbes Infect. 8(1),
- 452 17-28. <u>https://doi.org/10.1080/22221751.2018.1558960</u>
- 453 Borucki, M.K., Peppin, J.D., White, D., Loge, F., Call, D.R., 2003. Variation in biofilm formation among strains of *Listeria monocytogenes*. Appl.
- 454 Environ. Microbiol. 69, 7336-7342. <u>https://doi.org/10.1128/AEM.69.12.7336-7342.2003</u>
- 455 Kalmokoff, M.L., Austin, J.W., Wan, X.D., Sanders, G., Banerjee, S., Farber, J.M., 2001. Adsorption, attachment and biofilm formation among
- 456 isolates of *Listeria monocytogenes* using model conditions. J. Appl. Microbiol. 91, 725-734. <u>https://doi.org/10.1046/j.1365-2672.2001.01419.x</u>
- 457 Sumrall, E.T., Shen, Y., Keller, A.P., Rismondo, J., Pavlou, M., Eugster, M.R., Boulos, S., Disson, O., Thouvenot, P., Kilcher, S., Wollscheid, B.,
- 458 Cabanes, D., Lecuit, M., Gründling, A., Loessner, M.J., (2019). Phage resistance at the cost of virulence: *Listeria monocytogenes* serovar 4b
- 459 requires galactosylated teichoic acids for InIB- mediated invasion. PLoS Pathog. 15(10), e1008032.
- 460 <u>https://doi.org/10.1371/journal.ppat.1008032</u>
- 461 Filipello, V., Mughini-Gras, L., Gallina, S., Vitale, N., Mannelli, A., Pontello, M., Decastelli, L., Allard, M.W., Brown, E.W., Lomonaco, S., 2020.
- 462 Attribution of *Listeria monocytogenes* human infections to food and animal sources in Northern Italy. Food Microbiol. 89, 103433.
- 463 https://doi.org/10.1016/j.fm.2020.103433

- 464 Van Stelten, A., Roberts, A.R., Manuel, C.S., Nightingale, K.K., 2016. Listeria monocytogenes isolates carrying virulence-attenuating mutations in
- 465 internalin A are commonly isolated from ready-to-eat food processing plant and retail environments. J. Food Prot. 79 (10), 1733.1740.
- 466 https://doi.org/10.4315/0362-028X.JFP-16-145
- 467 Charpentier, E., Gerbaud, G., Jacquet, C., Rocourt, J., Courvalin, P., 1995. Incidence of antibiotic resistance in *Listeria* species. J. Infect. Dis. 172,
- 468 277-281. <u>https://doi.org/10.1093/infdis/172.1.277</u>
- 469 Walsh, D., Duffy, G., Sheridan, J.J., Blair, I.S., McDowell, D.A., 2001. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in
- 470 retail foods. J. Appl. Microbiol. 90, 517-522. <u>https://doi.org/10.1046/j.1365-2672.2001.01273.x</u>
- 471 Lekagul, A., Tangcharoensathien, V., Yeung, S., 2019. Patterns of antibiotic use in global pig production: A systematic review. Vet. An. Science 7,
  472 100058. https://doi.org/10.1016/j.vas.2019.100058
- 473 Scoppetta, F., Sensi, M., Franciosini, M.P., Capuccella, M., 2017. Evaluation of antibiotic usage in swine reproduction farms in Umbria region based
- 474 on the quantitative analysis of antimicrobial consumption. Ital J. Food Saf. 6:6886. <u>https://doi.org/10.4081/ijfs.2017.6886</u>
- 475 Kovacevic, J., Sagert, J., Wozniak, A., Gilmour, M.W., Allen, K.J., 2013. Antimicrobial resistance and co-selection phenomenon in *Listeria* spp.
- 476 recovered from food and food production environments. Food Microbiol. 34(2), 319-327. <u>https://doi.org/10.1016/j.fm.2013.01.002</u>
- 477 Safdar, A., Armstrong, D., 2003. Listeriosis in patients at a comprehensive cancer center, 1955-1997. Clin. Infect. Dis. 37, 359-364.
  478 <u>https://doi.org/10.1086/376631</u>

479 Ayaz, N.D., Erol, I., 2010. Relation between serotype distribution and antibiotic resistance profiles of *Listeria monocytogenes* isolated from ground

480 turkey. J.Food Protect. 73(5), 967-972. <u>https://doi.org/10.4315/0362-028X-73.5.967</u>

# Table 1. Occurrence of *Listeria monocytogenes* in the different areas, typology and sources investigated in two plants considered in this study and serotype and pulsed-field gel electrophoresis results of the 98 isolates included in this study.

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				Plant B					
Sampling area	Sample types	Sources	No. pos/tot samples(%)	Serotype(n)	P-type	No. pos/tot samples(%)	Serotype(n)	P-type	
		faeces	0/135(0) <sup>a</sup>			2/150(1.3) <sup>a</sup>	1/2a(1);1/2c(1)	P03;P05	
	Slaughtered pigs	amygdales	3/135(2.2)ª	1/2a(1);1/2b( 2)	P27;P28;P2 9	7/150(4.6) <sup>a</sup>	1/2a(5);1/2c(2)	P02;P03;P06;P07;P08;	
Claughtarhausa		ileo-cecal lymphonodes	n.p.	-		4/114(3.5)	1/2a(1);1/2c(3)	P04;P09;P20	
Slaughternouse		total	3/270(1.1) <sup>a</sup>			13/414(3.1) <sup>a</sup>			
		MCS	0/9(0) <sup>a</sup>			0/10(0) <sup>a</sup>			
	environment	gloves, knives, bib, splitting saw	0/18(0)ª			1/20(5)ª	1/2c(1)	P04	
		total	$0/27(0)^{a}$			1/30(3.3) <sup>a</sup>			
	total		3/297(1) <sup>a</sup>			14/444(3.1) <sup>a</sup>			
	food	HAC	0/9(0) <sup>a</sup>			$4/10(40)^{a}$	1/2b(3);1/2c(1)	P15;P16	
	environment	MCS	$1/18(5.5)^{a}$	1/2a(1)	P35	6/20(30) <sup>a</sup>	1/2b(3);1/2c(3)	P01;P15;P16	
Cutting		gloves, knives	0/9(0) <sup>a</sup>			7/10(70) <sup>b</sup>	1/2b(3);1/2c(4)	P01;P04;P12;P16	
Cutting		NMCS	0/9(0) <sup>a</sup>			3/10(30) <sup>a</sup>	1/2c(3)	P01;P23	
		total	1/36(2.7)ª			16/40(40) <sup>b</sup>			
	total		1/45(2.2)ª			20/50(40) <sup>b</sup>			
Cooling Room	environment	NMCS	0/9(0) <sup>a</sup>			1/10(10) <sup>a</sup>	1/2c(1)	P13	
	food	HBT	0/9(0) <sup>a</sup>			8/10(80) <sup>b</sup>	1/2b(4);1/2c(4)	P01;P13;P16	
		MCS	3/9(33.3)ª	1/2c(3)	P26;P31;P3 3	6/10(60) <sup>a</sup>	1/2b(3);1/2c(3)	P01;P13;P16;P19	
Trimming	environment	gloves and knives	3/9(33.3)ª	1/2c(3)	P26;P32;P3 4	5/10(50)ª	1/2b(3);1/2c(3)	P04;P16	
		NMCS	3/9(33.3)ª	1/2c(3)	P01;P26;P3 0	4/10(40) <sup>a</sup>	1/2b(1);1/2c(3)	P01;P04;P13;P16	
		total	9/27(33.3) <sup>a</sup>			15/30(50) <sup>a</sup>			
	total		9/36(25) <sup>a</sup>			23/40(57.5) <sup>b</sup>			
Storage Chill Room	food	НВС	0/100ª			27/100(27) <sup>b</sup>	1/2a(4);	P01;P04;P10;P11;P12;P14;	

				1/2b(14);1/2c(9 )	P16;P17;P18;P21;P22;P24;P2 5						
Total		13/487(2.6) <sup>a</sup>	85/644(13.2) b								
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485	n.p.: not performed; MCS: meat contact surface swabs, namely ham conveyor for cutting and trimming areas, and hams rack for cooling room;										
486	NMCS: non-meat contact surface swabs, namely drain; HAC: hams after cutting; HBT: hams before trimming; HBC: ham post-trimming and										
487	before curing. <mark>Number of isolates in rows bearin</mark>	g different letters are significantly differe	nt (p< 0.05) b	etween plants A a	ind B.						
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492	Table 2. Minimum Inhibitory Concentration d	listribution of the 98 <i>Listeria monocyto</i>	genes strains	s isolated in the	present study.						

	MIC												
Antimicrobials	0,06	0,12	0,25	0,5	1	2	4	8	16	32	64	128	1000
Erythromycin		94*		2				2*					
Clindamycin	1*		10	31	36	18	2*						
Quinupristin/dalfopristin			1	44	50	3							
Daptomycin					1	17	57	23					
Vancomycin				98*									
Tetracycline					95*					3*			
Ampicillin	56*		42										
Gentamicin					98*								
Levofloxacin				6	58	33			1*				
Linezolid					21	73	4						
Ceftriaxone							9*		32	35	13	9*	
Streptomycin												98*	
Penicillin	5	18	70	5									
Rifampin			98*										
Gatifloxacin				98*									
Ciprofloxacin			26*		30	41	1*						
Trimethoprim/sulfamethoxazole			95*		3								
Oxacillin				1	3	59	35						

494 495 496	Black vertical lines indicate breakpoints for resistance. Gray shading indicates that the isolates were not tested for susceptibility to those concentrations of a given antimicrobial agent. Asterisked number indicates the number of isolates exhibiting MIC values equal to or higher or lower than concentration of the test range. No usable breakpoint was for ceftriaxone.
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507	Table 3. Antimicrobial resistance, lineage, serotypes and origin of the 98 <i>Listeria monocytogenes</i> strains isolated in the present
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				No. of resistant isolates (%)										
Lineage	Serotype	Origin	N. of isolate	Macrolides	Lincosamides	Lipopeptides	Fluoroqu	iinolones	Tetracycline	Folate Pathway Inhibitors	Oxazolidinone s	Penicillins	R at least	MDR
			S	ERY	CLI	DAP	LEVO	CIP	TET	SXT	LZD	OXA	UNIC THE	
		Slaughtered pigs	2	-	1(50)	-	1(50)	1(50)	-	-	-	-	2(100)	-
Ι	1/2b	Environmen t	<sup>1</sup> 13	-	-	5(38.5)	-	-	-	-	-	13(100)	13(100)	-
		Food	21	-	4(19)	11(52.4)	1(4.8)	3(14.3)	-	-	-	15(71.4)	20(95)	1(4.8)
	Total		36	-	5(13.8) <sup>a,1</sup>	16(44.4) <sup>a,1</sup>	2(5.5) <sup>a,1</sup>	4(11) <sup>a,1</sup>	-	-	-	28(77.7) <sup>a,1</sup>	35(97.2) <sup>a,1</sup>	1(2.7) <sup>a,1</sup>
	1/2a	Slaughtered pigs	8	2(25)	6(75)	3(37.5)	4(50)	3(37.5)	2(25)	2(25)	1(12.5)	1(12.5)	8(100)	3(37.5)
		Environmen t	<sup>1</sup> 1	-	-	-	-	-	-	-	-	-	-	-
		Food	4	-	-	1(25)	-	-	-	-	-	4(100)	4(100)	-
II		Total	13	2(15.3)	6(46.1) <sup>b</sup>	4(30.7) <sup>a</sup>	4(30.7) <sup>a</sup>	3(23) <sup>b</sup>	2(15.3) <sup>a</sup>	2(15.3) <sup>a</sup>	1(7.6) <sup>a</sup>	5(38.4) <sup>a</sup>	12(92.3) <sup>a</sup>	3(23) <sup>b</sup>
		Slaughtered pigs	6	-	6(100)	2(33.3)	3(50)	5(83.3)	-	-	2(33.3)	2(33.3)	6(100)	4(66.7)
	1/2c	Environmen t	<sup>1</sup> 29	-	25(86)	1(3.4)	17(58.6)	20(68.9)	1(3.4)	-	1(3.4)	-	27(90)	2(6.7)
		Food	14	-	14(100)	-	8(57.1)	10(71.4)	-	1(7.1)	-	-	14(100)	1(7.1)

	Total	49	-	45(91.8) <sup>c</sup>	3(6.1) <sup>b</sup>	28(57.1) <sup>b</sup>	35(71.4) c	1(2) <sup>a</sup>	1(2) <sup>a</sup>	3(6.1) <sup>a</sup>	2(4) <sup>b</sup>	47(95.9) <sup>a</sup>	7(14.2) <sup>b</sup>
	Total	62	2(3.2)	51(82.2) <sup>2</sup>	7(11.2) <sup>2</sup>	32(51.6) 2	38(61.2) 2	3(4.8)	3(4.8)	4(6.4)	7(11.2) <sup>1</sup>	59(95.1) <sup>1</sup>	10(16.1) 2
Тс	otal	98	2(2.04)	56(57.1)	23(23.5)	34(34.7)	42(42.9)	3(3.1)	3(3.1)	4(4)	35(35.7)	93(94.9)	11(11.2)
510	ERY: erythromycin; D	AP: dapto	mycin; CLI	: clindamycin	; LEVO: levo	floxacin; CI	P: ciproflox	xacin; TET: t	etracycline;	SXT: trimeth	oprim/sulfame	ethoxazole;	
511	LZD: linezolid; OXA: o	xacillin; A	A: antimici	robial agent; I	MDR: multid	rug resista	nt strains; ·	- : not detect	ted; <mark>number (</mark>	of isolates in	<mark>columns beari</mark>	ng different	
512	numbers and letters a	re signific	cantly diffe	<mark>rent (p&lt; 0.05</mark> ]	) in relations	ship to resp	ectively lin	leage and se	<mark>rotypes</mark>				
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Table 4. Antimicrobial resistance and origin of the 98 *Listeria monocytogenes* strains isolated in the present study.

	Slaughtered		Feed				
AA	pigs	MCS	Utensil	NMCS	Total	ruuu	
Erythromycin	2(12.59)	-	-	-	-	-	
Clindamycin	13(81.3)	10(58.8)	8(50)	7(70)	25(58.1)	18(46.2)*	
Daptomycin	5(31.3)	1(5.9)	3(18.8)	2(20)	6(14)	12(30.8)	
Levofloxacin	8(50)	7(41.2)	7(43.8)	3(30)	17(39.5)	9(23.1)	
Ciprofloxacin	9(56.3)	7(41.2)	9(56.3)	4(40)	20(46.5)	13(33.3)	
Tetracycline	2(12.5)	-	1(6.3)	-	1(2.3)	-	
Trimethoprim/sulfamethoxazole	2(12.5)	-	-	-	-	1(7.1)	
Linezolid	3(18.8)	-	1(6.3)	-	1(2.3)	-	
Oxacillin	3(18.8)	6(35.3)	6(37.5)	1(10)	13(30.2)	19(48.7)	
R at least one AA	16(100)	16 (94)	16(100)	8(80)	40(93)	38(97.4)	
MDR	7(63)*	_	1(9)	1(9)	2(18)	2(18)	

526 MCS: meat contact surface swabs, namely ham conveyor for cutting and trimming areas, and hams rack for chilling room; NMCS: non-meat contact

surface swabs, namely drain; AA: antimicrobial agent; - : not detected; number of isolates bearing \* are significantly different (p< 0.05) in relationship to</li>
 the source

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Table 5. Antimicrobial resistance and pulsed-field gel electrophoresis results of the 98 Listeria monocytogenes strains isolated in the present study.

		No. of resistant isolates (%)									R at least	мпр
Cluster	Isolates	ERY	CLI	DAP	LEVO	CIP	TET	SXT	LZD	OXA	one AA	MDK
1	2	-	2(3.6)	1(4.3)	2(5.8)	1(2.3)	-	-	1(25)	-	2	1
2	2	-	1(1.7)	-	-	-	-	-	-	-	1	-
3	27	-	26(46.4)	-	18(52.9)	20(47.6)	-	-	-	-	26	-
4	13	-	12(21.4)	-	10(29.4)	13(30)	-	1(33.3)	2(50)	-	13	3
5	2	2(100)	2(3.6)	1(4.3)	2(5.8)	2(4.7)	2(66.7)	2(66.7)	-	-	2	2
6	34	-	-	16(69.6)	-	-	-	-	-	32(91.4)	33	-
7	3	-	3(5.4)	2(8.6)	-	2(4.7)	-	-	-	-	3	2
8	1	-	-	1(4.3)	-	-	-	-	-	1(2.8)	1	-
9	2	-	2(3.6)	2(8.6)	-	1(2.3)	-	-	-	2(5.7)	2	2
10	2	-	2(3.6)	-	-	-	-	-	-	-	2	-
11	1	-	-	-	1(2.9)	1(2.3)	1(33.3)	-	1(25)	-	1	1

	98	2	56	23	34	42	3	3	4	34	93	11
16	1	-	-	-	1(2.9)	1(2.3)	-	-	-	-	1	-
15	2	-	2(3.6)	-	-	-	-	-	-	-	2	-
14	1	-	-	-	-	-	-	-	-	-	-	-
13	2	-	2(3.6)	-	-	-	-	-	-	-	2	-
12	3	-	2(3.6)	-	-	1(2.3)	-	-		-	2	-

552 ERY: erythromycin; DAP: daptomycin; CLI: clindamycin; LEVO: levofloxacin; CIP: ciprofloxacin; TET: tetracycline; SXT: trimethoprim/sulfamethoxazole;
 553 LZD: linezolid; OXA: oxacillin