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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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Abstract

Listeria monocytogenes contamination in raw pork and ready to eat foods is an important food safety concern, also for the increasing detection of antimicrobial-resistant isolates. Data on *L. monocytogenes* occurrence, persistence, distribution and genetic characterization in two different plants,

namely in continuum from slaughtered pigs, environment and unfinished products (fresh hams) were observed by one-year monitoring and were integrated with their antimicrobial resistance patterns. A total of 98 samples out of the overall 1,131 (8.7%) were positive for *L. monocytogenes*, 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 classified in 35 pulsotypes and 16 clusters by PFGE; a unique P-type was highlighted according to the detection of virulence genes. The contamination flow of *L. monocytogenes* has a low occurrence in slaughterhouse (Plant A=1.1%, Plant B: 3.1%; $p>0.05$) and increased throughout the processing 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% in hams before trimming in plant B). The dominant role of environmental contamination in post-slaughter processing is confirmed to be a significant cause of meat contamination by *L. monocytogenes.* Very high levels of resistance were observed for clindamycin (57%) and high resistance levels (>20-50%) to ciprofloxacin, oxacillin, levofloxacin and daptomycin, confirming the *L. monocytogenes* resistance trend to a wide range of antimicrobial agents. A total of 11 *L. monocytogenes* isolates were multidrug resistant and 7 out of them were isolated from slaughtered pigs. An interesting significant ($p<0.05$) statistical correlation has been found between resistance to some antimicrobial agents and lineage/serotypes. 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 strains characterization is necessary for industries to target specific control measures, like the enforcement 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 considered as the conclusive assessment of a potential risk for public health, also in terms of emerging and emerged antimicrobial resistances.

Keywords

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

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 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* 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 which causes listeriosis mainly among the so called YOPIs (young, old, pregnant, immunocompromised) sensitive groups. Although still relatively 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.

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-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 initial *L. monocytogenes* load in intermediate fresh hams may lead to a significant reduction in the likelihood of *L. monocytogenes* contamination downstream the meat production chain.

Moreover, resistance of *L. monocytogenes* to many antimicrobial agents has emerged and evolved during the past few decades (Olaimat et al., 2018). 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 and significant percentages of resistance against ampicillin, penicillin G, and tetracycline were reported in *L. monocytogenes* strains isolated from meat, fish, and dairy production chains (Caruso et al., 2019). However, among this increasing number of studies on the emergence of antibiotic 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., 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 usually belong to strain collections or to different food sources considered all together, with any correlation to a specific scenario and/or phase within the food chains. No correlation of these antimicrobial resistance levels with information about presence, distribution and genomic characterization is 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 with annexed cutting and trimming plants, from slaughtered pigs, environment and unfinished products (fresh hams); ii) trace the relevant sources of

87 contamination for fresh hams by PFGE typing method; iii) characterize the *L. monocytogenes* isolates in relation to their serotyping, profiles of
88 virulence genes and antimicrobial resistance patterns.
89
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
94 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
95 monthly sampled from April 2014 to September 2015 with a sampling plan including food and environment samples collected in continuum at
96 slaughtering, cutting, cooling, trimming areas and storage chill room, respectively. One variable day within the week for each sampling was scheduled.
97 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
98 slaughterhouses came for most part (88.9% of pigs and 89.4% of batches) from Emilia–Romagna or from nearby northern Italian regions (Piedmont
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
100 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
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
103 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

in pool) and hams before trimming (HBT) (4 swabs in pool) were randomly sampled; environment and facilities, namely meat contact surface swabs (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 collected from hams belonging to 10 different batches. Plants A and B used different cleaning and disinfection protocols: i) a preliminary cleaning aimed at achieving "visual cleanliness" of the workplace was performed by both plants by washing down with a low-pressure hose, but using cold 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 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 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 citric acid and hydrogen peroxide or quaternary ammonium compound in plant B. In the latter plant, an amphoteric disinfectant based on acetic acid 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 pressure in plant B.

The environmental samples were collected by swabbing 30x30 cm of the surface, during processing. Overall, a total of 1,131 samples were collected, 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 the Experimental Institute for Zooprophylaxis of Lombardy and Emilia Romagna, and processed within 12 h after collection.

2.2. Isolation and identification of *Listeria monocytogenes*

For the microbiological analysis, the ileo-cecal lymph nodes and amygdales were separated from the fat and surrounding tissues, respectively, and both were processed using 95% alcohol to flame on specimen surface and then disrupted using a hammer to expose the interior before analysis. Fecal 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.

The detection of *L. monocytogenes* was performed according to the international standard method ISO 11290-1:1996\Amd 1:2004. Only culture-confirmed samples by biochemical tests (Gram staining, catalase, hemolysis and CAMP tests, carbohydrate utilization test for xylose, rhamnose and mannitol) were deemed positive.

One *L. monocytogenes* isolate for each positive sample was considered and preserved in brain heart infusion broth (Biolife) with glycerol (15%, vol/vol) at -80°C. All the considered isolates underwent their characterization by serotyping and by the presence of putative virulence genes. Bacterial DNA extracted from the selected collection of *L. monocytogenes* isolates was subjected to multiplex PCR-based serotyping assay targeting genes *lmo*0737, *lmo*1118, ORF2819, ORF2110, and *prs*, as previously described (Doumith et al., 2004), and confirmed with classical agglutination serotyping according to the method described in the Bacteriological Analytical Manual using commercial anti O and H antisera (Denka Seiken, Tokyo, Japan).

2.3. Antibiotics Susceptibility Testing

All the isolates were tested for their antimicrobial resistance using a commercial microbroth dilution method, namely Sensititre™ 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

(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), 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* infections or for antibiotic therapy that is usually applied against Gram-positive bacteria. The minimal inhibitory concentration (MIC) records were manually read. Antimicrobial resistance of the isolates was determined as first choice according to epidemiological cut-off values for *L. monocytogenes* proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), following by EUCAST clinical breakpoints for *L. monocytogenes* and *Staphylococcus* spp., and lastly by Clinical and Laboratory Standards Institute (CLSI) breakpoints for *L. monocytogenes* and *Staphylococcus* spp. (CLSI, 2012; CLSI, 2016). Isolates were considered multidrug resistant (MDR) when showing resistance to three or more antimicrobial classes (Schwarz et al., 2010).

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-Latem, 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

level ≥90% were assigned to the same pulsotype. Isolates showing pulsotypes with a similarity level of >80% were grouped in the same "PFGE cluster" and were identified by progressive Roman numbers. The definition of the clusters was performed by evaluating the two plants separately. The reliability of cluster analysis was evaluated by calculating the cophenetic correlation coefficient. The discriminatory index (DI) of the PFGE analysis was calculated using the Simpson's diversity index described by Hunter and Gaston (Hunter and Gaston 1988), considering the combined PFGE *Asc*I and *Apa*I profiles, and using the Comparing Partitions Website (http://www.comparingpartitions.info).

For the identification of virulence genes, DNA was amplified by PCR to detect the *inl*A, *inl*B, *inl*C, and *inl*J genes (Liu et al., 2007), which code for internalin proteins A, B, C, J, respectively, and of *plc*A, *hly*A, *act*A, and *iap* genes (Rawool et al., 2007). According to the presence of these genes, different pathotypes (P-types) were determined.

2.5. Statistical analysis

Descriptive statistics (absolute frequencies and percentages) have been provided regarding the occurrence and characteristics (serotype and P-type) of *L. monocytogenes* isolates. Findings were presented for the two plants by sampling area, sample types and sources. Descriptive statistics (absolute frequencies and percentages) have been also provided for their antimicrobial resistance patterns, namely the number of isolates resistant to, at least, 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 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. PRISM 5.0 software was used.

3. Results

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 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; $p<0.05$), reaching a maximum level of occurrence of 80% for HBT samples. For the other areas, not negligible differences in *L. monocytogenes* 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 area, cooling area and in HBC, respectively. The environment resulted more contaminated than food, with the only exception of the slaughterhouse 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 the occurrence, distribution of *L. monocytogenes* in the two plants and their statistical comparisons are reported in Table 1.

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, 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 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 in relation to lineage distribution, serotyping, and their source are summarized in Table 1.

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, quinupristin/dalfopristin, vancomycin, ampicillin, gentamicin, rifampin, penicillin, streptomycin and gatifloxacin. Ceftriaxone was not considered because *L. monocytogenes* is intrinsically resistant to cephalosporins (CRAB, 2020). A total of 93 isolates (94.9%) showed resistance to at least one 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 pigs. The percentage of MDR isolates was significant higher ($p<0.05$) in slaughtered pigs in comparison to environment and food, and significant 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 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; 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 susceptibility findings of *L. monocytogenes* isolates.

Different resistance levels were observed in relation to the lineage and serotype, and in relation to the origin: i) the percentage of resistant *L. monocytogenes* was significantly higher ($p<0.05$) in isolates of lineage II for clindamycin, levofloxacin and ciprofloxacin, whereas for daptomycin 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 ciprofloxacin, and of serotype 1/2a for daptomycin and oxacillin (see Table 3); iii) although the percentage of MDR isolates was significantly higher

206 (p<0.05) in slaughtered pigs, no significant differences were found between the percentage of resistant *L. monocytogenes* isolated from different
207 sources (slaughtered pigs, environment and food) for all the antimicrobial agents, with the exception of clindamycin for which the percentage of
208 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 *Asc*I and *Apa*I 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
214 predominant, namely pulsotypes P16, P01, P04. The most common pulsotype was that indicated as P16, exclusively associated with 29 isolates of
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
221 oxacillin and daptomycin, respectively. Cluster IV showed 18/34 (53%) and 20/42 (48%) resistant isolates to levofloxacin and ciprofloxacin, as well

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.

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

4. Discussion

The control of *L. monocytogenes* in meat processing facilities continues to be an important challenge consistent with the continuous, even if intermittent, introduction and/or reintroduction of the microorganism in the processing plants, and with the presence of persistent strains in the same environment and the parallel complexity of proper sanitization practices. The first objective of this study was to describe the occurrence and 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 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 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., 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% 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)
240 but with a high strain diversity (13 pulsotypes among 17 overall isolates) supporting the observations of Larivière-Gauthier and Colleague (2014) of
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
248 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
249 rather directly originating from the processing environment, our study highlighted the marginality of transfers of *L. monocytogenes* from primary
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
252 B in Figure 2), even if the initial microbial load as well as the environmental conditions favoring the microbial growth (nutrients from meat and
253 temperature) could have also played an increase in the *L. monocytogenes* level of contamination in both plants (Camargo et al., 2017; Larivière-
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
257 plants and a significant risk factor for the application and selection of the appropriate food plant sanitization procedures. The presence of *L.*
258 *monocytogenes* belonging to these predominant pulsotypes in cutting, trimming and storage chill room areas, and their circulation on plant B through
259 the transmission of indistinguishable or closely related pulsotypes between food and the environment (both MCS and NMCS), probably represent the
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
263 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
264 slaughterhouses (Prencipe et al., 2012) as well as in an Iberian pig slaughterhouse and processing plant underwent to a 3-year surveillance (Ortiz et
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,
267 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
268 hand, gloves and knives of workers were frequently contaminated and could be considered carriers for HBT and HBC contamination, and on the other,
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

disinfection (C&D) procedures. For example, no preventing crossover circuits for equipment and forklift, and the improper use of high-pressure hoses, both observed only in plant B (data not shown), could have respectively represented a possible source of environmental bacterial contamination and provoked the spreading of aerosol particles belonging to drainage water, as observed by Conficoni et al., 2016. These observations could justify the high number of *L. monocytogenes* strains isolated in HBT and HBC in plant B. Finally, besides within-plant strains similarity, pulsotype P01 (namely represented by isolate MN28 in plant A) was found in both the establishments, in line with two studies reporting the presence of indistinguishable strains in different plants, namely for the contamination pattern of *L. monocytogenes* in the environment of Cured Ham food chain (Morganti et al., 2016) and in finished pork-meat products of several unrelated factories (Autio et al., 2002).

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, 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 predominant in human listeriosis cases (Orsi et al., 2011): the 4b is responsible for the majority of human listeriosis outbreaks, while sporadic cases of *L. monocytogenes* gastroenteritis are typically caused by consumption of food products contaminated with high bacterial loads of serotype 1/2a and 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 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 (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 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 serotypes are unclear, strains belonging to serotype 1/2a and 1/2c have demonstrated to be more capable of biofilm formation when compared to 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.

In relation to data on antimicrobial resistance in *L. monocytogenes*, some studies are available in literature but most of them reported data generically 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 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., 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 our study very low resistance or full susceptibility were observed for ampicillin (treatment of choice for listeriosis), tetracycline, and trimethoprim-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 health, since most of these antibiotics are widely used in hospitals to treat Gram-positive infections. Our findings confirm the *L. monocytogenes* 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 to almost all antimicrobials. In addition, attention should be demanded for the risk of increasing multidrug resistance in *Listeria* and the possibility of its transfer to other bacteria (Moreno et al., 2014).

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 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 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) were the most common antimicrobials used; third-generation cephalosporins, penicillins, colistin and fluoroquinolones had the highest prevalence, while sulphonamides and trimethoprim, tetracyclines had the highest intensity of use (DDDs/animals) (Scoppetta et al., 2017).

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 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 particular with serotype 4b/4e, whereas intermediate clindamycin or ciprofloxacin resistances resulted higher in serotype 4b/4e (Caruso et al., 2019) 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 resistance profiles (Ayaz and Erol, 2010).

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 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

public health. Genotyping *L. monocytogenes* strains, mostly in cases in which a previous identification of the pathogen occurred within the plant or in unfinished products, is necessary for industries to target specific control measures, for example the enforcement of the hygiene program and of the control of operator activities, and may help reducing the risk of cross-contamination at the consumer level. *L. monocytogenes* microbiological sampling and its conjunction with a specific evaluation of virulence and antimicrobial resistance of the *L. monocytogenes* strains, at least for permanent strains, are necessary since it should result in a more comprehensive food safety vision and control, also in terms of emerging and emerged antimicrobial resistances.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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481 **Table 1. Occurrence of *Listeria monocytogenes* in the different areas, typology and sources investigated in two plants considered**
482 **in this study and serotype and pulsed-field gel electrophoresis results of the 98 isolates included in this study.**
483

			Plant A			Plant B		
Sampling area	**Sample types**	**Sources**	**No. pos/tot samples(%)**	**Serotype(n)**	**P-type**	**No. pos/tot samples(%)**	**Serotype(n)**	**P-type**
Slaughterhouse	**Slaughtered pigs**	faeces	0/135(0)[a]	1/2a(1);1/2b(2)	P27;P28;P29	2/150(1.3)[a]	1/2a(1);1/2c(1)	P03;P05
		amygdales	3/135(2.2)[a]			7/150(4.6)[a]	1/2a(5);1/2c(2)	P02;P03;P06;P07;P08;
		ileo-cecal lymphonodes	n.p.			4/114(3.5)	1/2a(1);1/2c(3)	P04;P09;P20
		total	3/270(1.1)[a]			13/414(3.1)[a]		
	environment	MCS	0/9(0)[a]			0/10(0)[a]		
		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)[a]		
	total		3/297(1)[a]			14/444(3.1)[a]		
Cutting	**food**	HAC	0/9(0)[a]			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)[a]	1/2b(3);1/2c(3)	P01;P15;P16
		gloves, knives	0/9(0)[a]			7/10(70)[b]	1/2b(3);1/2c(4)	P01;P04;P12;P16
		NMCS	0/9(0)[a]			3/10(30)[a]	1/2c(3)	P01;P23
		total	1/36(2.7)[a]			16/40(40)[b]		
	total		1/45(2.2)[a]			20/50(40)[b]		
Cooling Room	**environment**	NMCS	0/9(0)[a]			1/10(10)[a]	1/2c(1)	P13
Trimming	**food**	HBT	0/9(0)[a]			8/10(80)[b]	1/2b(4);1/2c(4)	P01;P13;P16
	environment	MCS	3/9(33.3)[a]	1/2c(3)	P26;P31;P33	6/10(60)[a]	1/2b(3);1/2c(3)	P01;P13;P16;P19
		gloves and knives	3/9(33.3)[a]	1/2c(3)	P26;P32;P34	5/10(50)[a]	1/2b(3);1/2c(3)	P04;P16
		NMCS	3/9(33.3)[a]	1/2c(3)	P01;P26;P30	4/10(40)[a]	1/2b(1);1/2c(3)	P01;P04;P13;P16
		total	9/27(33.3)[a]			15/30(50)[a]		
	total		9/36(25)[a]			23/40(57.5)[b]		
Storage Chill Room	**food**	HBC	0/100[a]			27/100(27)[b]	1/2a(4);	P01;P04;P10;P11;P12;P14;

		1/2b(14);1/2c(9) P16;P17;P18;P21;P22;P24;P25
Total	13/487(2.6)[a]	85/644(13.2)[b]

n.p.: not performed; MCS: meat contact surface swabs, namely ham conveyor for cutting and trimming areas, and hams rack for cooling room; NMCS: non-meat contact surface swabs, namely drain; HAC: hams after cutting; HBT: hams before trimming; HBC: ham post-trimming and before curing. Number of isolates in rows bearing different letters are significantly different ($p < 0.05$) between plants A and B.

Table 2. Minimum Inhibitory Concentration distribution of the 98 *Listeria monocytogenes* strains 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						

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.

Table 3. Antimicrobial resistance, lineage, serotypes and origin of the 98 *Listeria monocytogenes* strains isolated in the present study.

				No. of resistant isolates (%)										
Lineage	**Serotype**	**Origin**	**N. of isolates**	**Macrolides**	**Lincosamides**	**Lipopeptides**	**Fluoroquinolones**		**Tetracycline**	**Folate Pathway Inhibitors**	**Oxazolidinones**	**Penicillins**	**R at least one AA**	**MDR**
				ERY	**CLI**	**DAP**	**LEVO**	**CIP**	**TET**	**SXT**	**LZD**	**OXA**		
I	**1/2b**	**Slaughtered pigs**	2	-	1(50)	-	1(50)	1(50)	-	-	-	-	2(100)	-
		Environment	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)[a,1]	16(44.4)[a,1]	2(5.5)[a,1]	4(11)[a,1]	-	-	-	28(77.7)[a,1]	35(97.2)[a,1]	1(2.7)[a,1]
II	**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)
		Environment	1	-	-	-	-	-	-	-	-	-	-	-
		Food	4	-	-	1(25)	-	-	-	-	-	4(100)	4(100)	-
		Total	13	2(15.3)	6(46.1)[b]	4(30.7)[a]	4(30.7)[a]	3(23)[b]	2(15.3)[a]	2(15.3)[a]	1(7.6)[a]	5(38.4)[a]	12(92.3)[a]	3(23)[b]
	1/2c	**Slaughtered pigs**	6	-	6(100)	2(33.3)	3(50)	5(83.3)	-	-	2(33.3)	2(33.3)	6(100)	4(66.7)
		Environment	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)^{c}	3(6.1)^{b}	28(57.1)$_{b}$	35(71.4)$_{c}$	1(2)^{a}	1(2)^{a}	3(6.1)^{a}	2(4)^{b}	47(95.9)^{a}	7(14.2)^{b}
Total	62	2(3.2)	51(82.2)^{2}	7(11.2)^{2}	32(51.6)$_{2}$	38(61.2)$_{2}$	3(4.8)	3(4.8)	4(6.4)	7(11.2)^{1}	59(95.1)^{1}	10(16.1)$_{2}$
Total	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)

ERY: erythromycin; DAP: daptomycin; CLI: clindamycin; LEVO: levofloxacin; CIP: ciprofloxacin; TET: tetracycline; SXT: trimethoprim/sulfamethoxazole; LZD: linezolid; OXA: oxacillin; AA: antimicrobial agent; MDR: multidrug resistant strains; - : not detected; number of isolates in columns bearing different numbers and letters are significantly different ($p < 0.05$) in relationship to respectively lineage and serotypes

Table 4. Antimicrobial resistance and origin of the 98 *Listeria monocytogenes* strains isolated in the present study.

AA	Slaughtered pigs	Environment				Food
		MCS	Utensil	NMCS	Total	
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)

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 the source

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 one AA	MDR
Cluster	**Isolates**	**ERY**	**CLI**	**DAP**	**LEVO**	**CIP**	**TET**	**SXT**	**LZD**	**OXA**		
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**

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

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