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Occurrence and phenotypic and molecular characterization of *Listeria monocytogenes* and *Salmonella* spp. in slaughterhouses in southern Brazil

Mariana Almeida Iglesias[a]; Isabela Schneid Kroning[b]; Luana Tombini Decol[b]; Bernadette Dora Gombossy de Melo Franco[c]; Wladimir Padilha da Silva[a,b*]

[a]Centro de Desenvolvimento Tecnológico, Núcleo de Biotecnologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil

[b]Departamento de Ciência e Tecnologia Agroindustrial, Faculdade de Agronomia, Universidade Federal de Pelotas, Pelotas, RS, Brazil

[c]Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil

*Corresponding author: Laboratório de Microbiologia de Alimentos, Departamento de Ciência e Tecnologia Agroindustrial, Faculdade de Agronomia Eliseu Maciel, UFPel. Phone: 55 53 32757284. Mail: silvawp@ufpel.edu.br

ABSTRACT

This study addressed the occurrence of *Listeria monocytogenes* and *Salmonella* spp. in bovine carcasses at two slaughterhouses in southern Brazil. Then, the antimicrobial susceptibility profile and the virulence potential of the isolates were evaluated. Two hundred carcasses were sampled at four steps of the slaughter process, with *L. monocytogenes* being isolated in 12 and *Salmonella* spp. in 17 carcasses. All *L. monocytogenes* isolates carried the *hlyA*, *prfA*, *plcA*, *plcB*, *actA*, *iap*, *mpl*, *inlA*, *inlB*, *inlC*, and *inlJ* genes, while *Salmonella* spp. carried *invA* and *hilA*. Among the *L. monocytogenes* isolates, all of them presented virulence determinants and one showed multi-drug resistance. In relationship to *Salmonella* spp. isolates, many serogroups frequently related to outbreaks of foodborne diseases were identified and four isolates showed resistance to more than one antimicrobial agent. This data highlights the importance of a rigid hygienic-sanitary control during the slaughter process to reduce the risk of cross-contamination and lower the consumer exposure to *L. monocytogenes* and *Salmonella* spp. infections.

Keywords: *Listeria monocytogenes; Salmonella* spp.; bovine carcasses; multi-drug resistance

1 Introduction

The bacterial contamination of beef is facilitated in several stages of the slaughter process, for example during skinning, evisceration and pre-cooling, which are

the main steps where contamination needs to be controlled (Gill, Bryant, & Landers, 2003; Pointon, Kiermeier, & Fegan, 2012). In addition, surfaces within the industrial plants and of transport means of the animals can have an influence on carcass contamination (Nørrung & Buncic, 2008).

According to the Brazilian Ministry of Health (MS - Ministry of Health, 2014), products of animal origin are the main cause for foodborne diseases (FBD) and prevention depends mainly on the microbiological quality of the raw material. Among the pathogenic microorganisms that can contaminate beef cattle are *Salmonella* spp., *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli* and *Campylobacter* spp., which have been frequently involved in cases and outbreaks of FBD (Antic et al., 2010).

In the last decades an overall increase in the incidence of antimicrobial resistance in foodborne strains has been reported. This is an important aspect related to foodborne diseases which, together with the different virulence factors and mechanisms of pathogenicity of bacteria, makes difficult to control these diseases (Conter et al., 2009; Granier et al., 2011; Haubert, Mendonça, Lopes, Cardoso, & Silva, 2015; Kovacevic, Sagert, Wozniak, Gilmour, & Allen, 2013).

Considering this, the purpose of this study was therefore to assess the occurrence of *L. monocytogenes* and *Salmonella* spp. in bovine carcasses at two slaughterhouses in southern Brazil, as well as an evaluation of the antimicrobial susceptibility profile and the phenotypic and genotypic characterization of the virulence potential of the isolates.

2 Material and Methods

2.1. Sampling

Two hundred bovine carcasses were sampled in 2 slaughterhouses at 4 steps of the slaughter process (step 1 - after bleeding, step 2 - after skinning, step 3 - after

evisceration, and step 4 - after pre-cooling washing), totaling 800 samples. All the animals analyzed were males and belonged to different breed of European origin. Both slaughterhouses receive animals from all southern regions of the state of Rio Grande do Sul, Brazil. The slaughterhouse A (SH-A) has a daily slaughter capacity of 150 head of cattle, on average, while the slaughterhouse B (SH-B) slaughter 650 cattle per day. Of these 800 samples, 452 were collected in SH-A and 348 in SH-B. The carcasses were sampled according to the recommendations in force of the European Community - Commission Regulation EC (2007), using the surface swab technique (hides – sampling step 1, and carcasses - sampling steps 2, 3 and 4). Each carcass was sampled with a set of 4 previously moistened 3M™ sponges (3M Microbiology, St. Paul, MN, USA), and each sponge was applied in an area of 100 cm^2, resulting in a total sampled area of 400 cm^2 (Andrews and Hammack, 1998). The swab was performed in the region of the animal chest of the carcasses (sampling steps 1 and 2) and half carcasses (sampling steps 3 and 4).

Thereafter, 200 mL of buffered peptone (1%) saline (0.85%) solution (BPS, Oxoid Ltd., Basingstoke, England) was added to each sponge set. A 40 mL aliquot of each sample from the carcass surfaces was transferred to Falcon tubes, which were centrifuged at 1000 x *g* for 15 min, at 5 °C (Eppendorf 5430 R). The resulting supernatant was discarded and the pellets were used to evaluate the presence of *L. monocytogenes* and *Salmonella* spp..

2.2 Isolation and identification of *L. monocytogenes*

L. monocytogenes was isolated and identified according to the methods described by the International Organization for Standardization (ISO 11290-1). After centrifugation, the pellet was suspended in 10 mL half Fraser broth (Oxoid Ltd., Basingstoke, England), followed by incubation at 30 °C ±2 for 24 h (Solab, B.O.D SL –

200/364). After the incubation period, 0.1 mL of each culture was transferred to test tubes containing 10 mL of Fraser broth (Oxoid Ltd., Basingstoke, England), and incubated at 37 °C ±2 for 48 h (Solab, B.O.D SL – 200/364). Later, inoculation was carried out on Oxford (Oxoid Ltd., Basingstoke, England) and Chromogenic Listeria agar (Oxoid Ltd., Basingstoke, England), which were incubated at 37 °C ±2 for 48 h (Solab, B.O.D SL – 200/364). Typical *Listeria* spp. colonies were selected and inoculated on Tryptone-Soy agar (TSA, Oxoid Ltd., Basingstoke, England) enriched with 0.6% yeast extract (YE, Himedia Laboratories Pvt. Ltd., Mumbai, India) (TSA-YE) and incubated at 37 °C ±2 for 24 h (Solab, B.O.D SL – 200/364). Then they were subjected to phenotypic identification based on the production of catalase and β-hemolysis, motility at 25 °C ±2 and fermentation of dextrose, rhamnose, mannitol, and xylose. In all analyses, the *L. monocytogenes* ATCC 7644 strain was used as positive control.

2.3 Isolation and identification of *Salmonella* spp.

The *Salmonella* spp. isolation was performed as described by the International Organization for Standardization (ISO 6579:2002), with modifications. After centrifugation, the pellet was subject to selective enrichment in 10 mL of Muller-Kauffmann Tetrathionate broth with novobiocin (MKTTn, Oxoid Ltd., Basingstoke, England) and 10 mL of Rappaport-Vassiliadis broth with soybean (RVS, Oxoid Ltd., Basingstoke, England), and the respective tubes incubated for 24 h at 37 °C ±2 (Solab, B.O.D SL – 200/364) and 41.5 °C ±2 (Quimib, Mod. 0215M2). Thereafter, two selective media were used: Xylose Lysine Deoxycholate agar (XLD, Oxoid Ltd., Basingstoke, England) and Mannitol Lysine Crystal Bright Green Violet agar (MLCB, Oxoid Ltd., Basingstoke, England), incubated at 37 ºC ±2 for 24 h (Solab, B.O.D SL – 200/364). The colonies with typical characteristics of *Salmonella* spp. were subjected to

phenotypic confirmation on triple-sugar-iron agar (TSI, Acumedia Manufacturers, Inc. Lansing, Michigan), Lysine-Iron agar (LIA, Acumedia Manufacturers, Inc. Lansing, Michigan) and Urea broth (Labsynth Ltd., São Paulo), incubated at 37 °C ±2 for 24 h (Solab, B.O.D SL – 200/364).

The isolates with typical *Salmonella* spp. reactions in the phenotypic tests were subjected to serology tests with somatic polyvalent anti-*Salmonella* serum (Probac, Brazil) and flagellar polyvalent anti-*Salmonella* serum (Probac, Brazil). In all analyses, the *Salmonella* Typhimurium ATCC 14028 strain was used as positive control.

2.4 Molecular typing

2.4.1 DNA extraction

Genomic DNA was extracted according to the protocol recommended by Green & Sambrook (2012) with minor adaptations.

2.4.2 Detection of virulence genes by PCR assay

After confirmation of *Listeria* genus by the presence of *prs* gene and of *L. monocytogenes* by the presence of *inlA* gene, the isolates were subjected to molecular serogrouping identification by the presence of *lmo1118*, *lmo0737*, *ORF2110* and *ORF2819* genes. The other species of *Listeria* were phenotypically identified.

The virulence of *L. monocytogenes* isolates was characterized based on the presence of LIPI-1 (*hlyA*, *prfA*, *plcA*, *plcB*, *actA*, *mpl)*, *iap*, *inlA*, *inlB*, *inlC*, and *inlJ* genes. The *pfrA* gene is a central virulence regulator. The *iap* (encoding the invasion-associated protein) *and actA* (encoding the surface actin polymerisation protein actA) genes are required for invasiveness and *hlyA* (encoding a haemolysin) is responsible for hemolytic activity of *L. monocytogenes.* The *plcA* (encoding phosphatidylinositol phospholipase-C (PI-PLC)) and *plcB* (phosphatidycholine phospholipase-C) genes are required for primary and secondary vacuole lysis during the infection. The *mpl*

(encoding the metalloprotease) gene is involved in the PI-PLC activation in the new cycle of infection. Finally, the internalin genes (*inlA*, *inlB*, *inlC*, and *inlJ*), encoding a inlA, inlB, inlC and inlJ proteins, are involved in cell invasion and in post-intestinal infection.

For the *Salmonella* isolates, the presence of *hilA*, *pefA*, *invA*, *sefA*, and *spvC* genes was evaluated. The *hil*A (Hyper Invasibility) gene is located in a highly conserved genome region of *Salmonella* spp. and is required to regulate cell invasion and macrophage apoptosis. The *invA* gene is required to increase the capacity of invasion of *Salmonella* in epithelial cells. The *spvC* (*Salmonella Plasmid Virulence*) gene is responsible for systemic spread of the pathogen. Finally, the *sefA* and *pefA* genes are involved, respectively, in fimbria expression and cell adhesion. The oligonucleotide sequences and references used in this study are shown in Table 1. In all analyses, the *L. monocytogenes* ATCC 7644 strain was used as positive control.

PCR assays were performed using a final volume of 25 µL using 12.5 µL Gotaq® Green Master Mix (Promega®), 10 pmol of each primer and 10 ng DNA template. A reaction mixture without DNA template was included as a negative control for each primer. The isolates of *L. monocytogenes* were subjected to PCR for molecular serogrouping identification according to Doumith, Buchrieser, Glaser, Jacquet, & Martin (2004), under the following conditions: 3 min at 94 °C, followed by 35 cycles of 94 °C for 40 sec, 53 °C for 1.15 min, and 72 °C for 1.15 min, with a final cycle at 72 °C for 7 min (Bioer, Life Express Thermal Cycler TC-96/G/H(b)).

For the internalin genes (*inlA*, *inlB*, *inlC*, and *inlJ*), initial denaturation was performed at 94 °C for 2 min, 30 cycles of 94 °C for 20 s, 55 °C for 20 and 72 °C for 50 s, followed by a final extension at 72 °C for 2 min. For the *prfA, plcA, hlyA, iap, and actA* genes, initial denaturation was performed at 95 °C for 2 min, 35 cycles of 95 °C

for 15 s, 60 °C for 30 s, and 72 °C for 1 min and 30 s, followed by a final extension at 72 °C for 10 min. For the *mpl* and *plcB* genes, initial denaturation was used at 94 °C for 3 min, 35 cycles of 94 °C for 1 min, 60 °C for 2 min, and 72 °C for 1 min, with a final extension at 72 °C for 5 min (Bioer, Life Express Thermal Cycler TC-96/G/H(b)).

For the *Salmonella* isolates, the PCR program consisted, for the *hilA* gene, of initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 63 °C for 45 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. For the *pefA, invA, sefA,* and *spvC* genes, initial denaturation at 94 ºC for 2 min followed by 35 cycles of 94 ºC for 30 s, 53 ºC for 45 and 72 ºC for 1 min, with a final extension at 72 °C for 7 min (Bioer, Life Express Thermal Cycler TC-96/G/H(b)).

The PCR products were submitted to electrophoresis on 1.5% agarose gel (Invitrogen®) with a 1Kb molecular weight marker (Invitrogen®). The amplification products were visualized under UV light in a transilluminator (Loccus®, L-Pix Touch).

2.5 Evaluation of antimicrobial susceptibility

The isolates were tested for their susceptibility to antimicrobial agents, which are currently used in veterinary and human therapy, according to the Clinical and Laboratory Standards Institute (CLSI, 2015). Briefly, the isolates were grown in TSA-YE agar at 37ºC ±2 for 24 h. After the incubation period, the cultures are adjusted to approximately $1{,}4\times10^{8}$ CFU/mL, a turbidity equivalent to a 0.5 McFarland standard. Then, the cultures were spread on the surface of Mueller-Hinton agar plates (MH, Acumedia Manufacturers, Inc. Lansing, Michigan) and antimicrobial disks (Laborclin Produtos para Laboratórios Ltda, Brazil) were added. To determine the extent of antimicrobial susceptibility, the diameter of the inhibition zone around each antimicrobial disk was measured after an incubation period of 24h at 37 °C ±2 using CLSI guidelines.

L. monocytogenes isolates were tested against 15 antimicrobial agents: ampicillin (10 μg), vancomycin (30 μg), kanamycin (30 μg), gentamicin (10 μg), streptomycin (10 μg), tobramycin (10 μg), erythromycin (15 μg), tetracycline (30 μg), minocycline (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), clindamycin (2 μg), rifampicin (5 μg), trimethoprim (5 μg), and sulfonamides (300 μg). *Staphylococcus aureus* ATCC 25923 was used as control.

Salmonella isolates were tested against 15 antimicrobial agents: ampicillin (10 μg), cefoxitin (30 μg), cephalothin (30 μg), cefotaxime (30 μg), imipenem (10 μg), chloramphenicol (30 μg), amikacin (30 μg), cyclosporine (30 μg), gentamicin (10 μg), kanamycin (30 μg), clindamycin (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (25 μg), and sulfonamide (300 μg). *Escherichia coli* ATCC 25922 was used as control.

3 Results

3.1 Occurrence of *L. monocytogenes* and *Salmonella* spp. in bovine carcasses

The occurrence of *L. monocytogenes* in bovine carcasses was 6% (12/200), being 7 isolates (L99, L106, L108, L109, L110, L130, and L159) detected at step 1 and 5 (L17, L18, L97, L152, and L154) at step 4. Of these, only L130 was isolated from SH-B. In relation to serogroups, the 12 isolates belong to the lineages I and II, represented, respectively, by the serotypes 4b, 4d or 4e and 1/2c or 3c (Table 2).

Aside from contamination by *L. monocytogenes*, 22.5% (45/200) of the carcasses were contaminated by *L. innocua.* Of these, 40% (18/45) from SH-A (17 from step 1 and 1 from step 3) and 60% (27/45) from SH-B (16 from step 1, 1 from step 3 and 10 from step 4). Moreover, *L. grayi* (1/200) was isolated at step 3 in SH-A and *L. welshimeri* (2/200) at step 1 in SH-B.

Of the 200 sampled carcasses, 8% (16/200) presented contamination by *Salmonella* spp., of which 10 isolates (S338, S340, S359, S360, S560, S566, S572, S574, S577, and S582) were detected in the slaughterhouses SH-A and six (S307, S612, S618, S639, S634, and S654) in SH-B, all at step 1 of the slaughter line. Eight serovars were identified, with prevalence of *S.* Senftenberg (43.7%), followed by *S.* Muenster, *S.* Anatum, *S.* Enterica (O:6.7) (12.5%), *S.* Ohio (6.2%), *S.* Derby (6.2%) and *S.* Livingstone (6.2%) (Table 3).

3.2 Presence of the LIPI-1, *inlA, inlB, inlC, inlJ,* and *iap* genes among *L. monocytogenes* isolates

All *L. monocytogenes* isolates carried genes for LIPI-1 *(prfA, plcA, hlyA, mpl, actA, plcB*) as well as *inlA, inlB, inlC, inlJ* and *iap* genes.

3.3 Presence of the *hilA, invA, sefA, pefA, and spvC* genes among *Salmonella* spp. isolates

The expected fragments of the *hilA* and *invA* genes, used for confirmation of *Salmonella* at the genus level, were amplified in all isolates. However, the fragments of the *spvC, sefA* and *pefA* genes were not amplified.

3.4 Antimicrobial susceptibility

The *L. monocytogenes* isolates were resistant to gentamicin (1/12), kanamycin (1/12) and sulfonamides (10/12). Intermediate resistance was observed against clindamycin (7/12) and erythromycin (1/12). Isolate L18 was resistant to four classes of antimicrobials (gentamicin, erythromycin, sulfonamides, and kanamycin) and classified as multi-drug resistant.

All *Salmonella* spp. isolates were susceptible to cefotaxime, imipenem, chloramphenicol, amikacin, gentamicin, nalidixic acid, ciprofloxacin, trimethoprim-

sulfamethoxazole, and to kanamycin. Four isolates showed resistance to more than one antimicrobial agent. *S.* Derby showed resistance to the antimicrobials tetracycline and sulfonamides, whereas an isolate of *S.* Enterica (O:6.7) and all *S.* Anatum isolates presented resistance to ampicillin, cefoxitin and cephalothin. Only one *S.* Anatum isolate was moderately resistant to streptomycin.

4 Discussion

Carcass contamination in slaughterhouses can occur at various steps along the slaughter line, through the feces, hair and intestinal tract of the animals, as well as through handling, surfaces and equipment. The identification and monitoring of these steps are extremely important for public health for underlying the definition of adequate control measures, minimizing the presence of pathogens such as *L. monocytogenes* and *Salmonella* spp. in beef.

The occurrence of *L. monocytogenes* in bovine carcasses at two slaughterhouses in the southern region of the state of Rio Grande do Sul, Brazil, was 6% (12/200). This result is close to that found by Loiko et al. (2016) in the central region of the same Brazilian state, who found this microorganism in 6.48% (7/108) of the carcasses. However, Camargo et al. (2014) found a low incidence of *L. monocytogenes* (0.95%) when evaluating 209 bovine carcasses in slaughterhouses in the Minas Gerais state, Brazil.

Eight-five percent (17/200) of the carcasses were positive for *Salmonella* spp., as similarly reported by Cossi et al. (2014), who detected *Salmonella* spp. in 9.6% (20/209) of the evaluated carcasses in Minas Gerais state, Brazil. On the other hand, Loiko et al. (2016) isolated *Salmonella* spp. in only 0.93% (1/108) of the evaluated carcasses in Rio Grande do Sul state, Brazil.

Four critical steps of the slaughter line at two slaughterhouses were evaluated in this study. *L. monocytogenes* and *Salmonella* spp. were isolated at step 1 (after bleeding) and just *L. monocytogenes* at step 4 (after the final wash). *Salmonella* spp. was only isolated at step 1, where 10 isolates (S338, S340, S359, S360, S560, S566, S572, S574, S577, and S582) from SH-A and 6 (S307, S612, S618, S639, S634, and S654) from SH-B were found. Seven isolates of *L. monocytogenes* (L99, L108, L108, L109, L110, L130, and L159) were obtained at step 1 and 5 isolates (L17, L18, L97, L152, and L154) at step 4, of which only isolate L130 was from SH-B.

The occurrence of *L. monocytogenes* and *Salmonella* spp. on animal hides after bleeding (step 1) was 3.5% (7/200) and 8.5% (17/200), respectively, demonstrating that animal hides are an important vehicle of pathogen introduction into the slaughter line. After their entry, these microorganisms can adhere and persist in the slaughter environment, promoting recurrent contamination in the carcass throughout its processing (de Oliveira, Brugnera, Alves, & Piccoli, 2010).

It is noteworthy that the occurrence of *L. monocytogenes* in the carcass immediately before cooling was 5%, which is a concern because, due to its psychotrophic characteristic, this pathogen can multiply during storage in the cold chamber (Sofos & Geornaras, 2010). Similar results were obtained by Guerini et al. (2007) in the United States of America, as well as by Camargo et al. (2014) and Loiko et al. (2016) in Brazil, who have also found *L. monocytogenes* in bovine carcasses before cold storage.

The identity of *L. monocytogenes* isolates (n=12) evaluated by phenotypic tests was confirmed by PCR, targeting the species-specific *inlA* gene. The evaluation of the virulence potential of the isolates was based on the presence of internalin genes (*inlA*, *inlB*, *inlC*, and *inlJ*), of the genes present in LIPI-1 (*prfA*, *plcA*, *hlyA*, *mpl*, *actA*, and

plcB) and of the *iap* gene. The 12 isolates carried the genes *inlA*, *inlB*, *inlC* and *inlJ*, as also observed in previous studies (Camargo et al.,2014; Gelbíčová & Karpíšková, 2012; (Indrawattana et al., 2011; Jamali, Radmehr, & Thong, 2013; Liu, Lawrence, Austin, & Ainsworth, 2007; Lomonaco, Patti, Knabel, & Civera, 2012; Mammina et al., 2009; Sant'Ana, Igarashi, Landgraf, Destro, & Franco, 2012). The *inlA* gene encodes a product that is responsible for the entry of the bacterium into the host cell and the genes *inlB, inlC* and *inlJ* are directly involved in the subsequent infection stages (Doumith et al., 2004; Liu et al., 2007). The detection of the *inlC* and *inlJ* genes is an important result, since Liu et al. (2007) reported the absence of these genes in non-pathogenic isolates.

The genes located in LIPI-1 are related with many steps of *L. monocytogenes* infection, as well as the *iap* gene, which has an indirect function in their pathogenesis (Vázquez-boland et al., 2001) codifying a product that is responsible for entering the host cell. These genes were detected in all *L. monocytogenes* isolates, suggesting their virulence potential (Bueno et al., 2010; Camargo et al., 2014; Jaradat, Schutze, & Bhunia, 2002; Moreno et al., 2012).

Salmonella Senftenberg was the most prevalent serovar (43.7%) among the isolates, followed by *S.* Muenster, *S.* Anatum and *S.* Enterica (12.5% each). The least prevalent serovars were *S.* Ohio, *S.* Derby, *S.* Livingstone, and *S.* Diarizonae (6,2% each). Studies in other countries, such as those of Stevens et al. (2008) and Ghafir et al. (2005) detected *S.* Muenster and *S.* Enteritidis as the most prevalent serovars in bovine carcasses and meat, respectively.

All *Salmonella* isolates (17) carried the *hilA* gene, responsible for macrophage invasion and apoptosis, and the *invA* gene, used as standard for *Salmonella* spp. identification. However, none carried the *sefA, pefA* and *spvC* genes involved in fimbria

expression (Porwolik and McClelland, 2003), cell adhesion (Knodler et al., 2009), and systemic infection (Paesold et al., 2002), respectively. The absence of these genes may possibly be due to the different serovars found in this study, since these genes are normally found in *S.* Typhimurium and *S.* Enteritidis. Similar results were obtained by Crăciunaş et al. (2012), who evaluated 39 isolates of different *Salmonella* spp. serovars, and found that only *S.* Enteritidis isolates amplified the *sefA* and *spvC* genes. On the other hand, Bolton et al. (2013) evaluated 29 isolates of *Salmonella* spp. for the presence of the *spvC* and *pefA* genes, and observed that 96.6% amplified the *spvC* gene, all *S.* Typhimurium, whereas none amplified the *pefA* gene.

The frequency of antimicrobial resistance among the isolates was relatively low. Regarding to *L. monocytogenes* isolates, resistance to gentamicin (1/12), kanamycin (1/12) and sulfonamides (10/12) were observed. Intermediate resistance was observed for clindamycin (7/12) and erythromycin (1/12). Similar results were described by Camargo et al. (2014); Conter et al. (2009); Harakeh et al. (2009); Yücel et al. (2005); and Zulema Ruiz-Bolivar (2011), showing that the incidence of antimicrobial resistance in *L. monocytogenes* from food is still low. It is noteworthy that isolate L18 was resistant to gentamicin, erythromycin, sulfonamides, and kanamycin, and was therefore classified as multi-drug resistant, since resistance to three antimicrobial classes was observed (EFSA/ECDC, 2013). The multi-drug resistance of the L18 isolate reinforces the importance of monitoring the antimicrobial resistance profile of *L. monocytogenes* isolated from food, since an overall increase in the incidence of antimicrobial resistance in foodborne strains has been reported (Granier et al., 2011; Haubert et al., 2015; Kovacevic et al., 2013; Loiko et al., 2016).

Among the *Salmonella* isolates, 23.5% (4/17) were resistant to antimicrobial agents. The isolates belonging to the serovars *S.* Anatum, *S.* Derby and *S.* Enterica were

resistant to more than one antimicrobial agent, as described in previous studies (Favier et al., 2013, Alemu et al., 2012; Brichta-Harhay et al., 2011; Yan et al., 2010). In our study, the antimicrobial agents most related to multi-drug resistance belong to the β-lactams, aminoglycosides and tetracyclines, which have different action spectra, but are usually used in veterinary and human medicine.

4 Conclusion

There is occurrence of *L. monocytogenes* and *Salmonella* spp. in bovine carcasses slaughtered in southern Brazil, being the hides an important vehicle of these pathogens introduction into the slaughter lines. Among the *L. monocytogenes* isolates, all of them presented virulence determinants and one showed multi-drug resistance, characterizing a public health issue. In relationship to *Salmonella* isolates, many serovars frequently related to outbreaks of foodborne diseases were identified. This data highlights the importance of a rigid and systematic hygienic-sanitary control during the stages of the slaughter process to reduce the risk of cross-contamination and lower the consumer exposure to *L. monocytogenes* and *Salmonella* spp. infections.

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Table 1 Sequences of primer sets used in this study

Gene target	Primer sequence (5'- 3')	Amplicon size (bp)	Reference
InlA-1	ACGAGTAACGGGACAAATGC	800	Liu et al. (2007)
InlA-2	CCCGACAGTGGTGCTAGATT		
InlB-1	TGGGAGAGTAACCCAACCAC	884	Liu et al. (2007)
InlB-2	GTTGACCTTCGATGGTTGCT		
InlC-1	TGGGAGAGTAACCCAACCAC	471	Liu et al. (2007)
InlC-2	GTTGACCTTCGATGGTTGCT		
InlJ-1	TGTAACCCCGCTTACACAGTT	597	Liu et al. (2007)
InlJ-2	AGCGGCTTGGCAGTCTAATA		
hly-1	GCAGTTGCAAGCCTTGGAGTGTGAA	*456*	Kaur, Malik, Vaidya
hly-2	GCAACGTATCCTTCCAGAGTGATCG		& Barbuddhe. (2007)
mpl-1	TTG TTC TGG AAT TGA GGA TG	502	Conter et al. (2009)
mpl-2	TTA AAA AGG AGC GGT GAA AT		
plcA-1	CTC GGA CCA TTG TAG TCATCTT	326	Lomonaco et al. (2012)
plcA-2	CACTTTCAGGCGTATTAGAAACGA		
plcB-1	GGG AAA TTT GAC ACA GCG TT	261	Vazquez-Boland et al. (1992)
plcB-2	ATT TTC GGG TAG TCC GCT TT		
actA-1	CCAAGCGAGGTAAATACGGGA	650	Lomonaco et al. (2012)
actA-2	GTCCGAAGCATTTACCTCTTC		
prfA-1	ACCAATGGGATCCACAAGA	467	Bubert et al. (1999)
prfA-2	CAGCTGAGCTATGTGCGAT		
iap-1	ACAAGCTGCACCTGTTGCAG	*131*	Kaur et al. (2007)
iap-2	TGACAGCGTGTGTAGTAGCA		
hilA-1	GCGAGATTGTGAGTAAAAACACC	*413*	Craciunas et al. (2012)
hilA-2	CTGCCCGGAGATATAATAATCG		
invA-1	TTGTTACGGCTATTTTGACCA	*521*	(Swamy, Barnhart, Lee
invA-2	CTGACTGCTACCTTGCTGATG		& Dreesen, 1996)
sefA-1	GCAGCGGTTACTATTGCAGC	*330*	Woodward & Kirwan. (1996)
sefA-2	TGTGACAGGGACATTTAGCG		

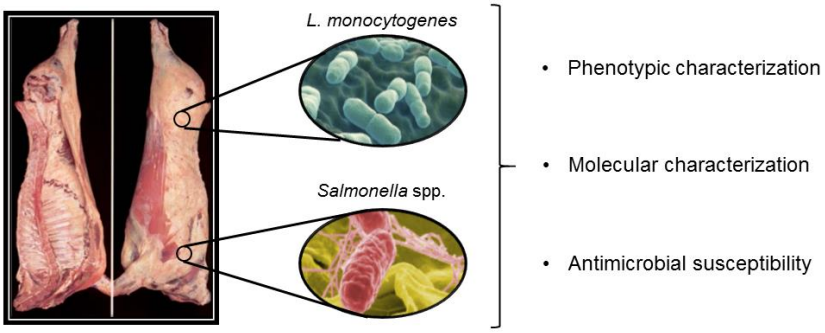
spvC-1	CGGAAATACCATCTACAAATA	*669*	Swamy et al. (1996)
spvC-2	CCCAAACCCATACTTACTCTG		
pefA-1	TTCCATTATTGCACTGGGTG	*497*	Haneda, Okada, Nakazawa,
pefA-2	AAGCCACTGCGAAAGATGCC		Kawakami & Danbara. (2001)
Imo0737-1	AGGGCTTCAAGGACTTACCC	*691*	Doumith et al. (2004)
Imo0737-2	ACGATTTCTGCTTGCCATTC		
Imo1118-1	AGGGGTCTTAAATCCTGGAA	*906*	Doumith et al. (2004)
Imo1118-2	CGGCTTGTTCGGCATACTTA		
ORF2110-1	AGTGGACAATTGATTGGTGAA	*597*	Doumith et al. (2004)
ORF2110-2	CATCCATCCCTTACTTTGGAC		
ORF2819-1	AGCAAAATGCCAAAACTCGT	*471*	Doumith et al. (2004)
ORF2819-2	CATCACTAAAGCCTCCCATTG		
prs-1	GCTGAAGAGATTGCGAAAGAAG	*370*	Doumith et al. (2004)
prs-2	CAAAGAAACCTTGGATTTGCGG		

Table 2 Identification, origin and lineages of *Listeria monocytogenes* isolates

Isolate	Slaughterhouse	Lineage
L17	A	I
L18	A	II
L97	A	II
L99	A	II
L106	A	I
L108	A	I
L109	A	I
L110	A	I
L130	B	I
L152	A	II
L154	A	II
L159	A	I

Table 3 Identification, origin and serovars of *Salmonella* spp. isolates

Isolate	Slaughterhouse	Serovar
S307	B	*S.* Derby
S338	A	*S.* Enterica (O:6,7)
S340	A	*S.* Livingstone
S359	A	*S.* Ohio
S360	A	*S.* Enterica (O:6,7)
S560	A	*S.* Senftenberg
S566	A	*S.* Senftenberg
S572	A	*S.* Senftenberg
S575	A	*S.* Senftenberg
S577	A	*S.* Senftenberg
S582	A	*S.* Senftenberg
S612	B	*S.* Senftenberg
S618	B	*S.* Muenster
S639	B	*S.* Anatum
S644	B	*S.* Muenster
S654	B	*S.* Anatum



Graphical abstract

March, 2017

Dear Editor Anderson Sant'Ana ,

Food Research International

Highlights to accompany the manuscript submitted to for **Food Research International** consideration as a Research paper. The manuscript is entitled:

"Occurrence and phenotypic and molecular characterization of *Listeria monocytogenes* and *Salmonella* spp. in slaughterhouses in southern Brazil"

Highlights

1) Occurrence of *L. monocytogenes* and *Salmonella* spp. in bovine carcasses slaughtered in southern Brazil
2) *L. monocytogenes* isolates presenting virulence potential
3) Just *hilA* gene was detected in *Salmonella* spp. isolates
4) One *L. monocytogenes* isolate presenting multidrug-resistance
5) *Salmonella* spp. isolates showing resistance to more than one antimicrobial agent