Meat Science 116 (2016) 193–200

Contents lists available at ScienceDirect

Meat Science

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

Genotypic and antimicrobial characterization of pathogenic bacteria at different stages of cattle slaughtering in southern Brazil

Márcia R. Loiko [a,b,*], Cheila M.D. de Paula [a], Ana C.J. Langone [a], Rochele Q. Rodrigues [a], Samuel Cibulski [b], Rogério de O. Rodrigues [c], Anderson C. Camargo [e], Luís A. Nero [e], Fabiana Q. Mayer [d], Eduardo C. Tondo [a]

[a] Laboratório de Microbiologia e Controle de Alimentos, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul — ICTA/UFRGS, Porto Alegre, Rio Grande do Sul, Brazil
[b] Laboratório de Virologia, Instituto de Pesquisas Veterinárias Desidério Finamor, Fundação Estadual de Pesquisa Agropecuária, Eldorado do Sul, Rio Grande do Sul, Brazil
[c] Laboratório de Leptospirose, Instituto de Pesquisas Veterinárias Desidério Finamor, Fundação Estadual de Pesquisa Agropecuária, Eldorado do Sul, Rio Grande do Sul, Brazil
[d] Laboratório de Biologia Molecular, Instituto de Pesquisas Veterinárias Desidério Finamor, Fundação Estadual de Pesquisa Agropecuária, Eldorado do Sul, Rio Grande do Sul, Brazil
[e] Universidade Federal de Viçosa, Centro de Ciências Biológicas e da Saúde, Departamento de Veterinária, Brazil

ARTICLE INFO

Article history:
Received 30 August 2015
Received in revised form 24 January 2016
Accepted 25 January 2016
Available online 26 January 2016

Keywords:
Escherichia coli O157:H7
Listeria monocytogenes
Salmonella spp.
Cattle contamination
Multidrug resistance
Foodborne pathogens

ABSTRACT

Meat can be contaminated in different stages of the slaughtering process and the identification of these stages is the starting point to implement adequate control measures. The objectives of this study were to assess the presence of pathogenic microorganisms in cattle carcasses, to identify the most important contamination points of the slaughtering process, and to evaluate the possible risk factors related to them in a cattle slaughterhouse. To this aim, 108 cattle carcasses were sampled at three stages of the slaughtering process: Point 1 (hides after bleeding); Point 2 (carcasses after hide removal); and Point 3 (carcasses immediately after division). *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Livingstone were isolated from the carcasses. Phenotypic and genotypic characterization indicated that there was cross-contamination among animals, since bacteria with identical genotypic and phenotypic profiles were isolated from different animals at the same sampling day. Furthermore, this is the first report about the isolation of *E. coli* O157:H7 in a bovine slaughterhouse from southern Brazil.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Pathogens can contaminate meat at different stages of the slaughtering process (Gill, 2007; Nouichi & Hamdi, 2009), and appropriate control measures must be in place in order to remove or prevent microbial contamination. Among pathogenic bacteria that can contaminate cattle meat, *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 are frequently pointed out as responsible for foodborne illnesses (Nouichi & Hamdi, 2009; Antic et al., 2010). According to Scallan et al. (2012) and the Ministry of Health/Brazil (MS - Ministry of Health, 2014), animal products are recognized as the major cause of foodborne diseases worldwide and the effective prevention of outbreaks depends on, among others factors, the microbiological quality of raw material used.

Another issue related to foodborne diseases is the pathogenic bacteria featuring antimicrobial resistance, which can be a serious threat to public health, as it makes the treatment of infectious diseases difficult (Arslan & Eyi, 2010). The increased use of antimicrobials in animal production and human medicine is a significant factor in the emergence of pathogens resistant to antibiotics (Silva, Hovarth, G., & Tondo, 2014; Nespolo, Saba, Rossatelli, Fairbrother, & Júnior, 2014). Based on these facts, information about sources of resistant pathogens that can contaminate meat is of great importance and is one of the main reasons of the present study, which aimed to assess the contamination of cattle hides and carcasses at different stages of the slaughtering process in southern Brazil. Moreover, we characterized the pathogenic organisms according to their antimicrobial profiles and genotypic characteristics.

2. Materials and methods

2.1. Industry characterization

The industry in which the present study was carried out was chosen due to its facilities and infrastructure management operating under the Brazilian Federal Inspection Service, with a slaughtering capacity of 500

* Corresponding author at: Laboratório de Microbiologia e Controle de Alimentos — ICTA/UFRGS, Avenida Bento Gonçalves, 9500, CEP: 43.212, Agronomia, Porto Alegre, Rio Grande do Sul, Brazil.
E-mail address: marcialoiko@gmail.com (M.R. Loiko).

http://dx.doi.org/10.1016/j.meatsci.2016.01.010
0309-1740/© 2016 Elsevier Ltd. All rights reserved.

to 700 animals/day. Besides, it was located in the central region of the Rio Grande do Sul State (RS), receiving cattle from several districts.

2.2. Sampling

From September 2010 to February 2012 (6 sampling days), samples from 108 animals of different Rio Grande do Sul regions and farms were collected. Three points of the cattle slaughtering process were assessed: Point 1 (P1): animal hides, just after bleeding; Point 2 (P2): carcasses, just after hide removal and before evisceration; and Point 3 (P3): after carcasses are cut into two large pieces for handling — totaling 324 samples. Carcasses were sampled using the sponge method, applied on the pectoral region according to the European Community guidelines (Commission Regulation — EC, 2007). Each point was sampled using four sponges, each of them scrubbed on a 100 cm^2 region (400 cm^2/point; 1200 cm^2/carcass) (Andrews & Hammack, 1998). After collection, the four sponges from each point were placed into a sterile plastic bag and stored at 4 °C until analysis. Table 1 shows the sample features and animals in this study.

2.3. Microbiological analyses

For each plastic bag containing the sponges, 200 mL of sterile 0.85% saline solution was added. The bags were then placed into a Stomacher (Seward, USA) at low speed for removal of the adhered material over the sponges.

2.3.1. Total count of aerobic mesophilic microorganisms

Aerobic Plate Count Petri films (3M Health Care, USA) were used to estimate the total count of mesophilic microorganisms. Five dilutions were plated in duplicate and incubated at 37 °C for 48 h. Red colonies were included in the count regardless of size or color intensity. The results were expressed as the log number of colony forming units (CFU) per unit area (cm^2). This method was performed in accordance with International Organization for Standardization (ISO) 4833:2003, 2003.

2.3.2. Counting of generic E. coli

Coliforms and *E. coli* Petri films (3M Health Care, USA) were used to estimate the count of generic *E. coli*. Five dilutions of the initial material were plated in duplicate and incubated at 35 °C for 48 h. Blue colonies with gas production were counted as *E. coli*. The results were expressed as log of CFU/cm^2. This method was performed in accordance with International Organization for Standardization (ISO) 21528-2:2004, 2004.

2.3.3. Isolation and identification of Salmonella spp.

During the pre-enrichment step, 25 mL of the suspension was mixed with 225 mL of buffered peptone saline water (BPW; Oxoid, England), and incubated for 18 h at 37 °C. Subsequently, 1 mL and 0.1 mL were transferred to 10 mL of Muller–Kauffmann tetrathionate–novobiocin (MKTTn, Oxoid, England) and Rappaport–Vassilliadis with soy broths (RVS, Oxoid, England) and incubated at 37 °C and 41.5 °C, respectively, for 24 h. Then, a loopful of each RVS and MKTTn broth was inoculated in xylose lysine deoxycholate (XLD, Oxoid, England) and mannitol lysine crystal violet brilliant green agar (MLCB, Oxoid, England) and incubated at 37 °C for 24 h. Candidate colonies were purified on nutrient agar and confirmed biochemically using API 20E kits (BioMérieux, USA) and serologically using poly "O" and "H" antisera (Probac, Brazil). This method was performed in accordance with International Organization for Standardization (ISO) 6579, 2001.

Table 1
Description of cattle samples collected in southern Brazil.

Variable	Legend	Score	Number of samples collected (%)
Sampling point	P1[a]	0	108 (33.33)
	P2[b]	1	108 (33.33)
	P3[c]	2	108 (33.33)
Animal age	Calf	0	57 (52.78)
	Adult	1	51 (47.22)
Region in RS	South	0	29 (26.85)
	Central	1	13 (12.04)
	West	2	48 (44.44)
	Midwest	3	18 (16.66)
Season of sampling	Spring	0	20 (18.51)
	Summer	1	32 (29.62)
	Autumn	2	25 (23.14)
	Winter	3	31 (28.70)

State of Rio Grande do Sul, southern Brazil.
[a] P1: hide after bleeding.
[b] P2: carcasses after hide removal but before evisceration.
[c] P3: carcasses immediately after division into two parts.

2.3.4. Isolation and identification of Listeria spp.

For *Listeria* spp. isolation, 40 mL of the homogenized suspension (after removal of adhered material over the sponges, as described above) was centrifuged at 2000 rpm for 15 min. The pellet was resuspended in half Fraser broth (Oxoid, England) and incubated at 30 °C for 24 h. After this, 0.1 mL was transferred to 10 mL Fraser broth (Oxoid, England) and incubated at 37 °C for 48 h. After the incubation, a loopful was inoculated on selective Agar Chromogenic and Agar Oxford (Oxoid, England) and incubated at 37 °C for 48 h. For biochemical characterization, three to five candidate colonies of *Listeria* spp. were transferred to trypticase soy agar plates (TSA, Oxoid, England) supplemented with 0.6% yeast extract (TSA–YE, Difco, USA). The plates were incubated at 37 °C for 48 h. The colonies were submitted to analysis at oblique light incidence transmitted at 45° (Henry method) and further subjected to biochemical identification using the following tests: catalase, carbohydrate fermentation, β-hemolysis, Voges–Proskauer (VP), methyl red (MR), gram staining, motility and CAMP test (McFaddin, 2000). After biochemical testing, the candidate isolates of *Listeria* spp. were tested by agglutination of "O" and "H" as recommended by Donker-Voet (1959) and Seeliger and Höhne (1979). This method was carried out in accordance with International Organization for Standardization (ISO) 11290-1 and 11290-2, 2002.

2.3.5. Isolation and identification of E. coli O157:H7

After processing on a Stomacher, 25 mL of the homogenized suspension was mixed to 225 mL of modified tryptone soy broth (Oxoid, England) containing 0.45 mg of novobiocin (Laborclin, Brazil) and incubated at 41.5 °C for 24 h. Target microorganisms were concentrated by immunomagnetic separation (IMS) using magnetic particles (Dynabeads, Invitrogen, USA) coated with anti-O157 antibodies. The pellet was inoculated onto cefixime tellurite sorbitol MacConkey agar and sorbitol MacConkey agar (both Oxoid, England). Sorbitol-negative colonies were characterized by the following biochemical tests: indol, beta-glucuronidase analysis and agglutination with *E. coli* O157 antiserum (Probac, Brazil). This method was applied in accordance with International Organization for Standardization (ISO) 16654-2001, 2001.

2.4. Molecular typing

2.4.1. DNA extraction

Salmonella spp. DNA was extracted using a Wizard® Genomic DNA kit (Promega, USA), following the manufacturer's instructions. Genomic DNA of *Listeria* spp. and *E. coli* O157:H7 were extracted by heat, as previously described (Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004; Border, Howard, Plastow, & Siggens, 1990; Paula, Geimba, Amaral, & Tondo, 2010).

2.4.2. Multiplex polymerase chain reaction (PCR)

Multiplex PCR was used to analyze the virulence of *Salmonella* spp., *Listeria* spp. and *E. coli* O157:H7 isolates. The sequences and references of the oligonucleotides used in this study are shown in Table 2. The presence of *inv*A, *sef*A and *spv*C genes was evaluated in *Salmonella* isolates. *Listeria* spp. serotype identification was carried out by the presence of *lmo*1118, *lmo*0737, *ORF* 2110, *ORF* 2819, *prs* and *hly*A genes. The

Table 2
Sequences of primer sets used in this study.

Gene target	Primer sequence (5′-3′)	Amplicon size (bp)	Serovar specificity	Reference
hlyA	For: CCTAAGACGCCAATCGAA Rev: AAGCGCTTGCAACTGCTC	702	*L. monocytogenes* serovars 1/2a and 4b	Border et al. (1990)
lmo0737	For: AGGGCTTCAAGGACTTACCC Rev: ACGATTTCTGCTTGCCATTC	691	*L. monocytogenes* serovars 1/2a, 1/2c, 3a, and 3c	Doumith et al. (2004)
ORF2110	For: AGTGGACAATTGATTGGTGAA Rev: CATCCATCCCTTACTTTGGAC	597	*L. monocytogenes* serovars 4b, 4d, and 4e	Doumith et al. (2004)
ORF2819	For: AGCAAAATGCCAAAACTCGT Rev: CATCACTAAAGCCTCCCATTG	471	*L. monocytogenes* serovars 1/2b, 3b, 4b, 4d, and 4e	Doumith et al. (2004)
Prs	For: GCTGAAGAGATTGCGAAAGAAG Rev: CAAAGAAACCTTGGATTTGCGG	370	All *Listeria* species	Doumith et al. (2004)
spvC	For: CGGAAATACCATCTACAAATA Rev: CCCAAACCCATACTTACTCTG	669	*Salmonella* spp.	Swamy et al. (1996)
invA	For: TTGTTACGGCTATTTTGACCA Rev: CTGACTGCTACCTTGCTGATG	521	*Salmonella* spp.	Swamy et al. (1996)
sefA	For: GCAGCGGTTACTATTGCAGC Rev: TGTGACAGGGACATTTAGCG	330	*Salmonella* spp.	Woodward & Kirwan (1996)
eaeA	For: GACCCGGCACAAGCATAAGC Rev: CCACCTGCAGCAACAAGAGG	384	*E. coli* O157:H7	Paton & Paton (1998)
stx2	For: GGCACTGTCTGAAACTGCTCC Rev: TCGCCAGTTATCTGACATTCTG	255	*E. coli* O157:H7	Paton & Paton (1998)
stx1	For: ATAAATCGCCATTCGTTGACTAC Rev: AGAACGCCCACTGAGATCATC	180	*E. coli* O157:H7	Paton & Paton (1998)

For: forward; Rev: reverse.

presence of virulence genes *eaeA*, *stx1* and *stx2* was evaluated in *E. coli* O157:H7 isolates.

2.4.3. E. coli O157:H7 genetic characterization

E. coli O157:H7 isolates were submitted to molecular typing by pulsed field gel electrophoresis (PFGE) using the PulseNet protocol (Ribot et al., 2006). Visualization and gel imaging were performed on an Image Quant 300 imager (Bio-Rad). Results were analyzed using BioNumerics software (Applied Maths, Belgium). Banding pattern similarities were compared using one-enzyme analysis with 1.5% band position tolerance. An international standard *Salmonella* Braenderup strain (H9812) was used as control. Dice similarity coefficients and the unweighted pair group method with averages were used to calculate similarity coefficients. *E. coli* strain O157:H7-INCQS 00171 and an *E. coli* O157:H7 isolate from a foodborne outbreak in Argentina were used as positive controls.

2.5. Cytotoxicity assay

Expression of Stx1 and Stx2 toxins was evaluated by cytotoxicity assay in Vero cells, performed according to World Organization for Animal Health recommendations (OIE, 2008). Two verotoxin-producing and two non-toxin producing *E. coli* O157:H7 strains were included in each test as positive and negative controls, respectively. Cell viability evaluation was performed as described by Mosmann (1983). Results were calculated based on the percentage of viable cells compared to cells incubated with a non-verotoxigenic bacteria supernatant. Isolates with toxicity higher than 70% in 72 h were considered verotoxic.

2.6. Antimicrobial susceptibility analysis

The isolates were tested for their resistance to antimicrobial agents, which are currently used in veterinary and human therapy, according to the National Committee for Clinical and Laboratory Standards Institute (NCCLS/CLSI, 2012). Antibiotic disks (NewProv, Brazil) with ampicillin (AMP, 10 μg), clindamycin (CLI, 2 mg), cefoxitin (CFX, 30 mg), cephalothin (CF, 30 mg), cefotaxime (CTX, 30 mg), imipenem (IPM, 10 mg), chloramphenicol (CL, 30 μg), gentamicin (GEN, 10 μg), kanamycin (K, 30 μg), streptomycin (STR, 10 μg), amikacin (AMI, 30 μg), ciprofloxacin (CIP, 5 μg), nalidixic acid (NAL,30 μg), tetracycline (TET, 30 μg), trimethoprim-sulfamethoxazole (SXT, 25 μg), and sulfonamide (SSS, 300 μg) were used in order to evaluate *E. coli* O157:H7 and *Salmonella* isolates. *E. coli* ATCC 8739 was used as control. In addition to these, vancomycin (VAN, 30 μg), tobramycin (TOB, 10 μg), erythromycin (ERY, 15 μg), minocycline (MIN, 30 μg) and trimethoprim (TRI, 5 g) were used to test *Listeria* spp. isolates. Since there are no standards or limits for testing *Listeria* spp. antimicrobials, the limits used were those established for *Staphylococcus* spp., as published by De Nes et al. (2010). *L. monocytogenes* ATCC 6477 strain was used as control.

2.7. Statistical analysis

Statistical analyses were performed using Stata software version 12.0 (Stata Corporation, College Station, Texas, USA). To compare the contamination frequencies with pathogenic bacteria among the different collection points, Fisher's exact test was carried out. For risk factor analysis, positive samples were considered as the ones with at least one isolated pathogen. Different scores were attributed to each independent variable to be evaluated (Table 1); then, a univariate analysis with dummy variables was created to test the effects of the interactions between them. Variables with $p < 0.05$ were selected to multivariate logistic regression analysis, which was performed stepwise.

3. Results

3.1. Mesophilic and E. coli counts

Mean mesophilic counts were 4.98, 2.90, and 2.30 log CFU/cm^2 in P1, P2 and P3, respectively. Regarding *E. coli*, means of 2.33, 0.64 and 0.09 CFU/cm^2 were observed at P1, P2 and P3, respectively. The mean counts observed in P1 were statistically different from those observed in other points for both groups ($p < 0.05$) (Table 3).

3.2. E. coli O157:H7, L. monocytogenes and Salmonella spp.

E. coli O157:H7, *Listeria* spp. and *Salmonella* Livingstone were isolated from 22 (20.37%), 11 (10.19%) and 1 (0.93%) of 108 carcasses, respectively. Most pathogens were isolated on bovine hide (Table 3). Among *Listeria* spp. isolates, 7 were identified as *L. monocytogenes*, two as *Listeria innocua* serovar 6a, and two remained unidentified at species level, being classified as *Listeria* spp.; *L. monocytogenes* isolates were identified as serotype 1/2a (by the gene *IMO* 0737) and 4b (by *ORF* 2110 and *ORF* 2819 genes) (Table 4).

Table 3
Bacterial contamination of 108 cattle carcasses, sampled in three points of the slaughter process in southern Brazil.

Microorganisms	P1[a]	P2[b]	P3[c]
Mesophylls (log CFU/cm^2)	4.98 ± 0.91*	2.90 ± 0.92	2.30 ± 0.89
E. coli (log CFU/cm^2)	2.33 ± 0.92*	0.64 ± 0.70	0.09 ± 0.17
E.coli O157:H7 (positivity frequency)	17 (77.27%)	4 (18.18%)	1 (4.55%)
Salmonella Livingstone (positivity frequency)	ND	ND	1 (100%)
Listeria spp. (positivity frequency)	7 (63.64%)	3 (27.27%)	1 (9.09%)

ND: not detected.
* $p < 0.0001$ when compared to P2 and P3. ANOVA with Dunn as post hoc.
[a] P1: hide after bleeding.
[b] P2: carcasses after hide removal but before evisceration.
[c] P3: carcasses immediately after division into two parts.

3.3. Virulence genes and antimicrobial resistance phenotypes

The *Salmonella* Livingstone isolate was positive for *invA* and *sefA* genes, and negative for the *spvC* gene. Among *L. monocytogenes* isolates, all of them were positive for the *hlyA* gene (Table 4). Most *E. coli* O157:H7 isolates were positive for *eae*, *stx1* and *stx2* genes (Table 5). Accordingly, cytotoxicity analysis revealed that 95.45% (21/22) of *E. coli* O157:H7 isolates were verotoxigenic.

The *Salmonella* Livingstone isolate showed resistance against six antimicrobials, i.e. ampicillin, clindamycin, cephalothin, cefoxitin, erythromycin, and vancomycin and was intermediately resistant to nalidixic acid. Regarding *L. monocytogenes*, all isolates were resistant to nalidixic acid and most showed resistance to cefoxitin (90.91%), clindamycin (90.91%), cephalothin (81.82%) and sulfonamide (54.55%). *L. monocytogenes* resistance patterns were classified into five profiles (a to e) (Fig. 1A and B). *E. coli* O157:H7 isolates had high levels of resistance, with 100% of them resistant to clindamycin (Fig. 1C). Resistance patterns were classified into seven profiles (a to g) (Fig. 1C and D).

3.4. Genotypic and phenotypic characterization of E. coli O157:H7 and Listeria spp.

E. coli O157:H7 isolates were classified into four genotypic patterns (A to D), according to PCR multiplex results (Table 5). Restriction enzyme analysis at PFGE with *XbaI* was able to discriminate six profiles (1 to 6; Table 5 and Supplementary Fig. 1). A general profile based on phenotypic and genotypic characteristics was created; thus, ten general profiles were identified (I to X). Isolates assigned to Profile I were recovered from hides of different animals sampled in the same day, at sampling Point 1 (isolates 9, 11, 12, 16 and 17). Profile I also grouped isolates from the same carcass, but at different sampling points (isolates 20, 21, and 22) (Table 5). Interestingly, isolates 7, 19, and C1 demonstrated the same Profile VI, but two of them were isolated in the present study on hide and carcass, respectively, and the third was isolated from a foodborne outbreak in Argentina in 2005.

Listeria spp. isolates were classified into four different genotype patterns (A to D) according to PCR results (Table 3). Based on genotypic and phenotypic characteristics, ten general profiles were created (I to X). As for *E. coli* O157:H7, there were isolates recovered from the same animal but at different sampling points (isolates 2 and 6 — profile VII) (Table 4), indicating cross-contamination on the slaughter process.

3.5. Longitudinal analysis of carcass contamination

The largest number of pathogenic isolates was recovered from P1 (Table3); however, when analyzing mesophilic counts, *L. monocytogenes* and *E. coli* O157:H7 by Fischer exact test, there was no statistical difference ($p > 0.05$) among the three points. For generic *E. coli* counts, there was a decrease on contamination in points P2 and P3, as compared to P1 ($p < 0.05$).

Univariate analysis showed that the sampling points 1 and 3, summer season, south and west regions and animal age were associated with a higher chance of contamination (Table 6). At multivariate analysis, the sampling Point 1 and summer season were risk factors for contamination with higher odds ratios (Table 7). Thus, despite the lack of difference on individual analysis, the multivariate data indicated that the presence of pathogens decreased in different slaughtering stages.

4. Discussion

Microorganisms can be present in cattle on animal hair, skin, and the intestinal tract, and may contaminate red meat during the slaughter process if appropriate good hygienic practices (GHP) were not applied (Gill & Landers, 2004; Shah, Shringi, Besser, & Call, 2010). The microbial contaminations during animal transport and from animal feed or contaminated surfaces are other factors that may influence the meat contamination inside slaughterhouses (Nørrung & Buncic, 2008). If these microorganisms are not inactivated before consumption, they can cause foodborne diseases; thus, meat processing control and maintenance of low microbial loads reduce the possibility of pathogen transmission (Sofos & Geornaras, 2010). Therefore, the present study aimed to monitor contamination during the slaughter process through

Table 4
Genotypic and phenotypic characteristics of *Listeria* spp. isolated from 108 cattle carcasses, sampled in three steps of slaughter process in southern Brazil.

Isolate identification	Sampling day	Animal identification	Collection point	PCR pattern[a]	Resistance pattern	General profile
1	1	1	1	B	A	I
2	1	7	1	ABD	D	VII
3	1	9	1	B	H	III
4	1	10	1	AD	B	VIII
5	1	3	1	ABD	E	IX
6	1	7	3	ABD	D	VII
7	3	22	2	ABCD	G	VI
8	3	25	2	ABCD	B	X
9	3	34	2	ABCD	C	IV
10	6	94	1	B	F	II
11	6	97	1	B	D	V

[a] PCR patterns: A — *hlyA* — *L. monocytogenes* 1/2a and 4b; B — *prs* — *Listeria* spp.; C — *ORF2110/ORF2819* — *L. monocytogenes* 4b; D — *Imo737* — *L. monocytogenes* 1/2a and 4b.

Table 5
Genotypic and phenotypic characteristics of *E. coli* O157:H7 isolated from 108 cattle carcasses, sampled in three points of the slaughter process in southern Brazil.

Isolate identification	Sampling day	Animal identification	Collection point	PCR pattern[c]	PFGE pattern	Cytotoxicity	Resistance pattern	General profile
1	4	47	NA	D	3	Negative	b	V
2	4	48	NA	B	2	Positive	a	IV
3	4	59	NA	B	2	Positive	a	IV
4	4	60	NA	B	2	Positive	a	IV
5	5	86	2	B	4	Positive	f	VIII
6	6	94	1	A	5	Positive	d	VII
7	6	95	1	A	5	Positive	c	VI
8	6	97	1	B	1	Positive	a	II
9	6	98	1	C	1	Positive	a	I
10	6	99	1	C	1	Positive	e	IX
11	6	100	1	C	1	Positive	a	I
12	6	101	1	C	1	Positive	a	I
13	6	102	1	B	1	Positive	a	II
14	6	104	1	B	1	Positive	a	II
15	6	105	1	A	1	Positive	a	III
16	6	106	1	C	1	Positive	a	I
17	6	107	1	C	1	Positive	a	I
18	6	92	2	B	1	Positive	a	II
19	6	96	2	A	5	Positive	c	VI
20	6	103	1	C	1	Positive	a	I
21	6	103	2	C	1	Positive	a	I
22	6	103	3	C	1	Positive	a	I
[a]C1	–	NA	NA	A	5	Positive	c	VI
[b]C2	–	NA	NA	A	6	Positive	g	X

NA: not applicable. Strains 1 to 4 were analyzed in pool from the three collection points.
[a] Positive control from Argentina.
[b] Positive control from Brazil (INCQS 00171).
[c] PCR patterns: A — *eae*, *stx1*, *stx2*, *rfb*O157; B — *eae*, *stx2*, *rfb*O157; C — *stx2*, *rfb*O157; D — *rfb*O157.

mesophilic microorganisms and generic *E. coli* quantification and also to detect and characterize pathogenic bacteria in cattle at an abattoir in southern Brazil. The results showed high mesophilic and generic *E. coli* counts mainly on animal hides and also the presence of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* Livingstone.

4.1. Longitudinal analysis of carcass contamination

High counts of microorganisms on animal hides are expected; however this is related to the likelihood of meat contamination in other points of the slaughter line (Gonzales-Barron, Piza, Xavier, Costa, & Cadavez, 2014; Khan et al., 2014). We observed that mesophilic counts were higher in P1, decreasing at subsequent assessed points. This suggests that dirt, feces, soil and dust were important sources of hide contamination during the slaughter process. Corroborating with this result, the majority of pathogens was isolated in P1. Several studies have shown that meat microbial contaminations in the abattoir occur during the skin cutting and opening, as well as during hide removal (Gill, McGinnis, & Bryant, 1998; McEvoy et al., 2004; Antic et al., 2010) with a decrease on contamination after this step (Gill et al., 1998; Phillips, Sumner, Alexander, & Dutton, 2001; Sumner et al., 2003).

The isolation of pathogenic microorganisms in the present study, besides providing information about their occurrence, which is a public health issue, also allowed us to survey the contamination in different points of the slaughterhouse; thus, the profiles created based on genotypic and phenotypic characteristics were used to evaluate cross-contamination in the abattoir. Therefore, of 10 *E. coli* O157:H7 general profiles, four were isolated at the same day, but on different animals (Table 5). The same was observed for two *L. monocytogenes* isolates (Table 4). This finding may strongly indicate an animal's contamination in the farm, during transport and/or at the beginning of the slaughter process. On the other hand, isolates with the same profile found at different points may indicate cross-contamination inside the slaughterhouse, as was observed here in the case of *L. monocytogenes* (isolates 2 and 6; Table 4), and *E. coli* 0157:H7 (isolates 20, 21 and 22; Table 5). This information may be used to monitor contamination or to guide implementation review of GHP procedures.

4.2. Pathogenic microorganisms

The pathogenic microorganisms isolated in this study belong to species identified as important food pathogens worldwide. Enterohemorrhagic *E. coli* (EHEC), in which *E. coli* O157:H7 is included, are currently considered some of the most important food pathogens. They can be transmitted from bovine feces to carcasses inside slaughterhouses, and the consumption of undercooked meat is the main cause of transmission to humans (Nataro & Kaper, 1998; Meng, Doyle, Zhao, & Zhao, 2007). *E. coli* O157:H7 strains have been isolated in more than 30 countries in the six continents (Bustamante, Sanso, Lucchesi, & Parma, 2010; Esumeh, Isibor, & Egbagbe, 2011; Oliveira, Viñas, Usall, Anguera, & Abadias, 2012; Velasco, Tydings, Boyer, Falkinham, & Ponder, 2012; Paula, Loiko, & Tondo, 2014); however, in Brazil, there is only one described foodborne disease outbreak, which was caused by ingestion of undercooked meat.

This study is the first report of isolation of *E. coli* O157:H7 in red meat of southern Brazil. The bacterium was isolated from 20.37% of the investigated animals, and although most of the isolates were from hide surfaces, which also had the major diversity of profiles, some of them were found on the carcasses. Interestingly, our findings showed phenotypic and genotypic similarity of 7 19 and C1 isolates (Supplementary Fig. 1; Table 5), suggesting possible transfer of *E. coli* O157:H7 clones between Brazil and Argentina. There are many reports of *E. coli* O157:H7 in cattle feces in Brazil, but just a few related to hide and beef carcass (Paula et al., 2014).

Most of the isolates were cytotoxic and obtained from animals 12–23 months of age (81.8%), which is in accordance with previously published data, which shows higher prevalence of *E. coli* O157:H7 in steers and calves than in adults (Nielsen, Tegtmeier, Andersen, Gronbaek, & Andersen, 2002; Moreira et al., 2003). Regarding cytotoxicity, few *E. coli* O157:H7 previously isolated from Brazilian cattle feces had the Shiga toxin (Stx) gene, with prevalence ranging from 1.2% to 95%, depending on the region (Moreira et al., 2003; Sandrini, Pereira, Brod, Carvalhal, & Aleixo, 2007; Stella et al., 2009; Vicente, Amaral, Nunes, & Lorenzon, 2010).

Salmonella Livingstone was isolated from one (0.93%) carcass at P3, after hide removal and before cooling. A similar result was found by

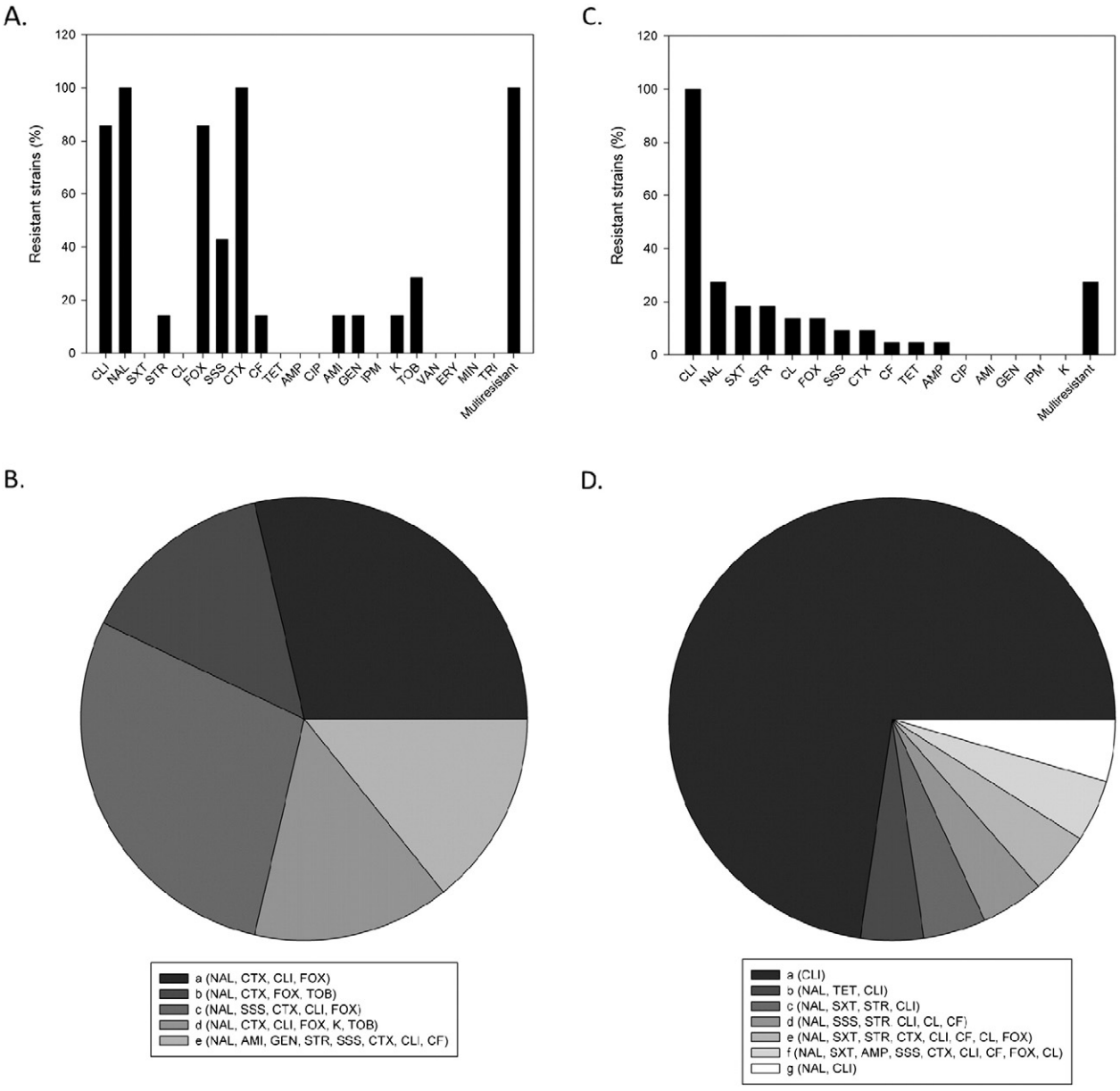

Fig. 1. Resistance patterns of *Listeria monocytogenes* and *E. coli* O157 strains isolated from 108 cattle carcasses in southern Brazil. A and C, percentage of resistant *L. monocytogenes* and *E. coli* O157:H7, respectively, to each tested antibiotic. B and D, profiles and frequency distribution of resistance patterns of *L. monocytogenes* and *E. coli* O157:H7, respectively.

Azevedo (2009), who isolated *Salmonella* spp. in 2% of bovine carcasses at the same process point. Low isolation of *Salmonella* spp. may indicate absence or low number of the bacteria on sampled areas, low sensitivity of the method used to detect stressed cells (Meyer, Thiel, Ullrich, et al., 2010), or may be related to high mesophilic counts, which can inhibit its proliferation.

Regarding *L. monocytogenes*, seven isolates were found at the three analyzed points. Moreover, two isolates of *L. innocua* and two of *Listeria* spp. were identified, which is indicative of suitable conditions for *L. monocytogenes* growth (Atil, Ertas, & Ozbey, 2011; Khan et al., 2014). The *L. monocytogenes* isolates were virulent, which is required for their survival and proliferation and is associated with hemolysis in the host. The presence of *L. monocytogenes* on carcasses is generally attributed to environmental contamination or fecal matter during slaughter, and it was estimated that 11–52% of healthy animals are carriers of these organisms (Rocourt, 1994). The maintenance of *L. monocytogenes* in the slaughterhouse is of great concern, since it is involved in severe foodborne illness outbreaks and due to its ability to proliferate, even at low temperatures (Sofos & Geornaras, 2010). *L. monocytogenes* serotypes 1/2a and 1/2b are mainly found in meat products and slaughtering environment and are the most prevalent serotypes found in foods (He Yan et al., 2010; Gianfranceschi et al., 2009). More than 95% of human listeriosis cases were caused by *L. monocytogenes* 4b and 1/2a serotypes (Fugett, Schoonmaker-Bopp, Dumas, Corby, & Wiedmann, 2007). In the present study, these serotypes were isolated from three analyzed points.

E. coli O157:H7 and *L. monocytogenes* isolates showed high levels of antimicrobial resistance (Fig. 1), which is important information for public health concerns. The use of antimicrobials as growth promoting agents in animals can modify their intestinal microbiota, creating a selection pressure favoring the development of antimicrobial resistant strains (Conter et al., 2009). Likewise, multidrug resistance is a problem in cases of foodborne outbreaks involving people infected with resistant bacteria (Conter et al., 2009; Diarra & Malouin, 2014). *Listeria* spp. resistance varies depending on the antibiotic use and the region; therefore, it is necessary to control antimicrobial resistance of *L. monocytogenes* worldwide (Wang et al., 2013). *E. coli* O157:H7 antimicrobial resistance poses a major challenge in both human and animal medicine, since the

Table 6
Univariate analysis with dummy variables of evaluated factors with potential influence in carcass contamination frequencies.

Variable	Influence	Odds ratio (95% CI)	p value
Sampling point			
P1	Yes	8.39 (4.92–14.30)	<0.001
P2	No	1.12 (0.70–1.78)	0.635
P3	Yes	0.06 (0.03–0.13)	<0.001
Age			
Calf	Yes	2.75 (1.74–4.35)	<0.001
Adult	Yes	0.36 (0.23–0.57)	<0.001
Region			
South	Yes	1.90 (1.15–3.13)	0.011
Central	No	1.26 (0.64–2.48)	0.486
West	Yes	0.41 (0.26–0.66)	<0.001
Midwest	No	1.49 (0.83–2.68)	0.178
Season			
Spring	No	2.08 (0.96–4.48)	0.060
Summer	Yes	3.44 (2.08–5.66)	<0.001
Autumn	No	0.95 (0.57–1.61)	0.865
Winter	Yes	0.24 (0.15–0.40)	<0.001

antibiotics used for this pathogen are commonly used in the treatment of human patients and in veterinary practice (WHO, 2000; Reuben & Owuna, 2013). The uncontrolled use of these drugs and their incorporation into animal feed contribute to development of resistance in humans and animals (Galland, Hyatt, Crupper, & Acheson, 2001; Reuben & Owuna, 2013).

5. Conclusion

The phenotypic and genotypic characterization in the present study showed virulent and multi-resistant isolates, which can be a public health issue. The results can be used as an alert to policymakers, highlighting the need to prevent multidrug resistance, as many antimicrobials can be used both in veterinary and human medicine. This study also showed that the bovine hides were the main source of bacterial contamination, but there was also isolation of pathogens in other assessed points. Based on these facts, the proper hide removal and implementation of GHP procedures to reduce cross-contamination are the most important control measures to be implemented in the cattle slaughter process.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meatsci.2016.01.010.

Acknowledgements

This study was supported by the project "Implementation of a Collaborating Centre for Agricultural Protection for Microbiological Risk Assessment in Animal Products (CDA-ARMPOA)" — CNPq/MAPA/SDA no. 64/2008, coordinated by Prof. Dr. Bernadette D.G.M. Franco, Universidade de São Paulo (USP) — Faculty of Pharmaceutical Sciences (Department of Food and Experimental Nutrition). We would like to thank the abattoir for having provided authorization for sample collection and the Laboratory of Zoonosis of the Department of Bacteriology of Instituto Oswaldo Cruz (FIOCRUZ), especially Dr. Dália Rodriguez, for helping with antigenic characterization, isolate identification and typing. The *E. coli* O157:H7 isolates from Argentina were kindly provided by Dra. Marta Rivas, Malbran Institute, from Argentina. We are grateful to Dr. Francisco Esmaile de Sales Lima for the English revision.

Table 7
Multivariate logistic regression. In bold are the variables which contribute to explain the overall carcass contamination ($p < 0.001$). Pseudo $R^2 = 0.3408$; N = 324; LR chi^2 (9) = 151.20; log likelihood = −146.24.

Variable	Odds ratio (95% CI)	Standard error	p value
Animal age	0.463 (0.228–0.942)	0.167	0.034
P1	4.804 (2.526–9.137)	1.575	<0.001
P3	0.082 (0.035–0.193)	0.035	<0.001
Summer	4.471 (1.904–10.304)	1.904	<0.001
Constant	0.766 (0.406–1.446)	0.248	0.412

References

Andrews, W., & Hammack, T. S. (1998). Food sampling and preparation of sample homogenate. *Food and Drug Administration bacteriological analytical manual* (8th ed.). Gaithersburg: AOAC International.
Antic, D., Blagojevic, B., Ducic, M., Nastasijevic, I., Mitrovic, R., & Buncic, S. (2010). Distribution of microflora on cattle hides and its transmission to meat via direct contact. *Food Control*, *21*, 1025–1029.
Arslan, S., & Eyi, A. (2010). Occurrence and microbial resistance profiles of *Salmonella* species in retail meat products. *Journal of Food Protection*, *73*, 1613–1617.
Atil, E., Ertas, H. B., & Ozbey, G. (2011). Isolation and molecular characterization of *Listeria* spp. from animals, food and environmental samples. *Veterinary Medicine*, *56*(8), 386–394.
Azevedo, A. P. (2009). Prevalência e características de Salmonella spp. em carne bovina brasileira para exportação: contribuição para uma avaliação de risco. *Faculdade de Ciências Farmacêuticas da Universidade de São Paulo – Ciências dos Alimentos. Dissertação de Mestrado.* (pp. 1–84).
Border, P., Howard, J., Plastow, G., & Siggens, K. (1990). Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. *Letters in Applied Microbiology*, *11*, 58–162.
Bustamante, A. V., Sanso, M. A., Lucchesi, P. M. A., & Parma, A. E. (2010). Genetic diversity of O157:H7 and non-O157 verotoxigenic *Escherichia coli* from Argentina inferred from multiple-locus variable-number tandem repeat analysis (MLVA). *International Journal of Medical Microbiology*, *300*, 212–217.
Commission Regulation (EC) No 1441/2007 (2007 Dec. 5). Amending regulation (EC) no 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union* (18 pp.).
Conter, M., Paludi, D., Zanardi, E., Ghidini, S., Vergara, A., & Ianieri, A. (2009). Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *International Journal of Food Microbiology*, *128*, 497–500.
De Nes, F., et al. (2010). Molecular analysis on *Listeria monocytogenes* in dairy products. *Revista da Sociedade Brasileira de Medicina*, *43*(4), 382–385.
Diarra, M. S., & Malouin, F. (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Frontiers in Microbiology*, *5*, 282.
Donker-Voet, J. A. (1959). Serological study on some strains of *Listeria monocytogenes* isolated in Michigan. *American Journal of Veterinary Research*, *20*, 176–179.
Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., & Martin, P. (2004). Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *Journal of Clinical Microbiology*, *42*(8), 3819–3822.
Esumeh, F. I., Isibor, J. O., & Egbagbe, I. D. S. (2011). Screening for *Escherichia coli* O157:H7 in diarrheic patients in Benin City, Nigeria. *Journal of Microbiology & Biotechnology*, *1*, 1–4.
Fugett, E., Schoonmaker-Bopp, D., Dumas, N., Corby, J., & Wiedmann, M. (2007). Pulsed-field gel electrophoresis (PFGE) analysis of temporally matched *Listeria monocytogenes* isolates from human clinical cases, foods, ruminant farms, and urban and nature environments reveals source-associated as well as widely distributed PFGE types. *Journal of Clinical Microbiology*, *45*, 865–873.
Galland, C. J., Hyatt, R. D., Crupper, S. S., & Acheson, W. D. (2001). Prevalence, antibiotic susceptibility and diversity of *E. coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. *Journal of Applied and Environmental Microbiology*, *4*, 67.
Gianfranceschi, M. V., et al. (2009). Distribution of serotypes and pulsotypes of *Listeria monocytogenes* from human, food and environmental isolates (Italy 2002–2005). *Food Microbiology*, *26*, 520–526.
Gill, C. O. (2007). Microbiological conditions of meats from large game animals and birds. *Meat Science*, *77*, 149–160.
Gill, C. O., & Landers, C. (2004). Microbiological conditions of detained beef carcasses before and after removal of visible contamination. *Meat Science*, *66*, 335–342.
Gill, C. O., McGinnis, J. C., & Bryant, J. (1998). Microbial contamination of meat during the skinning of beef carcass hindquarters at three slaughtering plants. *International Journal of Food Microbiology*, *42*, 175–184.
Gonzales-Barron, U., Piza, L., Xavier, C., Costa, E., & Cadavez, V. (2014). An exposure assessment model of the prevalence of *Salmonella* spp. along the processing stages of Brazilian beef. *Food Science and Technology International*, 1–11.
International Organization for Standardization (ISO) 11290-1 and 11290-2 (2002). *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Listeria monocytogenes.*
International Organization for Standardization (ISO) 16654-2001 (2001). Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157. *British standard, incorporating corrigendum. 1.*
International Organization for Standardization (ISO) 21528-2:2004 (2004). *Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriacea — Part 2: Colony-count method.*
International Organization for Standardization (ISO) 4833:2003 (2003). *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms colony count technique at 30 degrees C.*
International Organization for Standardization (ISO) 6579 (2001). *Microbiology of food and animal feeding stuffs – — Horizontal method for the detection of Salmonella spp.* Geneva, Switzerland: International Organization for Standardization.

Khan, B. K., Lynch, O. A., Carroli, J., McDowell, D. A., & Duffy, G. (2014). Occurrence, Antibiotic Resistente and Molecular Characterization of *Listeria monocytogenes* in the Beef chain in the Republic of Ireland 62. *Issue*, *1*, 11–17.

McEvoy, J. M., Doherty, A. M., Sheridan, J. J., Thomson-Carter, F. M., Garvey, P., McGuire, L., et al. (2004). The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. *Journal of Applied Microbiology*, *95*(2), 256–266.

McFaddin, J. F. (2000). (3rd ed.). *Biochemical tests for identification of medical bacteria*, *43*. Philadelphia, USA: Lippincott Williams & Wilkins, 544–545.

Meng, J., Doyle, M. P., Zhao, T., & Zhao, S. (2007). Enterohemorrhagic *Escherichia coli*. In M. P. Doyle, & L. R. Beuchat (Eds.), *Food microbiology: Fundamentals and frontiers* (pp. 249–269). Washington: ASM Press.

Meyer, C., Thiel, S., Ullrich, U., et al. (2010). *Salmonella* in raw meat and by-products from pork and beef. *Journal Food Protection*, *73*, 1780–1784.

MS - Ministry of Health (2014). Vigilância Epidemiológica das Doenças Transmitidas por Alimentos — VDE/DTA. http://www.anrbrasil.org.br/new/pdfs/2014/3.

Moreira, C. N., Pereira, M. A., Brod, C. S., Rodrigues, D. P., Carvalhal, J. B., & Aleixo, J. A. G. (2003). Shiga-toxin producing *Escherichia coli* (STEC) from healthy dairy cattle in southern Brazil. *Veterinary Microbiology*, *93*, 179–183.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55–63.

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, *11*, 142–201.

National Committee for Clinical Laboratory Standards International — CLSI (2012n). Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. *CLSI/NCCLS 2012-document M100 — S21, vol. 31, issue 1, January 2012*. Wayne, PA, USA: CLSI.

Nespolo, N. M., Saba, R. Z., Rossatelli, D. A., Fairbrother, J. M., & Júnior, O. D. R. (2014). Occurrence of *Escherichia coli* O157:H7 and O26 sorbitol negative in a cattle slaughterhouse and susceptibility to antimicrobials. *Arquivos do Instituto Biológico, São Paulo*, *81*(3), 209–217.

Nielsen, E., Tegtmeier, C., Andersen, H. J., Gronbaek, C., & Andersen, J. S. (2002). Influence of age, sex and herd characteristics on the occurrence of verocytotoxin-producing *Escherichia coli* O157 in Danish dairy farms. *Veterinary Microbiology*, *88*, 245–257.

Nørrung, B., & Buncic, S. (2008). Microbial safety of meat in the European Union. *Meat Science*, *78*, 14–24.

Nouichi, S., & Hamdi, T. M. (2009). Superficial bacterial contamination of ovine and bovine carcasses at El-Harrach Slaughterhouse (Algeria). *European Journal of Scientific Research*, *38*(3), 474–485.

Oliveira, M., Viñas, I., Usall, J., Anguera, M., & Abadias, M. (2012). Presence and survival of *E. coli* O157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. *International Journal of Food Microbiology*, *156*, 133–140.

Paula, C. M. D., Geimba, M. P., Amaral, P. H., & Tondo, E. C. (2010). Antimicrobial resistance and PCR-ribotyping of *Shigella* responsible for foodborne outbreaks occurred in southern Brazil. *Brazilian Journal of Microbiology*, *41*, 20–30.

Paula, C. M. D., Loiko, M. R., & Tondo, E. C. (2014). *Escherichia coli* O157:H7: Local epidemiology and disease spectrum in Brazil. *Clinical and Biological Research*, *34*(2), 113–121.

Paton, A. W., & Paton, J. C. (1998). Detection and Characterization of siga Toxigenic *Escherichia coli* by Using Multiplex PCR Assays for stx1, stx2, eaeA, Enterohemorrhagic E. *coli hlyA, rfb O111 and rfbO157, Journal of clinica Microbiology*, 598–602.

Phillips, D., Sumner, J., Alexander, J., & Dutton, K. (2001). Microbiological quality of Australian beef. *Journal of Food Protection*, *64*(5), 692–696.

Reuben, R. C., & Owuna, G. (2013). Antimicrobial resistance patterns of *Escherichia coli* O157:H7 from Nigerian fermented milk samples in Nasarawa state, Nigeria. *International Journal of Pharmaceutical Science Invention*, *2*(3), 38–44.

Ribot, E. M., Fair, M. A., Gautom, R., Cameron, D. N., Hunter, S. B., Swaminathan, B., & Barrett, T. J. (2006). Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella* and *Shigella* for PulseNet. *Foodborne Pathogens and Disease*, *3*, 59–67.

Rocourt, J. (1994). *Listeria monocytogenes* — The state of the science. *Dairy, Food and Environmental Sanitation*, *14*, 70–82.

Sandrini, C. N. M., Pereira, M. A., Brod, C. S., Carvalhal, J. B., & Aleixo, J. A. G. (2007). *Escherichia coli* verotoxigênica: Isolamento e prevalência em 60 propriedades de bovinos de leite da região de Pelotas, RS, Brasil. *Ciência Rural*, *37*, 175–182.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S. L., et al. (2012). Foodborne illness acquired in the United States: Major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://dx.doi.org/10.3201/eid1701.091101p1.

Seeliger, H. P. R., & Höhle, K. (1979). Serotyping of *Listeria monocytogenes* and Related Species - Chapter 11. *Methods in Microbiology*, *13*, 31–48.

Shah, D. H., Shringi, S., Besser, T. E., & Call, D. R. (2010). Escherichia. In D. LIU (Ed.), *Molecular detection of foodborne pathogens* (pp. 369–389). Boca Raton: CRC Press.

Silva, F. F. P., Hovarth, M. B., G., Silveira. J., & Tondo, E. C. (2014). Occurrence of *Salmonella* spp. and generic *Escherichia coli* on beef carcasses sampled at a Brazilian slaughterhouse. *Brazilian Journal of Microbiology*, *45*(1), 17–23.

Sofos, J. N., & Geornaras, I. (2010). Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. *Meat Science*, *86*, 2–14.

Stella, A. E., Rigobelo, E. C., Oliveira, A. C., Maluta, R. P., Marin, J. M., & Ávila, F. A. (2009). Ocorrência e sensibilidade microbiana de linhagens de *E. coli* enteropatogênicas isoladas de propriedades leiteiras na região de Ribeirão Preto — SP, Brasil. *Veterinária e Zootecnia*, *15*, 66–74.

Sumner, J., Petrenas, E., Dean, P., Dowsett, P., West, G., Wiering, R., & Raven, G. (2003). Microbial contamination on beef and sheep carcasses in South Australia. *International Journal of Food Microbiology*, *81*(3), 255–260.

Swamy, S. C., Barnhart, H. M., Lee, M. D., & Dreesen, D. W. (1996). Virulence Determinants *invA* and *spvC* in Salmonellae Isolated from Poultry products, Wastewater, and Human Sources. *Applied And Environmental Microbiology*, *62*, 37–3771.

Velasco, G. L., Tydings, H. A., Boyer, R. R., Falkinham, J. O., & Ponder, M. A. (2012). Characterization of interactions between *E. coli* O157:H7 with epiphytic bacteria in vitro and on spinach leaf surfaces. *International Journal of Food Microbiology*, *153*, 351–357.

Vicente, H. I. G., Amaral, L. A., Nunes, A. P., & Lorenzon, C. C. (2010). *Escherichia coli, produtoras de Shigatoxinas detectadas em fezes de bovinos leiteiros*. São Paulo: Arquivos do Instituto Biológico 77, 567–573.

Wang, X. M., Lü, X. F., Yin, L., Liu, H. F., Zhang, W. J., Si, W., et al. (2013). Occurrence and antimicrobial susceptibility of *Listeria monocytogenes* isolates from retail raw foods. *Food Control*, *32*, 153–158.

Woodward, M. J., & Kirwan, S. E. S. (1996). Detection of *Salmonella* Enteritidis in eggs by the Polymerase Chain reaction. *Veterinary Record*, *138*, 411–413.

World Health Organization (WHO) (2000). *Global principles for the containment of antimicrobial resistance in animals intended for food; report of WHO consultation with the participation of Food and Agriculture Organization of the United Nations and the Office International Des Epizooties, Geneva Switzerland 5–9 June 2000.* Department of Communicable Disease Surveillance and Response.

World Organization for Animal Health (OIE) (2008). (6th ed.). *Manual of diagnostic test and vaccines for terrestrial animals (mammals, birds and bees)*, *Vol. 2*.

Yan, H., Neogi, S. B., Mo, Z., Guan, Z., Shen, Z., Zhang, S., ... Zhong, N. (2010). Prevalence and characterization of antimicrobial resistance of foodborne *Listeria monocytogenes* isolates in Hebei province of Northern China, 2005–2007. *International Journal of Food Microbiology*, *144*, 310–316.