Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Listeria monocytogenes isolates from food and food environment harbouring *tetM* and *ermB* resistance genes

L. Haubert[1,2], M. Mendonça[2], G.V. Lopes[3], M.R. de Itapema Cardoso[3] and W.P. da Silva[1,2]

1 Departamento de Ciência e Tecnologia Agroindustrial, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil
2 Núcleo de Biotecnologia, Centro de Desenvolvimento Tecnológico, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil
3 Departamento de Medicina Veterinária Preventiva, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

Significance and Impact of the Study: *Listeria monocytogenes* is an important agent of foodborne diseases. The results of this study suggest a potential capacity of *L. monocytogenes* isolates from food and food environment to cause human infections. Antimicrobial multi-resistance profiles were detected in 10%, and two isolates harboured *tetM* and *ermB* resistance genes. Moreover, the present research can help to build up a better knowledge about antimicrobial resistance of *L. monocytogenes*. Additionally, we found one isolate carrying *tetM* resistance gene in a plasmid, that suggests a possible transmission between commensal and/or other pathogenic bacteria of food environment, thereby raising up concerns regarding bacterial resistance.

Keywords
antimicrobial resistance, internalin genes, *Listeria*, plasmid, pulsed field gel electrophoresis, virulence.

Correspondence
Wladimir Padilha da Silva, Departamento de Ciência e Tecnologia Agroindustrial; Núcleo de Biotecnologia, Centro de Desenvolvimento Tecnológico, Universidade Federal de Pelotas (UFPel), Campus Universitário, Capão do Leão, RS, Caixa Postal 354, 96160-000, Pelotas, RS, Brazil.
E-mail: wladimir@pq.cnpq.br

2015/0302: received 26 June 2015, revised 23 October 2015 and accepted 25 October 2015

doi:10.1111/lam.12516

Abstract

Listeria monocytogenes is a foodborne pathogen that has become an important cause of human and animal diseases worldwide. The purpose of this study was to evaluate the serotypes, virulence potential, antimicrobial resistance profile, and genetic relationships of 50 *L. monocytogenes* isolates from food and food environment in southern Brazil. In this study, the majority of *L. monocytogenes* isolates belonged to the serotypes 1/2b (42%) and 4b (26%), which are the main serotypes associated with human listeriosis. In addition, all isolates harboured internalin genes (*inl*A, *inl*C, *inl*J), indicating a virulence potential. The isolates were sensitive to most of the antimicrobial compounds analysed, and five isolates (10%) were multi-resistant. Two isolates harboured antimicrobial resistance genes (*tetM* and *ermB*) and in one of them, the gene was present in the plasmid. Moreover, according to the pulsed field gel electrophoresis assay, two multi-resistant isolates were a single clone isolated from food and the processing plant. The isolates were susceptible to the most frequently used antibiotics for listeriosis treatment. However, the presence of multidrug-resistant isolates and antimicrobial resistance genes including in the plasmid could even be transferred between bacterial species, suggesting a potential health risk to consumers and a potential risk of spreading multi-resistance genes to other bacteria.

Introduction

The genus *Listeria* consists of a group of Gram-positive, rod-shaped bacteria with 17 species known up to now (Weller *et al.* 2015). Among these, *Listeria monocytogenes* is the only one that can cause serious infections in animals and humans, and *L. ivanovii* has been related as pathogenic specie preferably to ruminants (Vázquez-Boland *et al.* 2001; Allerberger and Wagner 2010).

The consumption of contaminated food is the main transmission route of epidemic and sporadic human listeriosis (Bertrand *et al.* 2005). *Listeria monocytogenes* is frequently found in food products due to its ubiquitary occurrence in the environment, with varied contamination levels (Bertsch *et al.* 2013b).

To date, 13 *L. monocytogenes* serotypes are known (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4ab, 4c, 4d, 4e and 7) (Cartwright *et al.* 2013). However, 95% of the *L. monocytogenes*

strains isolated from food and human listeriosis samples belong to serotypes 1/2a, 1/2b and 4b, probably because these serotypes are the most virulent (Kathariou 2002; Orsi *et al.* 2011). Therefore, the determination of the serotype and detection of virulence genes are essential to predict possible risks and pathogenesis for consumers (Liu 2006).

Different virulence factors and mechanisms of pathogenicity act in *L. monocytogenes*, and a variety of virulence proteins were described. The internalin proteins can increase the invasion and consequently virulence of the pathogen, for example, the presence of *inl*A gene is required for internalization of the intestinal epithelial cells. Additionally, *inl*C and *inl*J genes, considered virulence markers, could be used to differentiate virulent from avirulent strains (Liu *et al.* 2007; De las Heras *et al.* 2011).

The lethality of severe listeriosis ranges from 20 to 30% (FAO/WHO 2004). Therefore, standard antibiotic therapy is required for an effective treatment of listeriosis. The first choice in the treatment of human listeriosis consists of the administration of β-lactam antibiotics, such as ampicillin or penicillin G, alone or in combination with aminoglycosides, although other antimicrobials can also be used in specific cases (Swaminathan and Gerner-Smidt 2007; Allerberger and Wagner 2010).

The strains of *L. monocytogenes* are considered relatively susceptible to a wide range of antibiotics, although in the last decades an overall increase in the incidence of antimicrobial resistance in foodborne strains has been reported (Conter *et al.* 2009; Granier *et al.* 2011; Kovacevic *et al.* 2013). In consideration of public health implications for a potential spread of antimicrobial resistant strains of *L. monocytogenes* in food products, antimicrobial resistance profiles must be determined and monitored. In the present study, we evaluate the presence of virulence genes and antimicrobial resistance profiles of *L. monocytogenes* isolates from food and food environment. Furthermore, the genetic background of the *L. monocytogenes* resistant to antimicrobials agents used in human and veterinary medicine was investigated.

Results and discussion

Listeria monocytogenes is one the most dangerous foodborne pathogens that can be found ubiquitously in the environment (De las Heras *et al.* 2011). Although any *L. monocytogenes* strain can be potentially virulent, the serotypes 1/2b and 4b, along with 1/2a, are the serotypes most frequently associated with human listeriosis (Orsi *et al.* 2011). In our study, the majority (68%) of the 50 *L. monocytogenes* isolates belonged to serotypes 1/2b and 4b. Furthermore, when we look at the serotypes isolated from food samples, the main serotype was 4b (40%), while serotype 1/2b (56%) was the most prevalent among food industry environment (Table 1). Once these serotypes are the most correlated with listeriosis, results herein suggested that consumers are exposed to potential risks of *L. monocytogenes* infection.

The internalin genes (*inl*A, *inl*C and *inl*J) were present in all 50 isolates, as also observed in other studies (Lomonaco *et al.* 2012; Jamali *et al.* 2013). The presence of these three internalin genes suggests the virulence potential of *L. monocytogenes* isolates from food and food-processing environments. The *inl*A gene is species-specific for detection of *L. monocytogenes*, expressing the surface protein InlA that plays an essential role in entry into host cells (Poyart *et al.* 1996; Torres *et al.* 2005). The *inl*C gene encodes a virulence protein of *L. monocytogenes,* which acts after the intestinal stages of infection, while *inl*J gene is involved directly in *L. monocytogenes* passage through the intestinal barrier and is also involved in the subsequent infection stages (Engelbrecht *et al.* 1996; Sabet *et al.* 2005). Studies reported that the genome of *L. monocytogenes* strains with potential virulence contains *inl*C and/or *inl*J genes, while these genes were not detected in naturally avirulent strains unable to cause mouse death (Liu *et al.* 2006, 2007).

The presence of antimicrobial resistance of *Listeria* in different food sources and its transmission to contaminated food may come to be an important public health concern. The antimicrobial resistance profile of *L. monocytogenes* isolates observed in this study is summarized in Table 2.

All 50 *L. monocytogenes* isolates were resistant to cefoxitin and nalidixic acid. Interestingly, these results were not surprising, since *Listeria* spp. were reported as naturally resistant to cephalosporins and constitutively resistant to nalidixic acid. The latter is regularly used in selective medium for this bacterium (Troxler *et al.* 2000). However, all isolates proved sensitive to other antibiotics, for example, ampicillin, penicillin G, gentamycin, amikacin, vancomycin, chloramphenicol and ciprofloxacin. Similarly, Kovacevic *et al.* (2013) found sensitivity of *Listeria* species to amikacin, ampicillin, gentamycin and

Table 1 Serotype identification of *Listeria monocytogenes* isolates from food and food industries in southern Brazil

	Sources		
Serotype	Food (%)	Food industries (%)	Total number (%)
	25 (100)	25 (100)	50 (100)
1/2a	3 (12)	2 (8)	5 (10)
1/2b	7 (28)	14 (56)	21 (42)
1/2c	5 (20)	6 (24)	11 (22)
4b	10 (40)	3 (12)	13 (26)

Table 2 Antimicrobial resistance profile of *Listeria monocytogenes* isolates

Antibiotic	Number (%)
Ampicillin	–
Penicillin G	–
Cefoxitin	50 (100)
Streptomycin	5 (10)
Gentamycin	–
Amikacin	–
Vancomycin	–
Erythromycin	3 (6)
Tetracycline	1 (2)
Chloramphenicol	–
Ciprofloxacin	–
Clindamycin	34 (68)
Trimethoprim–sulfamethoxazole	5 (10)
Nalidixic acid	50 (100)
Rifampicin	5 (10)
Meropenem	5 (10)

vancomycin. In a recent study of Wang *et al.* (2015), all *L. monocytogenes* isolates from ready-to-eat meat products were susceptible to penicillin, ampicillin, amikacin and gentamycin, whereas 33% were resistant to chloramphenicol and 30% to ciprofloxacin.

Our study found a high resistance to clindamycin, detected in 34 (68%) of the 50 *L. monocytogenes* isolates. These results disagree with Gómez *et al.* (2014), who reported 35% of clindamycin resistance. A high resistance level to this antibiotic in *L. monocytogenes* isolates from food was reported by Chen *et al.* (2010), in 100% of the isolates evaluated.

The resistance to streptomycin, meropenem, rifampicin and trimethoprim–sulfamethoxazole was observed in five (10%) of the *L. monocytogenes* isolates. A high susceptibility (98, 100 and 100%) of *L. monocytogenes* isolates to streptomycin, rifampicin and trimethoprim–sulfamethoxazole, respectively, was stated by Chen *et al.* (2010). Susceptibility to meropenem was also observed in all *Listeria* isolates from ready-to-eat meat products and food-processing environments in another study (Gómez *et al.* 2014).

Our results confirmed the susceptibility of *L. monocytogenes* isolates to the preferred antimicrobial agents to treat cases of human listeriosis (ampicillin, penicillin G and gentamycin). The second-choice therapy is the association of trimethoprim and a sulfonamide (e.g. trimethoprim–sulfamethoxazole), especially for β-lactam allergic patients. However, it is noteworthy that five (10%) of our evaluated isolates proved resistance to this drug association.

Other antibiotics used in cases of listeriosis are vancomycin and erythromycin, to treat, respectively, bacteraemia and pregnant women diagnosed with listeriosis. Although no *L. monocytogenes* isolates from food and food industries were vancomycin resistant, three isolates (6%) were resistant to erythromycin. Similarly, Wang *et al.* (2013) found erythromycin resistance in 4·5% of *L. monocytogenes* isolates from retail raw foods.

Despite the low use of rifampicin, tetracycline, chloramphenicol and fluoroquinolones in clinical cases, these antibiotics are also prescribed in cases of human listeriosis. We found resistance to rifampicin (10%) and tetracycline (2%), as similarly reported in other studies (Conter *et al.* 2009; Allerberger and Wagner 2010).

After the first multidrug-resistant *L. monocytogenes* strain was isolated from a patient in 1988, antimicrobial resistant strains were reported elsewhere (Poyart-Salmeron *et al.* 1990; Granier *et al.* 2011; Wang *et al.* 2015). The monitoring of antimicrobial resistance of *L. monocytogenes* is necessary, mainly in food products, because an increase in antimicrobial resistance of this bacterium is a major public health concern, owing to the high lethality rates associated with listeriosis and the possibility of spread of multi-resistance to other strains.

The results of our study demonstrated a low incidence of antimicrobial resistance in *L. monocytogenes* strains isolated from food and processing environments in the South of Brazil. However, multi-resistant profiles, that are characterized, herein, as isolates with resistance to three or more classes of antimicrobials agents (EFSA/ECDC 2013), were detected in five isolates (10%) and therefore analysed in more detail (Table 3).

Table 3 Characteristics of the multi-resistant *Listeria monocytogenes* isolates

Strain	Source	Year of isolation	Serotype	Resistance Phenotype	Resistance Genotype	PFGE Pattern
13	Fresh mixed sausage	2003	1/2a	STR-ERY-CLI-RIF-MER-SUT	–	I
14	Fresh mixed sausage	2003	4b	STR-CLI-RIF-MER-SUT	–	II
16	Fresh mixed sausage	2003	1/2a	STR-ERY-CLI-RIF-MER-SUT-TET	*tetM*	III
30	Processing line of fresh mixed sausage	2003	1/2a	STR-ERY-CLI-RIF-MER-SUT	–	I
46	Chicken slaughterhouse	2006	1/2b	STR-CLI-RIF-MER-SUT	*ermB*	IV

STR, streptomycin; ERY, erythromycin; CLI, clindamycin; RIF, rifampicin; MER, meropenem; SUT, trimethoprim–sulfamethoxazole; TET, tetracycline; PFGE, pulsed field gel electrophoresis.

Genetic methods can confirm the presence of specific genes conferring antimicrobial resistance. However, the phenotypic profile of resistance of the isolates should be also taken into consideration. To this end, we evaluated 13 resistance genes (*ereB*, *ermB*, *ermC*, *tetA*, *tetB*, *tetK*, *tetL*, *tetM*, *tetO*, Tn*916-1545*, *strA* and *strB*), of which only two (*tetM* and *ermB*) were detected and further confirmed by sequencing in multi-resistant *L. monocytogenes* isolates (Table 3). The isolates carrying antimicrobial resistance genes were also resistant to these antibiotics.

Tetracycline resistance in most bacteria and antimicrobial resistance dissemination are attributed to the acquisition of new genes, often associated with mobile elements such as conjugative plasmids and/or conjugative transposons found in several bacterial genera (Roberts 2005; Chen *et al.* 2010). According to most studies, the major genotype for tetracycline resistance found in *Listeria* strains is the *tetM* gene (Bertrand *et al.* 2005; Li *et al.* 2007; Chen *et al.* 2010; Wang *et al.* 2013), a ribosomal protection gene found most frequently in the chromosome related to the presence of the conjugative transposons Tn*916-1545* (Marra *et al.* 1999).

Bertsch *et al.* (2014) evaluated tetracycline-resistant *Listeria* isolates phenotypically, all of which carried the *tetM* gene. In 73% of the isolates, the gene was located on transposon Tn*916-1545.* This transposon family has the ability of transconjugation. In our study, the Tn*916-1545* family was not detected by the PCR analysis in the *L. monocytogenes* isolates. However, we found one tetracycline-resistant *L. monocytogenes* isolate from fresh mixed sausage harbouring the *tetM* gene in chromosome and plasmid. Thus, the presence of the *tetM* gene in the plasmid may increase the ability to spread the *tetM* gene to other species via conjugation, which will be investigated in future studies.

Among the antimicrobial resistance genes responsible for erythromycin resistance (*ereB*, *ermB* and *ermC*) evaluated in this study, only one isolate from a chicken slaughterhouse carried *ermB,* and no other antimicrobial resistance genes were found. Srinivasan *et al.* (2005) evaluated several antimicrobial resistance genes and found no *ereB* and *ermB* genes in any of the *L. monocytogenes* isolates. On the other hand, Roberts *et al.* (1996) and Bertsch *et al.* (2013a) found *ermC* and *ermA* genes in *Listeria* species respectively.

Interestingly, some of the *L. monocytogenes* isolates showed phenotypic multi-resistance to antimicrobials but did not harbour the antimicrobial resistance genes evaluated in this study. This fact suggests that other mechanisms can contribute as well to antimicrobial resistance phenotypes or that other genes are involved in the acquisition of antimicrobial resistance. For instance, through genes associated with the activation of efflux pump; mutations that confers antimicrobial resistance; genes which encode for enzymes with antimicrobial activities or even unknown resistance mechanisms, considering the limited information available about antimicrobial resistance of *Listeria* strains (Bertrand *et al.* 2005; Roberts 2005; Srinivasan *et al.* 2005; Bertsch *et al.* 2014).

Pulsed field gel electrophoresis (PFGE) assay was performed to characterize the genetic profiles of the five multi-resistant *L. monocytogenes* isolates and four distinct PFGE profiles were identified (listed in Figure S1). The analysis demonstrated that two isolates analysed had an identical genetic profile (100% of similarity), while the other three isolates belonged to different profiles. Considering the isolates with the same genetic profiling, is noteworthy that isolate 13 was obtained from a fresh mixed sausage and isolate 30 from a processing plant, where the sausage was produced. These isolates were obtained from samples collected on different dates (interval of 1 month), suggesting persistence in the processing environment (Thévenot *et al.* 2006; Mendonça *et al.* 2012). Furthermore, these two isolates belong to the same serotype (1/2a) and the antimicrobial resistance profiles were identical, although no antimicrobial resistance genes were found.

In summary, this study showed that all *L. monocytogenes* isolates belong to the main serotypes involved in outbreaks and sporadic cases of listeriosis and harbour virulence markers suggesting that these isolates may pose a potential threat to public health. The isolates were highly susceptible to the most commonly used antibiotics in listeriosis treatment, although some of them were multidrug-resistant. Multi-resistant *L. monocytogenes* isolates can serve as a source of resistance genes, and considering the presence of mobile genetic elements, further studies are needed to investigate the horizontal transferability of these antimicrobial resistance genes to other commensal and pathogenic bacteria. According to PFGE data, two multi-resistant isolates seem to be a single clone isolated from food and the processing plant where they were produced. Given these points, surveillance programmes are required to monitor epidemiological information and antimicrobial resistance profiles in pathogenic *L. monocytogenes* in Brazil.

Materials and methods

Bacterial strains

A total of 50 *L. monocytogenes* strains from the culture collection of the Laboratório de Microbiologia de Alimentos (DCTA/FAEM/UFPel) of southern Rio Grande do Sul, Brazil, between 2001 and 2010 were analysed. Table S1 shows the source of isolates evaluated.

Characterization of isolates

Listeria monocytogenes isolates were subjected to serogrouping by Polymerase Chain Reaction (PCR) using a multiplex PCR method with modifications for rapid discrimination of the major serotypes in each serogroup (Doumith *et al.* 2004). This method was proved with the conventional serotyping carried out at the Laboratório de Zoonoses Bacterianas, Departamento de Bacteriologia, Instituto Oswaldo Cruz (FIOCRUZ, Rio de Janeiro, Brazil). The *inl*A, *inl*C and *inl*J genes were detected in a multiplex PCR, using the primers and cycling conditions described by Liu *et al.* (2007).

Antimicrobial susceptibility tests

The agar disc diffusion method was applied and evaluated according to the specifications of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2014a,b). A panel of 16 antimicrobial agents and concentrations was used: ampicillin (10 μg), penicillin G (10 U), cefoxitin (30 μg), streptomycin (10 μg), gentamycin (10 μg), amikacin (30 μg), vancomycin (30 μg), erythromycin (15 μg), tetracycline (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), trimethoprim-sulfamethoxazole (1·25/23·75 μg), nalidixic acid (30 μg), rifampicin (5 μg) and meropenem (10 μg). Antimicrobial multi-resistance was defined as the resistance to three or more classes of antimicrobial agents (EFSA/ECDC 2013).

Antimicrobial resistance genes profiling

Listeria monocytogenes isolates with a multi-resistance profile were investigated for the presence of several resistance genes. Genes coding resistance to macrolides (*ereB*, *ermB* and *ermC*), tetracyclines (*tetA*, *tetB*, *tetK*, *tetL*, *tetM*, *tetO* and Tn*916-1545*) and aminoglycosides (*strA* and *strB*) were investigated by PCR assays, using specific primers listed in Table S2 and following recommendations of the cited studies. Plasmid DNA was extracted using NucleoSpin© Tissue Kits (Macherey-Nagel, Düren, Germany).

DNA sequencing

Representative amplicons were purified and sequenced to validate their identity. Purified samples and primers were subjected to sequencing at the genomic facility of the Unidade de Análises Moleculares e de Proteínas (Centro de Pesquisa Experimental, HCPA, Porto Alegre, RS, Brazil), using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Macrorestriction analysis (PFGE)

The multi-resistant isolates were subjected to macrorestriction analysis according to the CDC PulseNet standardized procedure for *L. monocytogenes* (Graves and Swaminathan 2001). The isolates were digested with restriction enzymes *Apa*I and *Asc*I (Thermo Scientific, Vilnius, Lithuania). The images were analysed by the unweighted pair-group method using arithmetic averages (UPGMA) using GEL COMPAR software (Applied Maths, Sint-Martens Latem, Belgium).

Acknowledgements

The authors thank the Brazilian Council for Scientific and Technological Development (CNPq) for financial support and for the scholarship of Louise Haubert.

Conflict of Interest

No conflict of interest declared.

References

Allerberger, F. and Wagner, M. (2010) Listeriosis: a resurgent foodborne infection. *Clin Microbiol Infect* **16**, 16–23.

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

Bertsch, D., Anderegg, J., Lacroix, C., Meile, L. and Stevens, M.J.A. (2013a) pDB2011, a 7.6 kb multidrug resistance plasmid from *Listeria innocua* replicating in Gram-positive and Gram-negative hosts. *Plasmid* **70**, 284–287.

Bertsch, D., Uruty, A., Anderegg, J., Lacroix, C., Perreten, V. and Meile, L. (2013b) Tn*6198*, a novel transposon containing the trimethoprim resistance gene *dfrG* embedded into a Tn*916* element in *Listeria monocytogenes*. *J Antimicrob Chemother* **68**, 986–991.

Bertsch, D., Muelli, M., Weller, M., Uruty, A., Lacroix, C. and Meile, L. (2014) Antimicrobial susceptibility and antibiotic resistance gene transfer analysis of foodborne, clinical, and environmental *Listeria* spp. isolates including *Listeria monocytogenes*. *Microbiologyopen* **3**, 118–127.

Cartwright, E.J., Jackson, K.A., Johnson, S.D., Graves, L.M., Silk, B.J. and Mahon, B.E. (2013) Listeriosis outbreaks and associated food vehicles, United States, 1998–2008. *Emerg Infect Dis* **19**, 1–9.

Chen, B.Y., Pyla, R., Kim, T.J., Silva, J.L. and Jung, Y.S. (2010) Antibiotic resistance in *Listeria* species isolated from catfish fillets and processing environment. *Lett Appl Microbiol* **50**, 626–632.

Conter, M., Paludi, D., Zanardi, E., Ghidini, S., Vergara, A. and Ianieri, A. (2009) Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *Int J Food Microbiol* **128**, 497–500.

De las Heras, A., Cain, R.J., Bielecka, M.K. and Vázquez-Boland, J.A. (2011) Regulation of *Listeria* virulence: PrfA master and commander. *Curr Opin Microbiol* **14**, 118–127.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. and Martin, P. (2004) Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J Clin Microbiol* **42**, 3819–3822.

EFSA/ECDC European Food Safety Authority, European Centre for Disease Prevention and Control (2013) The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2011. *EFSA J*, **11**, 1–359. Available at: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documedoc/3196.pdf.

Engelbrecht, F., Chun, S.K., Ochs, C., Hess, J., Lottspeich, F., Goebel, W. and Sokolovic, Z. (1996) A new PrfA-regulated gene of *Listeria monocytogenes* encoding a small, secreted protein which belongs to the family of internalins. *Mol Microbiol* **21**, 823–837.

EUCAST European Committee on Antimicrobial Susceptibility Testing (2014a) Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0. pp. 1–80. Available at: https://asmsig.files.wordpress.com/2014/11/breakpoint_table_v_4-01-2014.pdf.

EUCAST European Committee on Antimicrobial Susceptibility Testing (2014b) EUCAST disk diffusion method for antimicrobial susceptibility. Version 4.0. pp. 1–24. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Version_4/Reading_guide_v_4.0_EUCAST_Disk_Test.pdf.

FAO/WHO Food and Agriculture Organization of the United Nations and World Health Organization (2004) Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: technical report. *Microbiol Risk Assess*, **5**, 1–34. Available at: http://apps.who.int/iris/bitstream/10665/42875/1/9241562625.pdf?ua=1.

Gómez, D., Azón, E., Marco, N., Carramiñana, J.J., Rota, C., Ariño, A. and Yangüela, J. (2014) Antimicrobial resistance of *Listeria monocytogenes* and *Listeria innocua* from meat products and meat-processing environment. *Food Microbiol* **42**, 61–65.

Granier, S.A., Moubareck, C., Colaneri, C., Lemire, A., Roussel, S., Dao, T.T., Courvalin, P. and Brisabois, A. (2011) Antimicrobial resistance of *Listeria monocytogenes* isolated from food and the environment in France over a 10-year period. *Appl Environ Microbiol* **77**, 2788–2790.

Graves, L.M. and Swaminathan, B. (2001) PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int J Food Microbiol* **65**, 55–62.

Jamali, H., Radmehr, B. and Thong, K.L. (2013) Prevalence, characterisation, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk tanks. *Food Control* **34**, 121–125.

Kathariou, S. (2002) *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *J Food Prot* **65**, 1811–1829.

Kovacevic, J., Sagert, J., Wozniak, A., Gilmour, M.W. and Allen, K.J. (2013) Antimicrobial resistance and co-selection phenomenon in *Listeria* spp. recovered from food and food production environments. *Food Microbiol* **34**, 319–327.

Li, Q., Sherwood, J.S. and Logue, C.M. (2007) Antimicrobial resistance of *Listeria* spp. recovered from processed bison. *Lett Appl Microbiol* **44**, 86–91.

Liu, D. (2006) Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol* **55**, 645–659.

Liu, D., Lawrence, M.L., Wiedmann, M., Gorski, L., Mandrell, R.E., Ainsworth, A.J. and Austin, F.W. (2006) *Listeria monocytogenes* subgroups IIIA, IIIB, and IIIC delineate genetically distinct populations with varied pathogenic potential. *J Clin Microbiol* **44**, 4229–4233.

Liu, D., Lawrence, M.L., Austin, F.W. and Ainsworth, A.J. (2007) A multiplex PCR for species-and virulence-specific determination of *Listeria monocytogenes*. *J Microbiol Methods* **71**, 133–140.

Lomonaco, S., Patti, R., Knabel, S.J. and Civera, T. (2012) Detection of virulence-associated genes and epidemic clone markers in *Listeria monocytogenes* isolates from PDO Gorgonzola cheese. *Int J Food Microbiol* **160**, 76–79.

Marra, D., Pethel, B., Churchward, G.G. and Scott, J.R. (1999) The frequency of conjugative transposition of Tn*916* is not determined by the frequency of excision. *J Bacteriol* **181**, 5414–5418.

Mendonça, K.S., Michael, G.B., Von Laer, A.E., Menezes, D.B., Cardoso, M.R.I. and Silva, W.P.S. (2012) Genetic relatedness among *Listeria monocytogenes* isolated in foods and food production chain in southern Rio Grande do Sul, Brazil. *Food Control* **28**, 171–177.

Orsi, R.H., Den Bakker, H.C. and Wiedmann, M. (2011) *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. *Int J Med Microbiol* **301**, 79–96.

Poyart, C., Trieu-Cuot, P. and Berche, P. (1996) The *inlA* gene required for cell invasion is conserved and specific to *Listeria monocytogenes*. *Microbiology* **142**, 173–180.

Poyart-Salmeron, C., Carlier, C., Trieu-Cuot, P., Courtieu, A.L. and Courvalin, P. (1990) Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. *Lancet* **335**, 1422–1426.

Roberts, M.C. (2005) Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett* **245**, 195–203.

Roberts, M.C., Facinelli, B., Giovanetti, E. and Varaldo, P.E. (1996) Transferable erythromycin resistance in *Listeria* spp. isolated from food. *Appl Environ Microbiol* **62**, 269–270.

Sabet, C., Lecuit, M., Cabanes, D., Cossart, P. and Bierne, H. (2005) LPXTG protein InlJ, a newly identified internalin involved in *Listeria monocytogenes* virulence. *Infect Immun* **73**, 6912–6922.

Srinivasan, V., Nam, H.M., Nguyen, L.T., Tamilselvam, B., Murinda, S.E. and Oliver, S.P. (2005) Prevalence of antimicrobial resistance genes in *Listeria monocytogenes* isolated from dairy farms. *Foodborne Pathog Dis* **2**, 201–211.

Swaminathan, B. and Gerner-Smidt, P. (2007) The epidemiology of human listeriosis. *Microbes Infect* **9**, 1236–1243.

Thévenot, D., Delignette-Muller, M.-L., Christieans, S., Leroy, S., Kodjo, A. and Vernozy-Rozand, C. (2006) Serological and molecular ecology of *Listeria monocytogenes* isolates collected from 13 French pork meat salting–curing plants and their products. *Int J Food Microbiol* **112**, 153–161.

Torres, K., Sierra, S., Poutou, R., Carrascal, A. and Mercado, M. (2005) Patogenesis de *Listeria monocytogenes*, microorganismo zoonotico emergente. *Revista MVZ Córdoba* **10**, 511–543.

Troxler, R., Von Graevenitz, A., Funke, G., Wiedemann, B. and Stock, I. (2000) Natural antibiotic susceptibility of *Listeria* species: *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri* and *L. welshimeri* strains. *Clin Microbiol Infect* **6**, 525–535.

Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J. *et al.* (2001) *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* **14**, 584–640.

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

Wang, G., Qian, W., Zhang, X., Wang, H., Ye, K., Bai, Y. and Zhou, G. (2015) Prevalence, genetic diversity and antimicrobial resistance of *Listeria monocytogenes* isolated from ready-to-eat meat products in Nanjing, China. *Food Control* **50**, 202–208.

Weller, D., Andrus, A., Wiedmann, M. and den Bakker, H.C. (2015) *Listeria booriae* sp. nov. and *Listeria newyorkensis* sp. nov., from food processing environments in the USA. *Int J Syst Evol Microbiol* **65**, 286–292.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Characteristics of the *Listeria monocytogenes* isolates analysed in this study.

Table S2 Oligonucleotides used in this study to evaluate antimicrobial resistance genes.

Figure S1 Dendrogram of the genetic relationships among five multi-resistant strains of *Listeria monocytogenes* analysed by pulsed field gel electrophoresis, using *Apa*I and *Asc*I endonucleases.