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Original Article

Antimicrobial Resistance Profiles of *Listeria monocytogenes* and *Listeria innocua* Isolated from Ready-to-Eat Products of Animal Origin in Spain

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Abstract

The objective of this work was to investigate the antimicrobial resistance in *Listeria* spp. isolated from food of animal origin. A total of 50 *Listeria* strains isolated from meat and dairy products, consisting of 7 *Listeria monocytogenes* and 43 *Listeria innocua* strains, were characterized for antimicrobial susceptibility against nine antimicrobials. The strains were screened by real-time PCR for the presence of antimicrobial resistance genes: *tet M*, *tet L*, *mef A*, *msr A*, *erm A*, *erm B*, *lnu A*, and *lnu B*. Multidrug resistance was identified in 27 *Listeria* strains, 4 belonging to *L. monocytogenes*. Resistance to clindamycin was the most common resistance phenotype and was identified in 45 *Listeria* strains; the mechanisms of resistance are still unknown. A medium prevalence of resistance to tetracycline (15 and 9 resistant and intermediate strains) and ciprofloxacin (13 resistant strains) was also found. *Tet M* was detected in *Listeria* strains with reduced susceptibility to tetracycline, providing evidence that both *L. innocua* and *L. monocytogenes* displayed acquired resistance. The presence of antimicrobial resistance genes in *L. innocua* and *L. monocytogenes* indicates that these genes may be transferred to commensal and pathogenic bacteria via the food chain; besides this, antibiotic resistance in *L. monocytogenes* could compromise the effective treatment of listeriosis in humans.

Keywords: antimicrobial resistance, *Listeria monocytogenes*, *Listeria innocua*, genetic determinants, ready-to-eat products

Introduction

Antimicrobial resistance is currently revealed as a real and emerging threat to public health worldwide. The development of this antimicrobial resistance in bacteria appears to be associated with the use and misuse of antimicrobials in humans and animals (Aarestrup and Wegener, 1999; WHO, 2014). The result is a continuous positive selection of resistant bacterial clones that can be pathogenic, commensal, or even environmental bacteria and can spread to people via foods (EFSA, 2013). The emergence of pathogenic bacteria that are resistant to antimicrobials is of special concern since they might compromise the effective treatment of infections in humans; the severity of these diseases can vary from mild symptoms to life-threatening conditions (WHO, 2014; EFSA, 2015). It appears that these bacteria are rapidly acquiring a wide variety of antibiotic resistance genes, many of which may come from the commensal organisms found in food or in the gastrointestinal track of animals and humans (Gómez *et al.*, 2014). The monitoring of antimicrobial resistance in zoonotic and commensal bacteria in food-producing animals and food thereof is a prerequisite for understanding the development and diffusion of resistance, providing relevant risk assessment data, and evaluating targeted interventions (EFSA, 2015).

Human listeriosis is one of the most serious foodborne diseases under European Union (EU) surveillance due to high morbidity, hospitalization, and mortality in vulnerable populations (EFSA, 2015). It is among the most important causes of death from foodborne infections in industrialized countries (EFSA, 2010). A total of 2161 confirmed human cases of listeriosis were recorded in 2014 in the EU. Almost all (98.9%) reported were hospitalized, with 210 cases being fatal. This was the highest number of deaths observed between 2009 and 2014. The notification rate in the EU was 0.52 cases per 100,000 population, which represents a 30% increase compared with 2013. Spain was one of the four countries with highest notification rates (1.15 cases per 100,000 population). The largest

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outbreak caused by *Listeria monocytogenes* in the EU occurred in 2014, in Denmark, involving 41 human cases associated with consumption of cold meat cuts. Seventeen patients (41%) died within 30 days from the sample date (EFSA, 2015).

In the United States (US), the Center for Disease Control and Prevention (CDC) estimates that ~1600 illnesses, more than 1400 related hospitalizations and 260 deaths due to listeriosis, occur annually. During 2014, a total of 9 outbreaks were reported, which involved 55 human cases, 51 hospitalizations (>90%), and 13 deaths (23.6%) as indicated on the Foodborne Outbreak Online Database (FOOD) from CDC (2016; http://wwwn.cdc.gov/foodborneoutbreaks). The deadliest foodborne disease outbreak associated with *Listeria* in the United States in nearly 90 years took place in 2011, when 147 illnesses, 33 deaths, and 1 miscarriage occurred; the outbreak was associated with consumption of cantaloupe from a single farm (http://www.cdc.gov/listeria/statistics.html). The foods most commonly involved in recent listeria outbreaks in the EU and United States were dairy products and prepared foods. Many of these outbreaks have been linked to community food service (EFSA, 2015; Lomonaco *et al.*, 2015).

At the time of writing, the genus *Listeria* consists of 17 species with validly published names, two are considered pathogenic (*L. monocytogene*s and *L. ivanovii)*. Only *L. monocytogenes,* a foodborne pathogen, represents a significant public health threat (Weller *et al.*, 2015). *Listeria* infection usually requires antimicrobial treatment to heal and the outcome depends on the early administration of antibiotics having rapid and bactericidal activity against *L. monocytogenes* (Morvan *et al.*, 2010), recommendations are penicillin G or ampicillin combined or not with aminoglycoside for the treatment of invasive infections (Charpentier and Courvalin, 1999; Davis and Jackson, 2009). In cases where reduced sensitivity or resistance to beta-lactams is encountered, a number of agents active against Gram-positive bacteria may be used (Lecuit and Leclercq, 2009; Kovacevic *et al.*, 2013). With the exception of natural resistance to older quinolones, fosfomycin, and expanded-spectrum cephalosporins, *L. monocytogenes* is widely susceptible to clinically relevant classes of antibiotics active against Gram-positive bacteria (Morvan *et al.*, 2010; Granier *et al.*, 2011; Kovacevic *et al.*, 2013). Many studies of antimicrobial resistance in *L. monocytogenes* are focused on human isolates, but there is an urgent need to broaden the approach with surveillance data from different sources, including foods.

The first multiresistant strain of *L. monocytogenes* was isolated in France in 1988 (Poyart-Salmeron *et al.*, 1990), since then, other strains of *Listeria* spp. resistant to one or several antibiotics have been described (Rota *et al.*, 1996; Srinivasan *et al.*, 2005; Conter *et al.*, 2009; Filiousis *et al.*, 2009; Osaili *et al.*, 2011; Fallah *et al.*, 2012; Wang *et al.*, 2013; Gómez *et al.*, 2014). Emergence of resistance is not only the case for *L. monocytogenes* but also for other *Listeria* species such as *Listeria innocua* that can occur in similar habitats and that may represent reservoirs of antimicrobial resistance for the pathogenic species (Bertrand *et al.*, 2005).

In 2013, based on the proposals issued by the European Food Safety Authority, the EU put forward and discussed with the member states a new legislation on the harmonized monitoring of antimicrobial resistance in *Salmonella*, *Campylobacter*, and indicator bacteria in food-producing animals and food (EFSA, 2015), but there are relatively few epidemiological studies, and thus, only limited information on antibiotic resistance prevalence and spread concerning *Listeria* spp. Considering the high mortality rate of listeriosis in vulnerable populations, it is important to ensure the effectiveness of antimicrobials and monitor the emergence of antimicrobial-resistant *Listeria* strains (Gómez *et al.*, 2014).

Therefore, the objective of the present study was to evaluate the resistance phenotype and genotype in *L. monocytogenes* and *L. innocua* isolated from food of animal origin in Spain.

Materials and Methods

Strain collection

A total of 50 strains of *Listeria* spp. collected between 1994 and 2012 from ready-to-eat products of animal origin in Spain (meat and dairy products), including 7 *L. monocytogenes* and 43 *L. innocua*, were evaluated for antimicrobial susceptibility. Twenty-five *Listeria* strains were collected between 1994 and 2011 as previously described (Rota *et al.*, 1997) and the other 25 strains in 2012 were isolated according to the ISO-11290-1 (1996/Amd 1; 2004a) and ISO-11290-2 (1998/Amd 1; 2004b) standards as described in detail elsewhere (Gómez *et al.*, 2012). They were kept in cryovials (Vibakstore, Nirco S.L., Barcelona, Spain) and stored at −80°C.

The PCR assay was evaluated by using 5 different clinical strains selected to represent a variety of tetracycline-, clindamycin-, and/or erythromycin-resistant controls carrying the following genes: *tet M*, *tet L*, *mef A*, *msr A*, *erm A*, *erm B*, *lnu A*, and *lnu B*.

Antibiotic susceptibility testing

The 50 *Listeria* spp. strains were analyzed to determinate the susceptibility to a panel of 9 antibiotics currently used in human and veterinary therapy (ampicillin, ciprofloxacin, clindamycin, erythromycin, gentamicin, penicillin, rifampicin, tetracycline, and vancomycin). The 25 strains collected between 1994 and 2011 were analyzed by the microdilution method using breakpoints recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2001) for *Staphylococcus*, except for ampicillin and penicillin G where specific *Listeria* breakpoints are defined. The quality control strain included was *Enterococcus faecalis* ATCC 29212.

The other 25 *Listeria* spp. strains isolated in 2012 were analyzed by the disc diffusion method and the diameters of growth inhibition zones were measured and interpreted according to the breakpoints recommended by the Antibiogram Committee of the French Society for Microbiology (CA-SFM, 2010) for the various types of antibiotics. The following quality control strains were included with each batch: *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212 recommended by EUCAST (2010).

In both cases, the strains were classified as sensitive, intermediate (reduced susceptibility), or resistant.

Bacterial DNA isolation

DNA was isolated from bacterial cells grown overnight in brain heart infusion (Oxoid, Hampshire, United Kingdom) at 37°C. Total Genomic DNA and Plasmid DNA were extracted using PrepMan® Ultra Sample Preparation Reagent and

Plasmid DNA MiniPrepKit according to the recommendations of the manufacturer (Applied Biosystems and Norgen Biotek Corp., respectively). DNA concentration and purity were determined by measuring the absorbance at 260 and 280 nm in a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific).

Detection of antibiotic resistance genes by real-time PCR

The resistance genes for tetracycline (*tet M*, *tet L*), clindamycin (*lnu A*, *lnu B*), erythromycin (*mef A*, *msr A*), and macrolides–lincosamides–streptogramin B complex called MLSB (*erm A*, *erm B*) were tested by real-time PCR in all *Listeria* spp. strains that showed resistance or reduced susceptibility to these antibiotics. Primers used in this study are listed in Table 1.

Amplification of target genes for all bacteria was performed using a DNA thermal cycler (Mini Opticon™ Real-Time PCR Detection System; Bio-Rad). The master mix (25 μL total volume) consisted of 12.5 μL of FastStart SYBR Green Master (Roche Applied Science), 0.5 μL of each primer, 9 μL of sterile water, and 2.5 μL of template DNA. For every PCR, positive controls, negative extraction, and no DNA template controls included in each run to ensure that the PCR reagents were working properly. Thermal cycling conditions were adopted as follows: preincubation at 94°C for 5 min, 35 PCR cycles at 94°C 1 min, primer annealing at optimum temperature for 1 min (Table 1), and DNA extension at 72°C for 2 min.

Melting curve analysis allowed confirmation of amplicons, using the Tm value of the positive controls as reference (Table 1).

Validation of amplicons by agarose gel electrophoresis and sequence analysis

Validity of amplicons from the different bacterial strains was determined by agarose gel electrophoresis and sequence analysis. PCR products were purified using Dye Cleanup purification kit (IBIAN) and sequenced in the Biomedicine Institute of Valencia (IBV-CSIC). Nucleotide sequences of the PCR products were aligned and compared to GenBank sequences using BLAST of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Results

Antimicrobial susceptibility testing

The antibiotic resistance profiles of the 9 antibiotics tested in the 50 *Listeria* spp. strains are presented in Table 2.

Resistance or intermediate susceptibility to eight of nine antimicrobial agents tested in this study was observed. Resistance to clindamycin was the most common phenotype and was identified in 90% of *Listeria* spp. strains ($n = 45$, seven of them were *L. monocytogenes*). It is worth noting the prominent appearance of drug resistance to tetracycline, which reached prevalence levels from 30% ($n = 15$, one of them *L. monocytogenes*) to also 18% intermediate resistance ($n = 9$, two *L. monocytogenes*).

Other than the two antibiotics listed, resistance to ciprofloxacin ($n = 13$, three of them *L. monocytogenes*), ampicillin ($n = 8$, one *L. monocytogenes*), penicillin ($n = 5$, one *L. monocytogenes*), and gentamycin ($n = 1$) was observed at

TABLE 1. PRIMERS USED, OPTIMIZED CONDITIONS, AND MELTING TEMPERATURE OF REAL-TIME PCR FOR THE DETECTION OF GENES ENCODING RESISTANCE TO DIFFERENT ANTIMICROBIALS IN *LISTERIA* SPP. ISOLATED FROM FOOD

Gene	*Primer name*	*SEQUENCE 5′–3′*	*Amplicon size (pb)*	*T° annealing (°C)/ concentration (μM)*	*Melting temperature (°C)*	*Reference*
tet M (Ribosome protective protein)	Tet M (fw)	GTGGACAAAGGTACAACGAG	405	58/0.4	75	Morvan *et al.* (2010)
	Tet M (rv)	CGGTAAAGTTCGTCACACAC				
tet L (Tetracycline efflux pump)	Tet L (fw)	CCACCTGCGAGTACAAACTGG	739	61/0.4	78	Morvan *et al.* (2010)
	Tet L (rv)	TCGGCAGTACTTAGCTGGTGA				
mef A (Macrolide efflux)	Mef A (fw)	AGTATCATTAATCACTAGTGC	345	58/0.4	77.5–78	Morvan *et al.* (2010)
	Mef A (rv)	TTCTTCTGGTACTAAAAGTGG				
msr A (Macrolide streptogramin resistance)	Msr A (fw)	GCAAATGGTGTAGGTAAGACAACT	401	54/0.4	74.5	Morvan *et al.* (2010)
	Msr A (rv)	ATCATGTGATGTAAACAAAAT				
erm A (Erythromycin ribosome methylase)	Erm A (fw)	CTTCGATAGTTTATTAATATTAGT	645	51/1	75	Morvan *et al.* (2010)
	Erm A (rv)	TCTAAAAAGCATGTAAAAGAA				
erm B (Erythromycin ribosome methylase)	Erm B (fw)	GAAAAGGTACTCAACCAAATA	636	54/0.4	78	Morvan *et al.* (2010)
	Erm B (rv)	AGTAACGGTACTTAAATTGTTTAC				
lnu A (Lincomycin nucleotidyltransferase)	Lnu A (fw)	GGTGGCTGGGGGGTAGATGTATTAACTGG	323	59/0.4	74	Lina *et al.* (1999)
	Lnu A (rv)	GCTTCTTTTGAAATACATGGTATTTTTCGATC				
lnu B (Lincomycin nucleotidyltransferase)	Lnu B (fw)	CCTACCTATTGTTTGTGGAA	405	54/1	74–74.5	Bozdogan *et al.* (1999)
	Lnu B (rv)	ATAACGTTACTCTCCTATTC				

Table 2. Antimicrobial Susceptibility of *Listeria* spp. from Food to Nine Antimicrobials

	1994–2011 (MIC)			*2012 (disc diffusion)*		
Antibiotics	*S,* n *(%)*	*I,* n *(%)*	*R,* n *(%)*	*S,* n *(%)*	*I,* n *(%)*	*R,* n *(%)*
Ampicillin	17 (68)	—	8 (32)	25 (100)	—	—
Ciprofloxacin	12 (48)	—	13 (52)	25 (100)	—	—
Clindamycin	—	—	25 (100)	5 (20)	—	20 (80)
Erythromycin	12 (48)	13 (52)	—	25 (100)	—	—
Gentamycin	24 (96)	—	1 (4)	25 (100)	—	—
Penicillin	20 (80)	—	5 (20)	19 (76)	6 (12)	—
Rifampicin	25 (100)	—	—	25 (100)	—	—
Tetracycline	13 (52)	9 (36)	3 (12)	13 (52)	—	12 (48)
Vancomycin	24 (96)	1 (4)	—	25 (100)	—	—

I, intermediate susceptibility; R, resistant; S, sensitive.

considerably lower levels. Thirteen strains (four *L. monocytogenes*) showed intermediate susceptibility to erythromycin, six (one *L. monocytogenes*) to penicillin, and one to vancomycin (*L. monocytogenes*). With our approach, all strains were susceptible only to rifampicin.

Regarding multiresistance, 27 strains (54%) were resistant to more than one antibiotic. Eighteen strains (36%) of *Listeria* spp. (two of them were *L. monocytogenes*) showed resistance to two antibiotics, five strains (10%) (two *L. monocytogenes*) revealed resistance to three antibiotics, three strains (6%) to four antibiotics, and one strain (2%) proved resistant to five antibiotics. Only five strains (10%) were highly sensitive to the nine antibiotics tested (Table 3).

Presence of antimicrobial genes in Listeria *spp.*

Forty-five *Listeria* spp. evaluated in the present study were resistant to clindamycin and none of these strains contained antimicrobial resistance genes responsible for clindamycin resistance such *lnu A* and *lnu B.*

Other antimicrobial resistance genes (*mef A*, *msr A*, *erm A*, and *erm B)* were not found in any of the *Listeria* spp. strains that showed intermediate resistance to erythromycin.

Whereas all 15 strains resistant to tetracycline were encoded by *tet M* gene, one of them was *L. monocytogenes*. It should be pointed out that 7 strains, which showed intermediate susceptibility, contained also this gene (2 of them *L. monocytogenes*). In both cases, *Tet M* was detected, while *tet L* was not found in any *Listeria* spp. strains studied.

The PCR amplicons of *tet M* assays were sequenced, and sequences were blasted in NCBI database and confirmed. DNA sequence analysis of the *Listeria* spp. *tet M* gene showed 99% identity to the published GenBank sequences.

Discussion

The prevalence of antimicrobial resistance in *Listeria* spp. appears to vary considerably. Since the first report of antibiotic-resistant strains of *Listeria* spp. and *L. monocytogenes* (Poyart-Salmeron *et al.*, 1990), there has been a continuing

Table 3. Resistance Phenotypes in *Listeria* spp. Strains Studied

No. of antimicrobial resistance	*No. of antimicrobial intermediate*	*Resistant phenotype*	*No. of isolates*
0	0	—	5
1	0	CLI	13 (1 *Listeria monocytogenes*)
1	1	CLI + PEN	2
1	2	CLI + ERI + TET	2 (1 *L. monocytogenes*)
1	3	CLI + ERI + TET + VAN	1 (*L. monocytogenes)*
2	0	CLI + CIP	3 (1 *L. monocytogenes*)
2	0	CLI + PEN	1
2	0	CLI + TET	8
2	1	CLI + CIP + ERI	1 (*L. monocytogenes*)
2	2	CLI + CIP + ERI + TET	1
2	1	CLI + TET + PEN	4
3	0	CLI + CIP + PEN	1
3	0	CLI + PEN + TET	3 *(1 L. monocytogenes)*
3	1	CLI + AMP + CIP + ERI	1 (*L. monocytogenes*)
4	1	CLI + AMP + CIP + TET + ERI	2
4	2	CLI + AMP + CIP + PEN + ERI + TET	1
5	1	CLI + AMP + CIP + PEN + GEN + ERI	1

Underlined names represent intermediate susceptibility.

AMP, ampicillin; CIP, ciprofloxacin; CLI, clindamycin; ERI, erythromycin; GEN, gentamicin; PEN, penicillin; TET, tetracycline; VAN, vancomycin.

pattern of the emergence of resistant strains of *Listeria* spp. isolated from different sources (Walsh *et al.*, 2001; Conter *et al.*, 2009; Morvan *et al.*, 2010; Alonso-Hernando *et al.*, 2012). This wide variation of antimicrobial resistance patterns that has been found in the last 25 years may be due to the exposure to different antimicrobials in different geographical regions during different time periods (Srinivasan *et al.*, 2005; Klibi *et al.*, 2013; Moreno *et al.*, 2014).

The results of this study suggest the presence of a high level of resistance to clindamycin and to tetracycline. Both antimicrobial agents are classified as highly important for human medicine (WHO, 2011) and critically important for veterinary medicine in the case of tetracycline (OIE, 2015).

There are few studies demonstrating high levels of resistance to clindamycin especially in isolates from food. This antibiotic is widely used in hospitals and veterinary medicine to treat Gram-positive bacterial infections (Lozano *et al.*, 2011; Moreno *et al.*, 2014). The mechanism of activity is similar to that of erythromycin, owing to the fact that they share their binding site. Cross-resistance of these compounds has been observed previously in other Gram-positive bacteria (Weisblum, 1995; Levin *et al.*, 2005; Lozano *et al.*, 2012; Wendlandt *et al.*, 2013).

However, the present study reports only clindamycin resistance, finding negative results for erythromycin and clindamycin inducible resistance. Only 12 strains showed intermediate resistance to erythromycin but none of them carried *erm* genes, which confer resistance to macrolides, lincosamides, and streptogramins (complex MLSB). MLSB resistance has already been described in *Listeria* spp. isolated from food (Poyart-Salmeron *et al.*, 1990; Roberts *et al.*, 1996; Morvan *et al.*, 2010; Granier *et al.*, 2011). Macrolide resistance genes *mef A* and *msr A* were not found in these strains either, reporting that erythromycin intermediate phenotype is not related with efflux mechanism codified by these genes. In addition, another study has looked for *mef A* and *msr A* genes in *Listeria* spp. but these determinants were not detected (Granier *et al.*, 2011).

The clindamycin resistance that was reported in this study seems to be related with enzyme inactivation because cross-resistance was not observed with erythromycin. Recently, the detection of the unusual resistance, phenotype lincosamide resistance/macrolide susceptibility (L^R/M^S), has increased among staphylococcal strains of animal origin and seems to be related with *lnu A* and *lnu B* genes (Lozano *et al.*, 2012; Arana *et al.*, 2014). These genes are usually carried on small plasmids whose transference among bacterial genera of animal and human origin has been corroborated (Lozano *et al.*, 2012).

Conjugation experiments by Bertsch *et al.* (2013) demonstrated that isolates of *Listeria fleischmannii* harbored a transferable resistance to clindamycin, indicating a clindamycin resistance mechanism unknown in Gram-positive bacteria. In this study, *lnu A* and *lnu B* were not detected, and there is no information about lincosamide resistance genes in *Listeria* spp. strains.

Tetracycline was the next most frequent resistance trait in *Listeria* spp., among 50 strains, 15 were resistance and 9 intermediate. Similar results were also described by other researchers (Srinivasan *et al.*, 2005; Li *et al.*, 2007; Fallah *et al.*, 2012). Tetracycline resistance is the most frequently reported resistance phenotype in *Listeria* species from various origins (Poyart-Salmeron *et al.*, 1990; Charpentier and Courvalin, 1999); it could result from an extensive use of tetracycline, particularly in feed additives and in veterinary therapy (Gómez *et al.*, 2014). Resistance to tetracycline in many commensal and pathogenic bacteria is due to the acquisition of *tet* genes via self-transferable plasmids or conjugative transposons (Facinelli *et al.*, 1993; Charpentier *et al.*, 1995). In the present study, 100% of tetracycline-resistant strains harbored the *tet M* gene, and seven intermediate strains also showed this gene. The *tet L* gene was not found in any *Listeria* spp. isolated, suggesting that tetracycline resistance is produced by ribosome protection due to *tet M* gene. It is known that once a resistance determinant is disseminated, it is unlikely that another conferring a very similar phenotype will be acquired (Baquero *et al.*, 2009).

The *tet M* gene is highly prevalent in *Listeria* spp. strains resistant to tetracycline (Li *et al.*, 2007; Allen *et al.*, 2014) and is often associated with conjugative elements of the Tn916–Tn1545 family (Poyart-Salmeron *et al.*, 1989; Bertrand *et al.*, 2005). Other researches have suggested that the tetracycline resistance determinants (*tet M* and *tet L)* are common in *Enterococcus-Streptococcus* (Speer *et al.*, 1992; Charpentier *et al.*, 1995), these observations indicate that two types of movable genetic elements, conjugative plasmids and transposons originating from *Enterococcus-Streptococcus,* are responsible for the emergence of resistance to tetracycline in *Listeria* spp. (Poyart-Salmeron *et al.*, 1992).

The fact that the bacteria can develop resistance mechanisms or acquired resistance by transmission of genetic material from other bacterial species has led to an increase of antimicrobial resistance that threatens the treatment of infectious diseases.

It is therefore necessary to implement more comprehensive and continuous monitoring of the course and nature of the acquisition and dissemination of antibiotic resistance in *L. monocytogenes* and other members of the genus.

Conclusion

The inappropriate use of antibiotics in human medicine, veterinary medicine, and elsewhere has created selective pressure that favors the emergence of antibiotic-resistant bacteria with a direct impact on food safety.

Nonpathogenic microorganisms present in food may serve as reservoirs of potential antimicrobial resistance genes. These genes could be transferred to other bacteria, the foodborne pathogens being especially important. Of singular interest are those antibiotic-resistant bacteria that can survive and grow at refrigeration temperature such as *Listeria* spp. that can be present during food processing.

These results are consistent with the reports of emergence of resistance in *L. monocytogenes* and *L. innocua* and emphasize the need for further studies of the genotypic characterization of antibiotic resistance in *Listeria* spp. isolated from food. The fact is that *L. innocua* can be found in similar habitats and that *L. monocytogenes* could be a risk factor for the spread of antimicrobial resistance to the pathogenic species on the food chain and especially in ready-to-eat products. Due to the high morbidity, hospitalization, and mortality in vulnerable populations, *Listeria* infection usually requires antibiotic treatment. Consequently, it is necessary to control the prevalence and distribution of resistance in *Listeria* spp. to determine their potential as reservoirs of

transferable resistance genes via the food chain. The purpose is to establish measures to prevent the appearance and proliferation of new resistances that could compromise the effective treatment of listeriosis in humans.

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Disclosure Statement

No competing financial interests exist.

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