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Antimicrobial resistance of *Listeria monocytogenes* and *Listeria innocua* from meat products and meat-processing environment

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ABSTRACT

A total of 336 *Listeria* isolates from ready-to-eat (RTE) meat products and meat-processing environments, consisting of 206 *Listeria monocytogenes*, and 130 *Listeria innocua* isolates, were characterized by disc diffusion assay and minimum inhibitory concentration (MIC) values for antimicrobial susceptibility against twenty antimicrobials. Resistance to one or two antimicrobials was observed in 71 *L. monocytogenes* isolates (34.5%), and 56 *L. innocua* isolates (43.1%). Multidrug resistance was identified in 24 *Listeria* isolates, 18 belonging to *L. innocua* (13.9%) and 6 to *L. monocytogenes* (2.9%). Oxacillin resistance was the most common resistance phenotype and was identified in 100% *Listeria* isolates. A medium prevalence of resistance to clindamycin (39.3% isolates) and low incidence of resistance to tetracycline (3.9% isolates) were also detected. *Listeria* isolates from RTE meat products displayed higher overall antimicrobial resistance (31.3%) than those from the environment (13.4%). All the strains assayed were sensitive to the preferred antibiotics used to treat listeriosis. Results showed that although antimicrobial resistance in *L. monocytogenes* still occurs at a low prevalence, *L. innocua* can form a reservoir of resistance genes which may transfer between bacterial species, including transference to organisms capable of causing disease in humans.

1. Introduction

In the 1980s, *Listeria monocytogenes* infection (listeriosis) was recognized as a foodborne illness which can lead to invasive disease during vulnerable stages of life. Older adults, pregnant women and persons with immunocompromising conditions are at higher risk for *Listeria* bacteremia and meningitis, which can be fatal (Rocourt and Buchrieser, 2007; Drevets and Bronze, 2008). Outbreaks and sporadic cases of listeriosis have been associated with contamination of various food items including milk, soft cheese, meat and meat products, vegetables, seafood products, and ready-to-eat foods (Codex Alimentarius, 2007).

The average annual incidence of foodborne infections caused by *L. monocytogenes* is low in comparison with other pathogens such as *Salmonella*, *Campylobacter* and pathogenic *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC). However, *L. monocytogenes* is considered as an important foodborne pathogen due to its high mortality, with a case-fatality rate of up to 30% (Lecuit and Leclercq, 2012). During the year 2010, the number of human listeriosis cases reported in Europe was 1,601, 3.2% fewer than in 2009. The most widely affected population group was people over the age of 65 years (60.2% of cases) and the mortality rate was 17.0% (EFSA, 2012).

L. monocytogenes, a bacterium which is ubiquitous in the environment, is naturally susceptible to a range of antibiotics that act on Gram-positive bacteria (Charpentier and Courvalin, 1999). The *Comité de l'Antibiogramme de la Société Française de Microbiologie* (CA-SFM) (CA-SFM, 2010) and the National Reference Center for *Listeria* (NRCL) (Lecuit and Leclercq, 2012) indicated that human strains of *L. monocytogenes* are sensitive to a wide range of antibiotics that include penicillin, ampicillin, amoxicillin, gentamicin, erythromycin, tetracycline, rifampicin, co-trimoxazole, vancomycin and imipenem. However, most strains of *L. monocytogenes* show natural resistance to current fluoroquinolones and cephalosporins, especially third and fourth generation, such as cefotaxime and cefepime, and also to fosfomycin, oxacillin and licosamides. Clinicians typically use aminopenicillins (e.g., ampicillin or amoxicillin) in combination with an aminoglycoside, such as gentamicin, for the treatment of invasive infections. In cases where reduced sensitivity or resistance to beta-lactams is encountered, a number of agents active against Gram-positive bacteria may be used, though

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cotrimoxazole is generally regarded as the second-choice therapeutic option (Lecuit and Leclercq, 2009).

On the other hand, the application of antimicrobial agents is now seriously jeopardized by the emergence and spread of microbes that are resistant to affordable and effective first choice antibiotics. Increasing global trade and travel favor the spread of antimicrobial resistance between countries and continents. Therefore, antimicrobial resistance is a global public health concern (Doyle et al., 2013). One action to address threats posed by antimicrobial resistance includes the monitoring programs for antimicrobial-resistant microbes that integrate clinical settings and along the food chain. This is useful to identify trends in development and persistence of antimicrobial resistance among select foodborne pathogens. Studies performed by the NRCL with human strains and, to a lesser extent, with foodstuff strains do not reveal an increase in resistance to antibiotics by the circulating strains of *L. monocytogenes* (Morvan et al., 2010; Granier et al., 2011).

However, since the isolation of the first multi-resistant strain of *L. monocytogenes* in 1988, interspecies variation in antimicrobial susceptibilities has been reported in *Listeria* species (Charpentier et al., 1995; Margolles et al., 2001). The pattern of susceptibility between *L. monocytogenes* and *Listeria innocua* is important owing to the fact that both species are very often found in the same food or processing environment (Gómez et al., 2012). Balsalobre and Hernández-Godoy (2004) have suggested that species of *Listeria*, such as *L. innocua*, and other Gram-positive bacteria which are very often present in meat and meat products, show frequent resistance to antibiotics, with the possibility of a transfer of genetic information from one species to another through various mechanisms. This transfer has been demonstrated *in vitro* for streptomycin, erythromycin and chloramphenicol. Additionally, considering the high mortality rate of listeriosis, it is important to ensure the effectiveness of antimicrobials for listeriosis and monitor the emergence of antimicrobial-resistant *Listeria* strains.

The purpose of this study was to determine the resistance pattern of *Listeria* strains isolated from RTE meat products and food-contact surfaces in meat industries to various antibiotics that are widely used in human and veterinary medicine.

2. Material and methods

2.1. Bacterial isolates

A total of 336 *Listeria* isolates from ready-to-eat (RTE) meat products and food-processing environments, including 206 *L. monocytogenes*, and 130 *L. innocua* isolates, were characterized by antimicrobial susceptibility tests. The strains were isolated between May 2009 and April 2012 according to the ISO 11290-1 (ISO 11290-1: 1996/Amd 1, 2004a) and ISO-11290-2 (ISO 11290-2: 1998/Amd 1, 2004b) standards as described in detail elsewhere (Gómez et al., 2012). They were pre-identified (β-hemolysis and fermentation of rhamnose, xylose and mannitol) and finally identified with the gallery API-*Listeria*.

Table 1 shows the origin of the *Listeria* isolates used in this study. Of the 336 strains investigated, 103 (72 *L. monocytogenes* and 31 *L. innocua*) were isolated from environmental samples taken from 32 Spanish meat industries located across NE and SW Spain. The strains were isolated from food-contact surfaces made of stainless steel, high molecular weight polyethylene (HMWP) and polyvinyl chloride (PVC) conveyor belts. Another set of 147 strains (85 *L. monocytogenes* and 62 *L. innocua*) were isolated from the RTE meat products produced within the sampled industries. The remaining 86 strains of *Listeria* (49 *L. monocytogenes* and 37 *L. innocua*) were isolated from commercially available RTE meat products purchased in butcher's shops, supermarkets and hypermarkets in NE Spain (Table 1). The *Listeria* strains were kept in cryovials (Vibakstore, Nirco S.L., Barcelona, Spain) and stored at −80 °C.

Table 1
Strains of *Listeria* spp. used in the drug resistance study.

Source	Origin	Sample type	*L. monocytogenes*	*L. innocua*
Food industry	Surface	Stainless steel	53	29
		HMWP[a]	9	1
		Conveyor belt	10	1
		Subtotal	71	31
	RTE meat product	Cooked	21	12
		Raw-cured	53	42
		Dry-cured and ripened	9	8
		Marinated	2	0
		Subtotal	85	62
Market samples	RTE meat product	Cooked	35	25
		Raw-cured	10	10
		Dry-cured and ripened	4	2
		Subtotal	49	37
Total			206	130

[a] High molecular weight polyethylene.

2.2. Antimicrobial susceptibility testing: disk diffusion method and MIC values

Antimicrobial susceptibility testing of *Listeria* was performed by the disc diffusion method as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2012). Mueller-Hinton agar plates were used (Oxoid, Hampshire, UK) with incubation at 35 °C for 24 h. The inoculum was standardized by spectrophotometry (Spectronic 20, Bausch&Lomb, Rochester, N.Y., USA) inoculating the plates with a concentration of approximately 10^8 cfu/ml using a cotton swab.

The following 20 antimicrobials (and disk load), including those used to treat human listeriosis, were tested: amoxicillin-clavulanic acid (30 μg; AMC), ampicillin (10 μg; AMP), ciprofloxacin (5 μg; CIP), clindamycin (2 μg; DA), clarithromycin (15 μg; CLR), chloramphenicol (30 μg; C), gentamicin (10 μg; CN), imipenem (10 μg; IPM), levofloxacin (5 μg; LEV), linezolid (30 μg; LZD), meropenem (10 μg; MEM), moxifloxacin (5 μg; MXF), oxacillin (1 μg; OX), penicillin G (10 μg; P), rifampicin (30 μg; RD), trimethoprim–sulfamethoxazole [1.5 μg-23.5 μg; SXT], teicoplanin (30 μg; TEC), tetracycline (30 μg; TE), tigecycline (15 μg; TGC) and vancomycin (30 μg; VA). Additionally, in the case of *L. innocua*, resistance to five more antibiotics was assayed: erythromycin (15 μg; E), minocycline (30 μg; MH), streptomycin (10 μg; S), sulfamethoxazole (25 μg; RL) and trimethoprim (5 μg; W). 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.

The diameters of growth inhibition zones were measured and interpreted according to the breakpoints recommended by the EUCAST (2013) for the various types of antibiotics, and the strains were classified as sensitive, intermediate (reduced susceptibility) or resistant.

There were 62 *L. monocytogenes* and 85 *L. innocua* isolates for which the interpretation of the disc diffusion method was unclear for six antibiotics. These strains were confirmed by determining the minimum inhibitory concentrations (MIC) by graded-concentration antibiotic strips (M.I.C.E. strips; Oxoid). Simply, 0.5 McFarland inoculum of the *Listeria* isolates was swab-spread over Mueller–Hinton agar plates and, then, M.I.C.E. strips were aseptically placed on the dried surface within 15 min. Plates were incubated at 35 °C