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Antimicrobial Resistance of *Listeria monocytogenes* Isolates from Food and the Environment in France over a 10-Year Period[▽][†]

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In order to assess antimicrobial resistance in *Listeria monocytogenes*, 202 food and environmental isolates from 1996 to 2006 were tested. Only four strains displayed acquired resistance. Resistance to erythromycin, tetracycline-minocycline, and trimethoprim was evidenced, and the genes *erm*(B), *tet*(M), and *dfrD*, already found in *L. monocytogenes*, were detected.

Since the early 1980s, listeriosis has been considered a public health concern. In France, listeriosis cases must be reported and isolates are regularly molecularly subtyped for surveillance purposes, but antimicrobial resistance data are scarce. In 2008, 0.3 cases per 100,000 inhabitants were reported in the European Union. Listeriosis is among the most important causes of death from food-borne infections in industrialized countries (5). *Listeria* infection usually requires antimicrobial treatment to heal. Recommendations are penicillin G or ampicillin combined (or not) with an aminoglycoside (4).

Little is known about *Listeria monocytogenes* antimicrobial resistance, especially for non-human-origin isolates. Vela et al. (15) reported resistance to tetracycline at a low frequency in a sheep and feed study. Out of a collection of 210 isolates from various food items, Filiousis et al. (6) detected a unique isolate from beef meat resistant to tetracycline and carrying the *tet*(*M*) gene. Finally, Roberts et al. (13) collected, also from food samples, a unique isolate resistant to erythromycin and carrying an *erm*(C) gene. Such a small number of publications on this subject leads us to wonder if acquisition of resistance is a rare event in non-human-origin *L. monocytogenes* or if it is only poorly studied.

The aim of this study was to determine the resistance phenotype and genotype evolution over a 10-year period of *L. monocytogenes* isolated from food and the environment in France.

Strain collection. Two hundred two strains were collected between 1996 and 2006. They were selected on the basis of a unique pulsed-field gel electrophoresis (PFGE) profile after digestion by ApaI and AscI (14), performed as recommended by PulseNet Europe (10). Serotypes were 1/2a (55%), 4b (18%), 1/2b (13%), 1/2c (6%), 3a (2%), 3b (1%), 1/2 H=0 (0.5%), 3c (0.5%), 4a (0.5%), rough (0.5%), and not determined (1%). Half of the strains were isolated from food samples and a quarter from food processing plants; the balance was equally divided between natural-ecosystem and breeding-plant environmental samples. Major food channels were represented: dairy, 40%; sea products, 15%; pig, 10%; poultry, 5%; and cattle, 5%. Collection details are presented in Table S1 in the supplemental material.

Antibiotic susceptibility testing. Facing the lack of information about the appropriate antimicrobials to be tested and their expected range of concentrations, a generic Gram-positive commercial panel was used for MIC determination by the microdilution method. Ready-to-use Sensititre microplates (GPN2F) were inoculated with 100 μl Mueller-Hinton broth with lysed horse blood (Trek Diagnostic Systems, West Sussex, United Kingdom). The following antibiotics were tested: penicillin G, ampicillin, cefazoline, ceftriaxone, gentamicin, erythromycin, clarithromycin, clindamycin, linezolid, quinupristin-dalfopristin, levofloxacin, gatifloxacin, rifampin, co-trimoxazole, tetracycline, and vancomycin. Reading was performed using the Vizion system (Trek Diagnostic Systems).

Very limited guidelines for interpreting the MIC results for *L. monocytogenes* are available; the CLSI provides criteria only for penicillin G and co-trimoxazole (3). However, the mono-

TABLE 1. MIC ranges of antibiotics against susceptible *L. monocytogenes*

Antimicrobial agent	MIC range (mg/liter)
Ampicillin	<0.06–1
Penicillin G	0.125–1
Cefazoline	<1–8
Ceftriaxone	<4–32
Gentamicin	<1–2
Erythromycin	<0.125–0.25
Clarithromycin	<0.5–1
Clindamycin	<0.25–2
Linezolid	1–4
Quinupristin-dalfopristin	0.25–2
Levofloxacin	0.5–2
Gatifloxacin	<0.5–4
Rifampin	<0.25–0.5
Co-trimoxazole	<0.25
Tetracycline	<1–2
Vancomycin	<0.5–2

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TABLE 2. Acquired resistance among 202 isolates of French non-human-origin *L. monocytogenes*

Strain	Serotype	Origin	MIC (mg/liter) of:		
			Tetracycline	Co-trimoxazole	Erythromycin
LSEA 01-08	4b	Activated sludge			>4
LSEA 02-09	4b	Sludge			>4
03EB250LM	1/2a	Pork cheek	>8		
04CEB563LM	1/2a	Surface (beef processing plant)	>8	>2	

modal distribution of the MICs allowed delineation of the wild-type populations. MIC ranges are presented in Table 1. Most *L. monocytogenes* strains isolated from food and the environment during the period from 1996 to 2006 were fully susceptible to the penicillins, gentamicin, linezolid, rifampin, and vancomycin and were naturally resistant to cephalosporins.

As shown in Table 2, 4 strains (2% of the 202 isolates tested) were resistant: 2 to erythromycin, 1 to tetracycline-minocycline, and 1 to trimethoprim and tetracycline-minocycline. Those resistance phenotypes were confirmed by Etest (AB Biodisk, Solna, Sweden).

Acquired resistance to quinolones and chloramphenicol, reported previously (1), was not detected.

Molecular characterization of acquired resistance. The 4 resistant strains were screened by PCR for the resistance genes *erm*(A), *erm*(B), *erm*(C), *erm*(TR) [a subset of the *erm*(A) class], *mef*(A), and *msr*(A) for macrolide-lincosamide-streptogramin B (MLS_B), *dfrD* for trimethoprim, *tet*(M), *tet*(S), *tet*(K), and *tet*(L) for tetracyclines, and the *int*-Tn gene, encoding the integrase of the Tn*916*-Tn*1545* family of transposons, as previously described (11).

In *Listeria* spp., two genes for resistance to MLS_B, *erm*(B) and *erm*(C), have been reported (1). These *erm* (*e*rythromycin *r*ibosome *m*ethylase) genes encode methyltransferases that modify 23S rRNA. In Gram-positive bacteria, resistance to 14- and 15-membered-ring macrolides can also be mediated by drug efflux pumps belonging to the ATP-binding cassette transporter family [*msr*(A) gene] or to the major facilitator superfamily [*mef*(A) gene] (9). In this study, both erythromycin-resistant strains, LSEA 02-09 and LSEA 01-08, carried the *erm*(B) gene. The *erm*(A), *erm*(C), *erm*(TR), *mef*(A) and *msr*(A) determinants were not detected.

Dihydrofolate reductase (DHFR) is a key enzyme in the tetrahydrofolic pathway, in which it catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate (7). The most common mechanism of resistance to trimethoprim is plasmid-mediated production of an additional trimethoprim-resistant DHFR. The first strain of *L. monocytogenes* resistant to high levels of trimethoprim was isolated in 1995 from the environment in France and harbored the *dfrD* gene (2). Trimethoprim resistance in *L. monocytogenes* 04CEB563LM was of a high level (MIC = 1,024 mg/ml) and was due to *dfrD* acquisition.

Two known mechanisms of resistance to tetracyclines have been found in *Listeria* spp.: efflux by proton antiporters, conferring resistance to tetracycline only [*tet*(L)], and ribosome protection, conferring resistance to both tetracycline and minocycline [*tet*(M) and *tet*(S)] (1). Both strains 03EB250LM and 04CEB563LM were resistant to tetracycline and minocycline due to the acquisition of the *tet*(M) gene. This determinant is highly prevalent in *Listeria* isolates resistant to tetracyclines (1) and is often associated with conjugative elements of the Tn*916* family (12). Accordingly, the *int*-Tn gene, encoding the integrase of Tn*916*-Tn*1545*, was found in 03EB250LM and 04CEB563LM. The *tet*(S), *tet*(K), and *tet*(L) determinants were not detected.

Thus, only 2% of the *L. monocytogenes* strains studied displayed resistance due to acquisition of genes that had already been found in *L. monocytogenes* in the 1990s.

Even if the number of resistant strains is low, notably, three of the four resistant strains were isolated from environmental sources. Moreover, two isolates were of serotype 4b, the most frequently encountered serotype in human infections. Both strains were recovered from sludge from the same purification plant (8), at a 1-year distance, and even if they displayed different PFGE profiles, an epidemiological link between these strains is likely.

The present results are not in favor of dissemination of resistance determinants within the population of *L. monocytogenes* strains from nonhuman sources in France. No resistance gene related to the preferred treatment, β-lactams and aminoglycosides (4), has been detected. However, resistance to trimethoprim is a problem since co-trimoxazole is the second-line therapy in case of allergy to β-lactams.

Comparison of this study to that of Morvan et al. (11), on French human *L. monocytogenes* isolates from 1989 to 2007, shows that the global frequency of acquired resistance is not higher than 2% in each strain collection. Results from the two studies are similar except that fluoroquinolone resistance has been found only in clinical isolates from humans.

Facing the recent increase of human listeriosis in industrialized countries, regular surveillance of *L. monocytogenes* should be maintained for early detection of any shift in the antimicrobial resistance of food or environmental isolates.

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