

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

Sophie A. Granier, Carole Moubareck, Cécile Colaneri, Astrid Lemire, Sophie Roussel, Trinh-Tam Dao, Patrice Courvalin and Anne Brisabois

Appl. Environ. Microbiol. 2011, 77(8):2788. DOI: 10.1128/AEM.01381-10.

Published Ahead of Print 25 February 2011.

Updated information and services can be found at:
<http://aem.asm.org/content/77/8/2788>

SUPPLEMENTAL MATERIAL

These include:

[Supplemental material](#)

REFERENCES

This article cites 14 articles, 7 of which can be accessed free at:
<http://aem.asm.org/content/77/8/2788#ref-list-1>

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Antimicrobial Resistance of *Listeria monocytogenes* Isolates from Food and the Environment in France over a 10-Year Period^{∇†}

Sophie A. Granier,^{1*} Carole Moubareck,² Cécile Colaneri,¹ Astrid Lemire,² Sophie Roussel,¹ Trinh-Tam Dao,¹ Patrice Courvalin,² and Anne Brisabois¹

ANSES, Laboratory for Food Safety, Unité CEB, 23 Avenue du General de Gaulle, 94706 Maisons-Alfort Cedex, France,¹ and Institut Pasteur, Centre National de Référence de la Résistance aux Antibiotiques, 75724 Paris Cedex 15, France²

Received 10 June 2010/Accepted 16 February 2011

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 *dfpD*, 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, Filiouis 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 sam-

ples 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 μ l 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.....	<25
Tetracycline.....	<1–2
Vancomycin.....	<0.5–2

* Corresponding author. Mailing address: ANSES, Laboratory for Food Safety, Unité CEB, 23 Avenue du General de Gaulle, 94706 Maisons-Alfort Cedex, France. Phone: 33 1 49 77 13 00. Fax: 33 1 49 77 46 66. E-mail: sophie.granier@anses.fr.

† Supplemental material for this article may be found at <http://aem.asm.org/>.

∇ Published ahead of print on 25 February 2011.

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), *dfpD* for trimethoprim, *tet(M)*, *tet(S)*, *tet(K)*, and *tet(L)* for tetracyclines, and the *int-Tn* gene, encoding the integrase of the Tn916-Tn1545 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* (erythromycin ribosome methylase) 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 tetrahydrofolate 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 *dfpD* gene (2). Trimethoprim resistance in *L. monocytogenes* 04CEB563LM was of a high level (MIC = 1,024 mg/ml) and was due to *dfpD* 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 Tn916 family (12). Accordingly, the *int-Tn* gene, encoding the integrase of Tn916-Tn1545, 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.

We thank Dik Mevius and Kees Veldman for technical advice.

REFERENCES

1. Charpentier, E., and P. Courvalin. 1999. Antibiotic resistance in *Listeria* spp. *Antimicrob. Agents Chemother.* **43**:2103–2108.
2. Charpentier, E., and P. Courvalin. 1997. Emergence of the trimethoprim resistance gene *dfpD* in *Listeria monocytogenes* BM4293. *Antimicrob. Agents Chemother.* **41**:1134–1136.
3. Clinical and Laboratory Standards Institute. 2006. M45-A. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. Approved guideline. Clinical and Laboratory Standards Institute, Wayne, PA.
4. Davis, J. A., and C. R. Jackson. 2009. Comparative antimicrobial susceptibility of *Listeria monocytogenes*, *L. innocua*, and *L. welshimeri*. *Microb. Drug Resist.* **15**:27–32.
5. European Food Safety Authority. 2010. The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *EFSA J.* **8**:1496.
6. Filiouis, G., A. Johansson, J. Frey, and V. Perreten. 2009. Prevalence,

- genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. *Food Control* **20**:314–317.
7. **Huovinen, P., L. Sundstrom, G. Swedberg, and O. Skold.** 1995. Trimethoprim and sulfonamide resistance. *Antimicrob. Agents Chemother.* **39**: 279–289.
 8. **Kerouanton, A., et al.** 2009. Characterization of isolates of *Listeria monocytogenes* from sludge using pulsed-field gel electrophoresis and virulence assays. *J. Appl. Microbiol.* **108**:1380–1388.
 9. **Leclercq, R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.* **34**:482–492.
 10. **Martin, P., C. Jacquet, V. Goulet, V. Vaillant, and H. De Valk.** 2006. Pulsed-field gel electrophoresis of *Listeria monocytogenes* strains: the PulseNet Europe Feasibility Study. *Foodborne Pathog. Dis.* **3**:303–308.
 11. **Morvan, A., et al.** 2010. Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. *Antimicrob. Agents Chemother.* **54**:2728–2731.
 12. **Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, and P. Courvalin.** 1989. Molecular characterization of two proteins involved in the excision of the conjugative transposon Tn1545: homologies with other site-specific recombinases. *EMBO J.* **8**:2425–2433.
 13. **Roberts, M. C., B. Facinelli, E. Giovanetti, and P. E. Varaldo.** 1996. Transferable erythromycin resistance in *Listeria* spp. isolated from food. *Appl. Environ. Microbiol.* **62**:269–270.
 14. **Roussel, S., et al.** 2010. Semi-automated repetitive-sequence-based polymerase chain reaction compared to pulsed-field gel electrophoresis for *Listeria monocytogenes* subtyping. *Foodborne Pathog. Dis.* **7**:1005–1012.
 15. **Vela, A. I., et al.** 2001. Molecular typing by pulsed-field gel electrophoresis of Spanish animal and human *Listeria monocytogenes* isolates. *Appl. Environ. Microbiol.* **67**:5840–5843.