Antimicrobial Resistance of Listeria monocytogenes Strains Isolated from Humans in France

A. Morvan,1 C. Moubareck,2 A. Leclercq,1 M. Hervé-Bazin,1 S. Bremont,2 M. Lecuit,1,3,4,5,* P. Courvalin,6 and A. Le Monnier5,6,7,*

Institut Pasteur, Centre National de Référence des Listeria and World Health Organization Collaborating Centre for Foodborne Listeriosis, Paris, France; Institut Pasteur, Centre National de Référence de la Résistance aux Antibiotiques, Paris, France; Institut Pasteur, Groupe Microorganismes et Barrières de l’Hôpital, Paris, France; INSERM avenir U604, Paris, France; Université Paris Descartes, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France; and Laboratoire de Microbiologie du Centre Hospitalier de Versailles, Le Chesnay, France

Received 3 November 2009/Returned for modification 24 January 2010/Accepted 5 April 2010

Susceptibility to antibiotics of 4,816 clinical L. monocytogenes strains isolated since 1926 was studied, and the temporal evolution of susceptibility to antibiotics was analyzed through several decades. The mechanisms of resistance in each resistant strain were studied. The prevalence of resistant strains was estimated at 1.27% among isolates from humans. Resistance to tetracyclines+ and fluoroquinolones was more common and has recently emerged. Although acquired resistance in clinical L. monocytogenes did not implicate clinically relevant antibiotics, the possibility of resistance gene transfers, the description of the first clinical isolate with high-level resistance to trimethoprim, and the recent increase in penicillin MICs up to 2 μg/ml reinforce the need for microbiological surveillance.

Listeria monocytogenes is a food-borne pathogen widespread in the environment (8). It causes severe and life-threatening infection mainly in high-risk groups of patients (8, 11, 24). The outcome of listeriosis depends on the early administration of antibiotics having rapid and bactericidal activity against L. monocytogenes (11, 17, 24).

With the exception of natural in vitro 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 (35).

The reference treatment is currently based on a synergistic association of high doses of aminopenicillin (ampicillin or amoxicillin) and gentamicin (17, 34). Although rifampin, vancomycin, linezolid, and carbapenems have been proposed as possible alternatives (2, 11, 16, 17, 34), trimethoprim is generally used in case of intolerance of beta-lactams (17, 34).

L. monocytogenes rarely develops acquired resistance to antibiotics. However, some studies have recently reported an increased rate of resistance to one or several clinically relevant antibiotics in environmental isolates (1, 6, 7, 21, 33, 37) and less frequently in clinical strains (3, 9, 26, 31). Yet, this probably remains a marginal phenomenon for clinical strains, although only a limited number of studies have focused on the evaluation of antimicrobial resistance in Listeria (14, 15, 19, 22, 25, 31).

The present work evaluated the prevalence of resistance in a large collection of clinical L. monocytogenes strains isolated between 1989 and 2007 and studied the temporal evolution of susceptibility to antibiotics since the first characterization of L. monocytogenes in 1926.

Prevalence of resistance among L. monocytogenes strains isolated from humans. Prevalence of acquired resistance was determined for all L. monocytogenes strains isolated from humans between 1989 and 2007 that were not epidemiologically linked and that had been previously characterized (serovar and pulsovar) by the French National Reference Center (NRC) for Listeria (10, 23).

Susceptibility to a panel of 23 antibiotics (penicillin G, amoxicillin, ampicillin, imipenem, cefotaxime, tetracycline, erythromycin, clindamycin, nalidixic acid, moxifloxacin, levofloxacin, ciprofloxacin, kanamycin, streptomycin, gentamicin, rifampin, vancomycin, sulfonamides, trimethoprim, fosfomycin, chloramphenicol, linezolid, and fusidic acid) was determined by screening on breakpoint concentrations as previously described (6) between 1989 and 2005 and by disk diffusion since 2006. Reference strains of L. monocytogenes, including those with previously characterized resistance to antibiotics, were used as controls (4, 9, 26).

Among the 4,668 clinical L. monocytogenes strains tested, we detected 61 (1.27%) strains resistant to at least one clinically relevant antibiotic according to the breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for non-species-related bacteria (Table 1). The prevalence of acquired resistance in France determined from the largest collection of clinical isolates to date remains low, similar to reported results of previous epidemiological studies in other countries (14, 15, 19, 22, 25, 31). This contrasts with the higher prevalence of resistance reported for food and environmental Listeria species, possibly overstated due to the existence of a larger reservoir of resistance in Listeria innocua than in L. monocytogenes (6).

We reported two isolates with acquired multidrug resistance
TABLE 1. Resistance to antibiotics of *L. monocytogenes* strains isolated from humans between 1989 and 2007 (n = 4,668)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of resistant strainsa</th>
<th>Pulsotype</th>
<th>MIC or MIC range (μg/ml)</th>
<th>Resistance mechanism or gene (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim</td>
<td>1</td>
<td>1</td>
<td>1.024 dfrD (4)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>34</td>
<td>15</td>
<td>16–128</td>
<td>tet(M) (n = 34), int-Tn (n = 14)</td>
</tr>
<tr>
<td>Minocycline</td>
<td>8–16</td>
<td></td>
<td></td>
<td>tet(M) (n = 34), int-Tn (n = 14)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1</td>
<td>1</td>
<td>256 Putative chromosomal</td>
<td>Putative ribosomal mutation</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2</td>
<td>2</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
<td>1</td>
<td>48 cat</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20</td>
<td>14</td>
<td>6–32</td>
<td>lde (9)</td>
</tr>
</tbody>
</table>

a The two MDR *L. monocytogenes* strains are not presented. BM4210, isolated in 1988 from an 84-year-old patient with meningocencephalitis (26), was resistant to chloramphenicol (cat221), erythromycin (erm(B)), streptomycin (gene aad6), and tetracycline [tet(S)]; the second MDR strain, isolated in 1990 from a case of septic abortion, was resistant to chloramphenicol (cat221), erythromycin [erm(B)], and tetracycline [tet(S)].

(MDR) (Table 1) (26, 29). However, MDR remains exceptional in *L. monocytogenes*. Indeed, only two additional cases were reported worldwide: a Swiss patient with endocarditis (13) and a Greek newborn with neonatal meningitis (36).

Resistance to tetracyclines and fluoroquinolones was more common and has emerged since the late 1980s and 1990s, respectively. However, comparison of the pulsotypes (pulsed-field gel electrophoresis [PFGE]) rules out clonal spread of resistant strains (Table 1).

**Genetic basis and resistance mechanisms.** MICs of antibiotics for resistant strains were determined on Mueller-Hinton agar with or without reserpine by Etest or agar dilution. Total DNA was prepared with the Instagene matrix (Bio-Rad Laboratories, Hercules, CA) and used for screening the resistance genes listed in Table 2 by PCR.

**Tetracycline resistance.** Both multidrug-resistant strains and 34 additional isolates (15 pulsotypes) exhibited resistance to tetracycline (MICs, 16 to 128 μg/ml) and minocycline (MICs, 8 to 16 μg/ml), suggesting ribosome protection due to either the tet(M) or tet(S) gene (3). The tet(S) gene was detected in both MDR strains, whereas the remaining strains had acquired tet(M). The *int-Tn* gene for the integrase of Tn916-Tn1545 was found in 41% of strains harboring tet(M), confirming that tetracycline resistance in *L. monocytogenes* is only partly due to acquisition of conjugative transposons (27), as opposed to results previously obtained (28). The tet(K) and tet(L) determinants, conferring resistance to tetracycline only by efflux, were not detected (32).

**Fluoroquinolone resistance.** Twenty isolates (14 pulsotypes) were found to be resistant to fluoroquinolones. On the basis of a 3-fold or greater decrease of ciprofloxacin MIC in the presence of reserpine, resistance was attributed in all the tested strains to active efflux associated with overexpression of the *lde* gene (9).

**Streptomycin resistance.** Resistance to streptomycin was observed at low levels in two clinical strains (MICs, 4 to 6 μg/ml) and at a high level in BM4210, with an MIC of 256 μg/ml. BM4210 produces a 6-N-streptmycin adenyltransferase, encoded by the *aad6* gene (3). In the two other strains, the *aad6* gene was not detected, suggesting that this resistance could be due to ribosomal mutations.

**Chloramphenicol resistance.** Three chloramphenicol-resistant strains, including the MDR strains, carried a gene that belonged to the cat family. As previously described, chloramphenicol resistance is due to acquisition of a *cat* gene encoding an acetyltransferase which catalyzes acetyl-S-coenzyme A (CoA)-dependent acetylation of chloramphenicol at the 3-hydroxyl group (26).

**Macrolide resistance.** Three erythromycin-resistant strains have been detected, including the two multidrug-resistant strains (3). The erm(B) gene, encoding a 235 RNA methyltransferase that modifies the macrolide-lincosamide-streptogramin B (MLSb) antibiotic binding site, was detected in both MDR strains. However, neither *erm* genes nor *msr(A) and mef(A)* genes encoding previously described efflux pumps in Gram-positive bacteria were detected in the third resistant strain (5, 20, 30). Determination of the erythromycin MIC against this last strain in the absence and presence of reserpine did not lead to a decrease in the MIC, ruling out an efflux mechanism. Resistance in this case could be due to a chromosomal mutation.

**Trimethoprim resistance.** Trimethoprim resistance in *L. monocytogenes* U2A2348 was of high level (MIC, 1,024 μg/ml) and due to acquisition of the dfrD gene, encoding a resistant
TABLE 3. Evolution of median value of MICs of penicillins for clinical L. monocytogenes strains isolated in France between 1926 and 2007 according to four time periods

<table>
<thead>
<tr>
<th>Period (n)</th>
<th>Penicillin G</th>
<th>Amoxicillin</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% 90% Range</td>
<td>50% 90% Range</td>
<td>50% 90% Range</td>
</tr>
<tr>
<td>Total (436)</td>
<td>0.5 1 0.023–2</td>
<td>0.5 0.016–2</td>
<td>0.38 0.75 0.016–2</td>
</tr>
<tr>
<td>1926–1963 (13)</td>
<td>0.38 0.75 0.032–1</td>
<td>0.5 0.023–0.75</td>
<td>0.19 0.5 0.032–0.5</td>
</tr>
<tr>
<td>1964–1988 (61)</td>
<td>0.25 0.75 0.023–1</td>
<td>0.25 0.016–0.75</td>
<td>0.19 0.75 0.016–0.75</td>
</tr>
<tr>
<td>1989–2005 (145)</td>
<td>0.5 0.75 0.125–2</td>
<td>0.5 0.125–2</td>
<td>0.5 0.5 0.047–2</td>
</tr>
<tr>
<td>2006–2007 (217)</td>
<td>0.5 1 0.125–2</td>
<td>0.5 1 0.19–2</td>
<td>0.5 1 0.094–2</td>
</tr>
</tbody>
</table>

Evolution of the susceptibility to antibiotics since 1926. The temporal evolution of susceptibility to antibiotics, determined by disk diffusion, was analyzed for historical clinical L. monocytogenes strains isolated between 1926 and 1988 that originated from the collection of the NRC and the international Special Listeria Culture Collection (SLCC). Between 1926 and 1963, all L. monocytogenes strains isolated from humans in France (n = 13) or other countries (n = 51) from our collections were tested. During this period, we also tested a randomly selected set representative of all animal isolates (n = 23). For strains isolated between 1964 and 1988, we tested a randomly selected set of L. monocytogenes strains isolated from humans in France (n = 61/477 isolates) representative of each year, clinical form, and serovar.

As expected, the natural resistance preexisted marketing and use of major classes of antibiotics. However, no acquired resistance to the 23 antibiotics tested was detected for strains isolated between 1926 and 1988.

Although L. monocytogenes resistant to penicillins were not detected by disk diffusion according to the breakpoints of EUCAST, the MICs of penicillin, amoxicillin, and ampicillin were determined by Etest for a set of 436 L. monocytogenes strains isolated from humans in France between 1926 and 2007 and distributed in four successive time periods (Table 3).

Statistical analysis showed a significant difference in the distribution of MICs between 1926 and 2007 according to the selected time periods (P = 0.00001, Kruskal-Wallis test). The comparison of each period to each other showed that the MICs determined during the 1926 to 1963 and 1964 to 1988 periods differ from those observed during the 1989 to 2005 and 2006 to 2007 time periods (P = 0.0001, Wilcoxon test). The MIC50 of aminopenicillins for 1989 to 2007 were more than twice those for 1926 to 1988 (Table 3). Moreover, the number of strains with MICs greater than 1 μg/ml has increased since 1989. Strains with an MIC of 2 μg/ml, not observed before 1988, have recently emerged. Whereas this increase in MICs has already been reported for environmental strains (7, 33), we observed the same increase for L. monocytogenes strains isolated from humans in France since 1926. As has been observed for Streptococcus pneumoniae (12), this MIC creeping could be explained by increased use of beta-lactams from the middle of the 20th century, accentuated in recent years. Although we do not report resistance to penicillins associated with clinical failure, the results of this study justify systematic determination of MICs of aminopenicillins for adapting dosages. According to MICs, no differences in activity between ampicillin and amoxicillin have been observed, contrary to what had been suggested in previous studies (33).

Acquired resistance in L. monocytogenes from humans has no clinical consequence so far as it does not concern the first-line treatment of listeriosis. However, transfer of resistance genes from other bacteria and the recent increasing MICs of aminopenicillins underline the need for active and continuous surveillance of the susceptibility to antibiotics.

We thank Yves Pechine for statistical analysis.

This study was supported by the Institut Pasteur (Paris, France) and the Institut de Veille Sanitaire (Saint Maurice, France).

REFERENCES


