ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2003, p. 704–708 Vol. 47, No. 2
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.2.704–708.2003

Efflux Pump Lde Is Associated with Fluoroquinolone Resistance in *Listeria monocytogenes*

Sylvain Godreuil,[1] Marc Galimand,[1]* Guy Gerbaud,[1] Christine Jacquet,[2] and Patrice Courvalin[1]

Unité des Agents Antibactériens[1] and Laboratoire des Listeria,[2] Institut Pasteur, 75724 Paris Cedex 15, France

Received 6 March 2002/Returned for modification 20 September 2002/Accepted 29 October 2002

Five *Listeria monocytogenes* isolates (CLIP 21369, CLIP 73298, CLIP 74811, CLIP 75679, and CLIP 79372) were found to be resistant to fluoroquinolones during the screening for antibiotic resistance of 488 *L. monocytogenes* isolates from human cases of listeriosis in France. On the basis of a fourfold or greater decrease in the ciprofloxacin MIC in the presence of reserpine, fluoroquinolone resistance was attributed to active efflux of the drugs. The *lde* gene (*Listeria* drug efflux; formerly *lmo2741*) encodes a 12-transmembrane-segment putative efflux pump belonging to the major facilitator superfamily of secondary transporters that displayed 44% identity with PmrA from *Streptococcus pneumoniae*. Insertional inactivation of the *lde* gene in CLIP 21369 indicated that the corresponding protein was responsible for fluoroquinolone resistance and was involved in the level of susceptibility to dyes such as ethidium bromide and acridine orange.

Listeria monocytogenes is widely distributed in the environment and can cause serious human infections, such as bacteremia and central nervous system infections, primarily in neonates and immunocompromised adults, and abortions (22). Food-borne transmission is recognized as the main route of acquisition of the infection during epidemic and sporadic listerioses (3, 18). Listeriosis differs from most food-borne diseases by its high fatality rate (20 to 30% of cases), despite the administration of appropriate antibiotics (3, 9). *L. monocytogenes* is generally susceptible to a wide range of antibiotics but is not susceptible to cephalosporins and fosfomycin (8). However, during the last few years, increasing numbers of strains resistant to one or more antibiotics have been reported (1, 2, 8, 11, 19, 20).

Although indications for the use of fluoroquinolones do not include listeriosis, they can, due to their increasing use for other pathologies such as respiratory tract infections, select resistant *Listeria*. In gram-positive bacteria, resistance usually results from mutational alterations in the so-called quinolone resistance-determining regions (QRDRs) of the intracellular targets of fluoroquinolones, the type II DNA topoisomerases gyrase and topoisomerase IV, or active export of the drugs via efflux pumps (12). We have detected ciprofloxacin-resistant *L. monocytogenes* clinical isolates and characterized the efflux pump involved in resistance.

(An initial report of this work was presented at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy [S. Godreuil, M. Galimand, G. Gerbaud, and P. Courvalin, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. UL-11, 2001].)

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. Screening of 488 *L. monocytogenes* isolates responsible for human listeriosis in France for resistance to fluoroquinolones was carried out on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) containing 4 μg of ciprofloxacin per ml. Strains that were found to be resistant and susceptible *L. monocytogenes* EGD were studied (Table 1). The strains were serotyped with antibodies made in the Laboratoire des *Listeria* and by the agglutination technique, as described elsewhere (23). *Escherichia coli* TOP10 plasmid PCR2 (Invitrogen, Paisley, United Kingdom), which is a vector used for cloning of PCR products, and plasmid pKSV7, which is thermosensitive for replication (24), were used. The *E. coli* strains were grown at 37°C, and the *L. monocytogenes* strains were grown at 30, 37, or 43°C. The strains were grown in brain heart infusion broth and agar supplemented with ampicillin at 100 μg/ml and kanamycin at 20 μg/ml for *E. coli* and chloramphenicol at 8 μg/ml for *L. monocytogenes*.

Susceptibility testing. The isolates were tested for their antibiotic susceptibilities by disk diffusion on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France). A minimum of three independent determinations of the MICs of the antimicrobial agents and dyes were determined on Mueller-Hinton agar by the E-test (AB Biodisk France, Combourg, France) or by agar dilution with or without reserpine (10 μg/ml) with 10^4 CFU per spot after 24 h of incubation at 37 or 43°C (25).

DNA preparation and transformation. Purification of plasmid DNA was performed by using the Wizard minipreps DNA kit (Promega, Madison, Wis.). Total DNA of *L. monocytogenes* was prepared as described previously (20). Restriction with endonucleases was according to the recommendations of the supplier. Extraction of DNA fragments separated by agarose gel electrophoresis was carried out with a Sephaglas BandPrep kit (Amersham-Pharmacia Biotech, Saint Quentin en Yvelines, France). *L. monocytogenes* DNA in plugs was digested with *Asc*I or *Apa*I, and large restriction fragments were separated by electrophoresis in a CHEF-DRIII system (Bio-Rad Laboratories, Richmond, Calif.) by using an electric field of 6 V/cm and an angle of 120°. The initial and final pulse times were 4 and 40 s, respectively. Migration of the DNA fragments was performed in a 1% agarose gel in 0.5× Tris-borate-EDTA at 14°C (6).

Amplification of DNA was performed in a 2400 thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.), with *Pfu* DNA polymerase (Stratagene, La Jolla, Calif.), as recommended by the manufacturer. PCR elongation times and temperatures were adjusted according to the expected size of the PCR product and the nucleotide sequences of the primers, respectively.

The QRDRs of the *gyrA* and *gyrB* genes for DNA gyrase and the *parC* and *parE* genes for DNA topoisomerase IV were amplified by PCR with specific primers (Table 2) and chromosomal DNA of fluoroquinolone-resistant and -susceptible *L. monocytogenes* strains as the template.

E. coli transformation was performed by the protocol provided by Invitrogen, and electrotransformation of *L. monocytogenes* was as described previously (16) and was done with a Gene Pulser apparatus (Bio-Rad).

Insertional mutagenesis of *lde* gene. A fragment internal to the *lde* gene of *L. monocytogenes* CLIP 21369 was amplified with the specific oligodeoxynucleotides LDE-F (5′-GGGGTACCCCAGAATTTGTATGTTGTCTGGG-3′), which harbors a *Kpn*I site (underlined), and LDE-R (5′-CGGGATCCCGTACGAGGAA

* Corresponding author. Mailing address: Unité des Agents Antibactériens, Institut Pasteur, 25, rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: (33) 1 45 68 83 18. Fax: (33) 1 45 68 83 19. E-mail: galimand@pasteur.fr.

TABLE 1. Properties of the strains tested

L. monocytogenes	Serovar	Yr of isolation	MIC (μg/ml)[a]											
			Ciprofloxacin		Norfloxacin		Sparfloxacin		Moxifloxacin		Ethidium bromide		Acridine orange	
			−	+	−	+	−	+	−	+	−	+	−	+
EGDe	1/2a	1964	0.5–1	0.50–0.75	2–3	2–3	1–1.5	0.75–1	0.25–0.38	0.19–0.25	10	10	20	20
CLIP 21369	1/2b	1992	8–16	0.75–1	48–64	3–4	1–1.5	0.75–1	0.25–0.38	0.19–0.25	200	100	80	40
CLIP 73298	1/2a	1997	8–16	0.75–1	48–64	3–4	1–1.5	0.50–0.75	0.25–0.38	0.19–0.25	200	100	80	40
CLIP 74811	4b	1997	6–8	0.50–0.75	32–64	2–4	1–1.5	0.50–0.75	0.25–0.38	0.19–0.25	200	100	80	40
CLIP 75679	SND[b]	1997	6–8	0.50–0.75	32–48	2–3	1–1.5	0.50–0.75	0.25–0.38	0.19–0.25	200	100	80	40
CLIP 79372	1/2b	1999	6–8	0.50–0.75	16–24	2–4	1–1.5	0.75–1	0.25–0.38	0.19–0.25	200	100	80	40
BM4497	SND	NA[c]	0.5–1	0.50–0.75	2–4	2–3	1–1.5	0.50–0.75	0.25–0.38	0.19–0.25	100	100	40	40

[a] Values are ranges from three independent determinations; consistent values were obtained for dyes. −, without reserpine; +, with 10 μg of reserpine per ml.
[b] SND, serovar not designated.
[c] NA, not applicable.

GATCTCCGTA-3′), which contains a *Bam*HI site (underlined). After digestion of the 1,105-bp PCR product with *Kpn*I and *Bam*HI, the fragment was cloned into similarly digested pKSV7 DNA. The recombinant plasmid pAT778 DNA was introduced into CLIP 21369 by electrotransformation, and transformants were selected for chloramphenicol resistance at 30°C. At 43°C chloramphenicol resistance in transformants can be maintained only following integrative recombination of the plasmid. Total DNA from three chloramphenicol-resistant clones was analyzed by PCR with the M13 reverse and forward primers and with two specific primers whose sequences are complementary to the sequences of the flanking regions of the *lde* gene in the *L. monocytogenes* chromosome (Table 2). The sequences of the amplification products were determined.

DNA sequence determination and analysis. DNA sequencing was performed with a CEQ 2000 DNA analysis system automatic sequencer (Beckman Coulter, Palo Alto, Calif.). Nucleotide sequence data were analyzed with the GCG sequence analysis software package (version 10.1; Genetics Computer Group, Madison, Wis.). BLAST program searches were performed by using the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov). Transmembrane sequences (TMSs) were identified by using the TMHMM program at ExPASy (http://www.expasy.ch/). Multiple-sequence alignment was performed with the ClustalW program (version 1.82) at the Institut Pasteur website (http://www.bioweb.pasteur.fr/).

RESULTS AND DISCUSSION

Properties of the *L. monocytogenes* strains. Screening of clinical *L. monocytogenes* isolates for fluoroquinolone resistance led to the detection of five ciprofloxacin-resistant strains (Table 1). The disk agar diffusion test showed that the five isolates were susceptible to ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, minocycline, streptomycin, tetracycline, trimethoprim, and vancomycin (data not shown). The clinical isolates belonged to various serovars (Table 1), and pulsed-field gel electrophoresis of total DNA after digestion with the *Asc*I or *Apa*I restriction endonuclease revealed different profiles (data not shown). These results indicate that the resistant isolates were not clonally related.

The MICs of fluoroquinolones and dyes for the clinical isolates and susceptible strain EGDe were determined by the E-test and agar dilution, respectively (Table 1). The clinical isolates were resistant to ciprofloxacin and norfloxacin, and in the presence of reserpine, a fourfold or greater decrease in the level of resistance to these hydrophilic quinolones was observed. By contrast, the MICs of relatively hydrophilic agents (levofloxacin and moxifloxacin) and hydrophobic drugs (gatifloxacin, sparfloxacin, and trovafloxacin) (Table 1 and data not shown) were not significantly altered (onefold or less decrease). These data, together with the reproducible twofold reduction in the MICs of the dyes tested, suggested that efflux was responsible for the resistance.

Partial sequencing of *gyr* and *par* genes. Pairs of primers designed from the genome of *L. monocytogenes* EGDe (Table 2) were used to amplify and sequence the QRDRs of *gyrA*, *gyrB*, *parC*, and *parE*. No differences in the sequences of susceptible strain EGDe (5, 13) and the five ciprofloxacin-resistant isolates were found (data not shown).

TABLE 2. Primers used for PCR and sequencing

Primer	Sequence (5′-3′)[a]	Location
gyrA-F	AGTGTAATTGTTGCCCG	bp 82–98 of *gyrA*
gyrA-R	ATATCGCCATCAACCGA	bp 350–334 of *gyrA*
gyrB-F	AAGCGCGCGCGTGAAGT	bp 1192–1208 of *gyrB*
gyrB-R	CGAGATTTAGAAACGTC	bp 1499–1483 of *gyrB*
parC-F	GAACGTGCGCTTCCAGA	bp 91–107 of *parC*
parC-R	GTTGCATAACCAGCGGA	bp 539–523 of *parC*
parE-F	GGAAAATTAACGCCAGC	bp 1222–1238 of *parE*
parE-R	TCGGTCATGATAACTAC	bp 1499–1483 of *parE*
lmo-F	ATCGTGAACTTAATGGTGG	198 bp upstream from *lde* start codon
lmo-R	ATCCTCATATAACTCAAGCG	112 bp downstream from *lde* stop codon
M13-F	GTTGTAAAACGACGGCCAGT	Used for pCR2 and pKSV7 vectors
M13-R	TCACACAGGAAACAGCTATGA	Used for pCR2 and pKSV7 vectors

[a] The primers were designed from the sequence of *L. monocytogenes* EGDe (GenBank accession number AL591824).

TABLE 3. Identities between the proteins deduced from the *lmo* gene of *L. monocytogenes* EGDe and those from 12-TMS MFS-type efflux proteins from gram-positive bacteria

Sequence compared	% Identity[a]			
	Bmr	NorA	EmeA	PmrA
Lmo 0839	29	25	27	26
Lmo 0872	22	24	21	26
MdrL (Lmo 1409)	24	25	26	25
Lmo 2377	26	24	25	32
Lde (Lmo 2741)	25	24	29	44

[a] Bmr, from *B. subtilis* (GenBank accession number L25604); NorA, from *S. aureus* (GenBank accession number AB019536); EmeA, from *E. faecalis* (10); PmrA, from *S. pneumoniae* (GenBank accession number AJ007367); MdrL, from *L. monocytogenes* (14); Lde, from *L. monocytogenes* EGDe (this study and GenBank accession number AL591824).

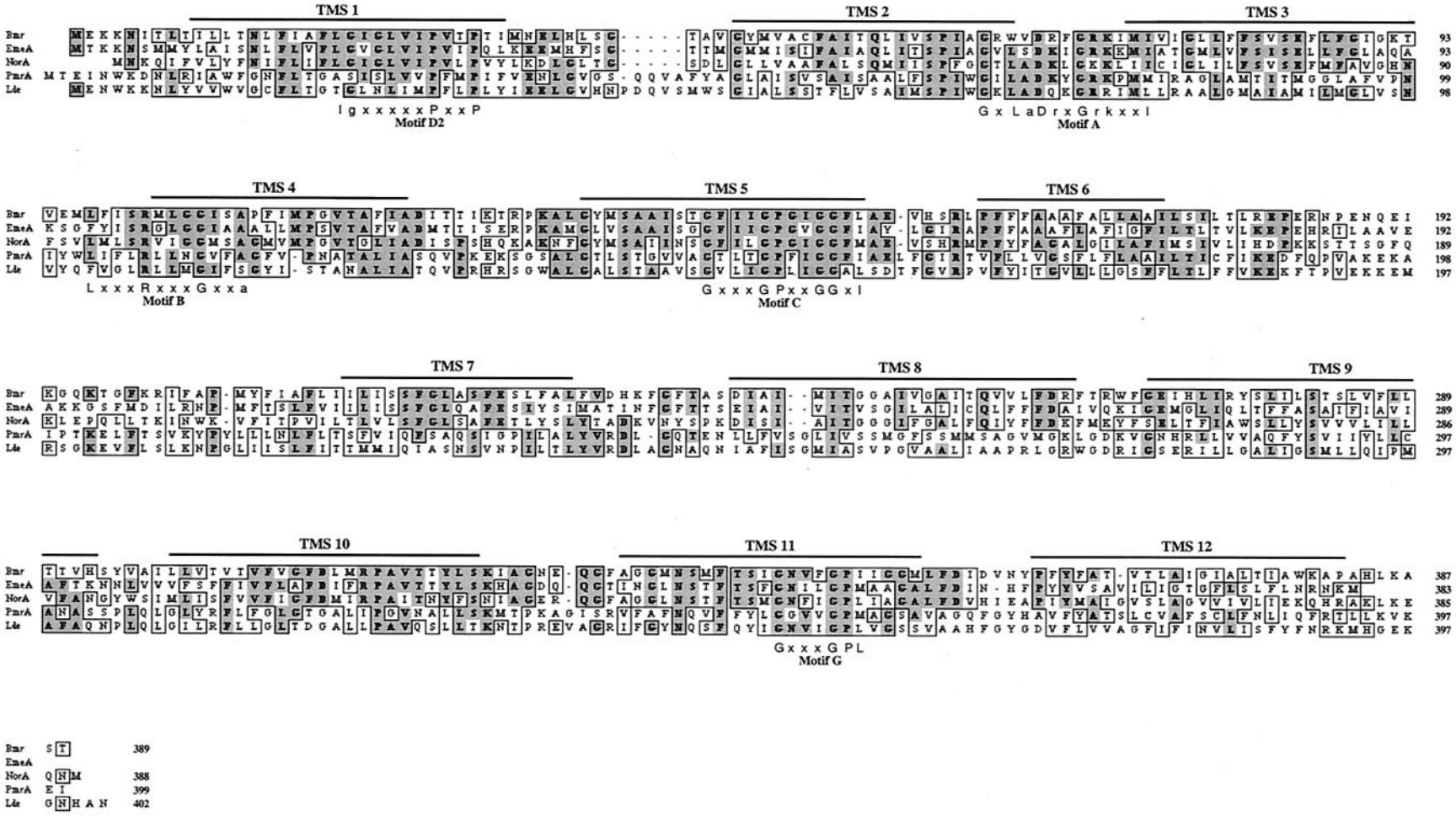

FIG. 1. Alignment of the deduced sequences of 12-TMS MFS proteins from gram-positive bacteria. Bmr, from *B. subtilis* (GenBank accession number L25604); EmeA, from *E. faecalis* (10); NorA, from *S. aureus* (GenBank accession number AB019536); PmrA, from *S. pneumoniae* (GenBank accession number AJ007367); Lde, from *L. monocytogenes* EGDe (this study and GenBank accession number AL591984). Identical amino acids are indicated by boldface letters on a black background, and conservative substitutions are indicated by letters on a grey background. The horizontal lines above the alignment indicate the locations of the 12 TMSs (1 to 12). Highly conserved motifs (motifs A, B, C, D2, and G) are displayed below the alignment.

Characterization of *lde* gene. In gram-positive bacteria, four multidrug efflux transporters that use the proton motive force present across the cytoplasmic membrane to extrude a variety of toxic compounds including fluoroquinolones and dyes have been described: Bmr from *Bacillus subtilis* (15), NorA from *Staphylococcus aureus* (26), PmrA from *Streptococcus pneumoniae* (4), and EmeA from *Enterococcus faecalis* (10). They belong to the major facilitator superfamily (MFS) of secondary multidrug transporters, share a common structure with 12 TMSs, and are closely related phylogenetically (17, 21).

The family-specific motifs of the MFS proteins were used to identify structural genes for putative multidrug exporter proteins in the genome of *L. monocytogenes* EGDe: (i) a search for the gxxxGPxxGGxl C motif (where x is any amino acid, a capital letter indicates that the amino acid occurs in >70% of the examined sequences, and a lowercase letter indicates that the amino acid occurs in <40% of the examined sequences) for multidrug transporters resulted in the identification of 10 genes for multidrug exporter proteins; (ii) Kyte-Doolittle hydropathy plots detected five 14-TMS and five 12-TMS exporters; and (iii) Blast comparison of the 12-TMS putative efflux pumps identified the *lde* gene (formerly *lmo2741* [5]), which encodes a 402-amino-acid protein that displayed 44% identity with PmrA from *S. pneumoniae* (Table 3).

The sequence alignments of the Bmr, NorA, EmeA, PmrA, and Lde proteins are shown in Fig. 1. TMS prediction with the TMHMM program indicated that the 12 TMSs of the Lde efflux pump were at positions similar to those of other 12-TMS proton-dependent efflux pumps (17), with the N and C termini located in the cytoplasm. The translocase consensus sequence (motif A) located in the loop between TMS 2 and TMS 3 and the drug extrusion consensus sequence (motif C) at the end of TMS 5 were conserved in all five sequences. Other motifs, in particular, D2 and G, that are exclusive to the 12-TMS family suggested that Lde is a new member of MFS family 2, which consists of 12-TMS exporters (17, 21).

Inactivation of Lde efflux system. To determine if the Lde pump is involved in resistance to fluoroquinolones and dyes, we inactivated the *lde* gene by insertion. Two specific primers, LDE-F and LDE-R, were used to amplify a 1,105-bp fragment internal to *lde* that was ligated into vector pKSV7, which is thermosensitive for replication, to generate pAT778. Plasmid pAT778 DNA was introduced into CLIP 21369 by electrotransformation, and at a high temperature homologous recombination in the chromosome for disruption of *lde* was favored. Transformants resistant to chloramphenicol at 43°C were found to be susceptible to fluoroquinolones by disk diffusion. Clones were analyzed by PCR and sequencing; and strain BM4497, which has a single integrated copy of pAT778 in the *lde* gene, was studied further (data not shown).

The MICs of fluoroquinolones and dyes for strain BM4497 were determined. The strain was three- to fourfold more sensitive than parental strain CLIP 21369 to ciprofloxacin and norfloxacin and two times more sensitive to dyes, and its susceptibility was not affected by the presence of reserpine (Table 1). These results indicate that *lde* is responsible for fluoroquinolone resistance and, in part, for acridine orange and ethidium bromide resistance in *L. monocytogenes* CLIP 21369. The *lde* gene was found by PCR in all six strains of *L. monocytogenes* examined, irrespective of their susceptibilities to quinolones. It is therefore likely that resistance results from an increase in the level of expression of the pump.

In summary, we have demonstrated that *lde* encodes a multidrug efflux pump associated with resistance to hydrophilic fluoroquinolones in *L. monocytogenes*. Because of the ubiquitous distribution of *L. monocytogenes*, this bacterial species is exposed to a wide variety of drugs, and thus, active efflux could contribute to the adaptation of *Listeria* to these environmental challenges (14). Trends in the use of antimicrobials in France between 1980-1981 and 1990-1991 revealed that the overall rate of antibiotic use increased by 3.7% a year. During that time, the rate of use of all quinolones increased by 5.7% per year, even when the rate of use of nonfluorated quinolones decreased by 4.7% (7). Our observations indicate that increasing use of fluoroquinolones can select resistant mutants in nontarget species.

ACKNOWLEDGMENTS

This work was supported in part by a Bristol-Myers Squibb Unrestricted Biomedical Research Grant in Infectious Diseases.

We thank P. Glaser, P. Cossart, and the *Listeria* Consortium for access to the *L. monocytogenes* EGDe sequence prior to publication.

REFERENCES

1. **Charpentier, E., and P. Courvalin.** 1999. Antibiotic resistance in *Listeria* spp. Antimicrob. Agents Chemother. **43:**2103–2108.
2. **Charpentier, E., G. Gerbaud, and P. Courvalin.** 1999. Conjugative mobilization of the rolling-circle plasmid pIP823 from *Listeria monocytogenes* BM4293 among gram-positive and gram-negative bacteria. J. Bacteriol. **181:** 3368–3374.
3. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55:**476–511.
4. **Gill, M. J., N. P. Brenwald, and R. Wise.** 1999. Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **43:**187–189.
5. **Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok, A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couvé, A. de Daruvar, P. Dehoux, E. Domann, G. Domínguez-Bernal, E. Duchaud, L. Durant, O. Dussurget, K.-D. Entian, H. Fsihi, F. Garcia-Del Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gómez-López, T. Hain, J. Hauf, D. Jackson, L.-M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueño, A. Maitournam, J. Mata Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. de Pablos, J.-C. Pérez-Diaz, R. Purcell, B. Remmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J.-A. Vázquez-Boland, H. Voss, J. Wehland, and P. Cossart.** 2001. Comparative genomics of *Listeria* species. Science **294:**849–852.
6. **Graves, L. M., and B. Swaminathan.** 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int. J. Food Microbiol. **65:**55–62.
7. **Guillemot, D., P. Maison, C. Carbon, B. Balkau, F. Vauzelle-Kervroëdan, C. Sermet, G. Bouvenot, and E. Eschwège.** 1998. Trends in antimicrobial drug use in the community—France, 1981–1992. J. Infect. Dis. **177:**492–497.
8. **Hadorn, K., H. Hächler, A. Schaffner, and F. H. Kayser.** 1993. Genetic characterization of plasmid-encoded multiple antibiotic resistance in a strain of *Listeria monocytogenes* causing endocarditis. Eur. Clin. Microbiol. Infect. Dis. **12:**928–937.
9. **Hof, H., T. Nichterlein, and M. Kretschmar.** 1997. Management of listeriosis. Clin. Microbiol. Rev. **10:**345–357.
10. **Jonas, B. M., B. E. Murray, and G. M. Weinstock.** 2001. Characterization of *emeA*, a *norA* homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **45:**3574–3579.
11. **Jones, E. M., and A. P. MacGowan.** 1995. Antimicrobial chemotherapy of human infection due to *Listeria monocytogenes*. Eur. J. Clin. Microbiol. Infect. Dis. **14:**165–175.
12. **Koehler, T., and J.-C. Pechere.** 1998. Bacterial resistance to quinolones: mechanisms and clinical implications, p. 117–142. *In* V. T. Andriole (ed.), The quinolones, 2nd ed. Academic Press, Inc., San Diego, Calif.
13. **Lampidis, R., D. Kostrewa, and H. Hof.** 2002. Molecular characterization of the genes encoding DNA gyrase and topoisomerase IV of *Listeria monocytogenes*. J. Antimicrob. Chemother. **49:**917–924.
14. **Mata, M. T., F. Baquero, and J. C. Pérez-Diaz.** 2000. A multidrug efflux transporter in *Listeria monocytogenes*. FEMS Microbiol. Lett. **187:**185–188.
15. **Neyfakh, A. A., V. E. Bidnenko, and L. B. Chen.** 1991. Efflux-mediated multidrug resistance in *Bacillus subtilis*: similarities and dissimilarities with the mammalian system. Proc. Natl. Acad. Sci. USA **88:**4781–4785.

16. **Park, S. F., and G. S. Stewart.** 1990. High-efficiency transformation of *Listeria monocytogenes* by electroporation of penicillin-treated cells. Gene **94:** 129–132.
17. **Paulsen, I. T., M. H. Brown, and R. A. Skurray.** 1996. Proton-dependent multidrug efflux systems. Microbiol. Rev. **60:**575–608.
18. **Pinner, R. W., A. Schuchat, B. Swaminathan, P. S. Hayes, K. A. Deaver, R. E. Weaver, B. D. Plikaytis, M. Reeves, C. V. Broome, J. D. Wenger, and the Listeria Study Group.** 1992. Role of foods in sporadic listeriosis. II. Microbiologic and epidemiologic investigation. JAMA **267:**2046–2050.
19. **Poyart-Salmeron, C., C. Carlier, P. Trieu-Cuot, A.-L. Courtieu, and P. Courvalin.** 1990. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. Lancet **335:**1422–1426.
20. **Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, A. MacGowan, J. McLauchlin, and P. Courvalin.** 1992. Genetic basis of tetracycline resistance in clinical isolates of *Listeria monocytogenes*. Antimicrob. Agents Chemother. **36:**463–466.
21. **Putman, M., H. W. van Veen, and W. N. Konings.** 2000. Molecular properties of bacterial multidrug transporters. Microbiol. Mol. Biol. Rev. **64:**672–693.
22. **Rocourt, J.** 1990. *Listeria* et listériose humaine. Ann. Inst. Pasteur **1:**25–30.
23. **Seeliger, H. P. R., and K. Höhne.** 1979. Serotyping of *Listeria monocytogenes* and related species, p. 33–48. *In* T. Bergan and J. Norris (ed.), Methods in microbiology. Academic Press, New York, N.Y.
24. **Smith, K., and P. Yougman.** 1992. Use of a new integrational vector to investigate compartment-specific expression of the *Bacillus subtilis spoIIM* gene. Biochimie **74:**705–711.
25. **Steers, E., E. L. Foltz, B. S. Graves, and J. Riden.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antimicrob. Chemother. (Basel) **9:**307–311.
26. **Yoshida, H., M. Bogaki, S. Nakamura, K. Ubukata, and M. Konno.** 1990. Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. J. Bacteriol. **172:**6942–6949.