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Genetic Basis of Tetracycline Resistance in Clinical Isolates of *Listeria monocytogenes*

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The genetic basis of tetracycline resistance was studied in 25 clinical isolates of *Listeria monocytogenes*. Resistance to tetracycline was associated with resistance to minocycline and due to the presence of the *tet*(M) gene in 24 strains. Association of *tet*(M) with *int-Tn*, the gene encoding the protein required for the movements of Tn*1545*-like conjugative transposons, was found in all strains. Cotransfer of *tet*(M) and *int-Tn* among *L. monocytogenes* cells and from *L. monocytogenes* to *Enterococcus faecalis* was detected in 7 of the 12 strains studied at frequencies similar to those obtained with the prototype element Tn*1545*. *tet*(L), the secondmost prevalent tetracycline resistance gene in enterococci and streptococci, was detected in the remaining strain, where it was borne by a 5-kb plasmid. These observations indicate that two types of movable genetic elements, transposons and plasmids, in enterococci and streptococci are responsible for emergence of drug resistance in *L. monocytogenes*.

Listeria monocytogenes is a gram-positive opportunistic pathogen responsible for severe infections (septicemia, meningitis, and meningoencephalitis) primarily in immunocompromised hosts, the elderly, neonates, and fetuses. Infections caused by *L. monocytogenes* are likely to be food borne and the intestinal tract is the most initial probable site of invasion (16). Prognosis of listeriosis remains serious, and even with appropriate antibiotic therapy, listeriosis is fatal in approximately one-third of all cases (10). Most strains of *L. monocytogenes* are uniformly susceptible to antibiotics (18, 19). Acquired resistance to chloramphenicol, erythromycin, streptomycin, and tetracycline in two clinical isolates of *L. monocytogenes* has been reported (27, 30). It is likely that the emergence of multiple-antibiotic resistance in this species results from acquisition of self-transferable plasmids originating in the genera *Enterococcus* and *Streptococcus* (27). Recently, the involvement of enterococcal and streptococcal Tn*1545*-like conjugative transposons in the acquisition of antibiotic resistance by *L. monocytogenes* was anticipated (7). All these transposons contain the tetracycline resistance (Tc^r) gene *tet*(M) alone (Tn*916*, Tn*918*, Tn*919*, and Tn*925*) or associated with other resistance genes (Tn*1545*) (3). These elements possess a broad host range of conjugal transposition and are responsible for acquired antibiotic resistance in *Streptococcus pneumoniae* (5, 29). We have demonstrated that a transposon-encoded protein designated Int-Tn is essential for transposition and conjugation of Tn*1545* and related elements (28, 37). By using probes specific for *int-Tn* (the gene encoding the integrase of Tn*1545* [28] and Tn*916* [4]), and *tet*(M) and dot blot hybridization, we have studied the distribution of this class of transposons and of this resistance gene in clinical isolates of *L. monocytogenes* resistant to tetracycline. The distribution of three other Tc^r determinants [*tet*(K), *tet*(L), and *tet*(O)] already found in gram-positive bacteria was also studied.

MATERIALS AND METHODS

Bacterial strains. Strains from 1,288 cases of human listeriosis which had occurred in the United Kingdom were collected in the Division of Microbiological Reagents and Quality Control of the Central Public Health Laboratory. The isolates were identified by using the following criteria: gram strain positive; motile at room temperature; hemolytic on horse blood agar; catalase positive; oxidase negative; Voges-Proskauer positive; urease negative; esculin positive; no reduction of nitrates to nitrites; production of acid but not gas from D-glucose; and production of acid from D-salicin, L-rhamnose, and α-methyl-D-mannitol but not from D-mannitol or D-xylose. All strains were serotyped in the Division of Microbiological Reagents and Quality Control. *L. monocytogenes* L017RF (26), resistant to fusidic acid and rifampin, *L. monocytogenes* EGDSmR (9), and *Enterococcus faecalis* BM4110 (5), both resistant to streptomycin, were used as recipients in conjugation experiments. *Escherichia coli* JM83 (22) was used in transformation experiments.

Media. Brain heart infusion (BHI) broth and agar (Difco Laboratories, Detroit, Mich.) were used for bacterial cultures. Sensitivity tests were done on Mueller-Hinton agar (Diagnostics Pasteur, Marnes-la-Coquette, France). All incubations were at 37°C.

Antibiotic susceptibility testing. Susceptibility to antibiotics was determined by either agar diffusion with disks impregnated with antibiotics (Diagnostics Pasteur) or a breakpoint multipoint inoculation technique. The method of Steers et al. (36) with 10^4 CFU per spot was used to determine the MICs of antibiotics.

Genetic techniques. Mating on filters was performed as described previously (38), and transfer frequencies were expressed as the number of transconjugants per donor CFU after the mating period. Transformation of *E. coli* (34) and

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TABLE 1. Probes used for hybridization

Probe	Reference
tet(K) (870-bp *Hin*cII fragment of pT181)	Khan and Novick (15)
tet(L) (310-bp *Cla*I-*Hpa*II fragment of pBC16)	Hoshino et al. (13)
tet(M) (850-bp *Cla*I-*Hin*dIII fragment of Tn*1545*)	Martin et al. (20)
tet(O) (1,458-bp *Hin*dIII-*Nde*I fragment of pIP1433)	Sougakoff et al. (35)
int-Tn (830-bp *Taq*I fragment of Tn*1545*)	Poyart-Salmeron et al. (28)
inlA (3.3-kb *Hin*cII chromosomal DNA fragment of *L. monocytogenes* EGDSmR)	Gaillard et al. (8)

electrotransformation of *E. faecalis* (6) were performed as described previously. The antibiotics and concentrations used for selection of transcipients were as follows: fusidic acid (Leo, Montigny-Le-Bretonneux, France), 10 μg/ml; rifampin (Merell Dow, Neuilly-sur-Seine, France), 20 μg/ml; streptomycin (Pfizer, Paris-la-Défense, France), 500 and 1,000 μg/ml of *L. monocytogenes* EGDSmR and *E. faecalis* BM4110, respectively; and tetracycline (Rhône-Poulenc, Vitry, France), 8 μg/ml.

Preparation of DNA. Total DNA from *Listeria* and *Enterococcus* cells was prepared as follows: cells from 5 ml of a culture grown overnight were harvested, suspended in 1 ml of TE buffer (0.01 M Tris-hydrochloride [pH 8.0], 0.001 M EDTA) containing 25% sucrose and lysozyme (25 mg/liter), and incubated at 37°C for 1 h. The resulting protoplasts were lysed by phenol-chloroform extraction. Total DNA was recovered from the supernatant by ethanol precipitation and dissolved in 100 μl of TE buffer. The presence of DNA was monitored by agarose gel electrophoresis. Aliquots (10 μl) were denatured for 10 min at 100°C and spotted on Nytran membranes (Amersham S.A., Les Ulis, France). Purification of plasmid DNA from *L. monocytogenes* (27) and *E. faecalis* (5) by ultracentrifugation in cesium chloride-ethidium bromide was performed as described previously. Plasmids from *E. coli* were purified by rapid alkaline lysis as described previously (34).

DNA-DNA hybridization. The probes used for hybridization experiments are listed in Table 1. Fragments were extracted from low-gelling-temperature agarose type VII (Sigma S.A., La Verpillière, France) as described previously (34). Purified restriction endonuclease-generated DNA fragments were labeled with [α-^{32}P]dCTP (Amersham S.A.) by nick translation (34). Dot blot hybridization under stringent conditions was performed as follows: prehybridization and hybridization for 5 and 18 h, respectively, at 65°C in a solution consisting of 6× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate (SDS), and 0.05% nonfat dry milk, two washings in 2× SSC–0.1% SDS at room temperature for 30 min each, and two washings in 0.2× SSC–0.1% SDS at 68°C for 45 min. Hybridization under low-stringency conditions was done in 6× SSC–0.5% SDS–0.05% nonfat dry milk at 37°C for 18 h, and then the probes were washed twice in 2× SSC–0.1% SDS at 45°C for 1 h. In dot blot experiments, self-hybridization of the probe and hybridization of the probe with total DNA from *L. monocytogenes* L017RF were used as positive and negative controls, respectively.

RESULTS

Antibiotic sensitivity testing and strain characteristics. Thirty-three (2.5%) of the 1,288 isolates of *L. monocytogenes* collected from cases occurring over the time periods 1967 to 1981, 1983 to 1986, and 1987 to 1990 were found to be resistant to tetracycline (Tc^r). The numbers of cases yielding strains resistant to tetracycline for these time periods were 13 of 265 (5%), 7 of 405 (2%), and 13 of 618 (2%), respectively. The results of antibiotic susceptibilities for the final group of strains (1987 to 1990) have been published previously (19). There was no obvious temporal or geographical clustering of cases due to the Tc^r *L. monocytogenes*. Twenty-five of the Tc^r strains were selected for further study. One strain was serovar 1/2a, one strain was serovar 1/2c, and the remaining 23 were serovar 4b, which is the most common cause of human listeriosis (21). Twenty-four of these strains were resistant to both tetracycline (MIC, 64 to 128 μg/ml) and minocycline (MIC > 4 μg/ml) (Tc^r Mc^r) (24). The remaining strain was resistant to tetracycline (MIC, 64 μg/ml) only (Tc^r Mc^s). The strains were uniformly susceptible to ampicillin, chloramphenicol, cotrimoxazole, erythromycin, gentamicin, imipenem, kanamycin, penicillin, rifampin, streptomycin, and vancomycin (24).

Hybridization. Five classes of tetracycline resistance determinants, *tet*(K), *tet*(L), *tet*(M), *tet*(O), and *tet*(P), have been detected in gram-positive bacteria (17). We tested the listerial strains for the presence of nucleotide sequences that were structurally related to *int-Tn*, *tet*(K), *tet*(L), *tet*(M), and *tet*(O) by dot blot hybridization using intragenic probes. The *tet*(P) gene, which is apparently specific for the anaerobic species *Clostridium perfringens* (1), was not included in this study. Homology with *tet*(M) and *int-Tn* was detected in the 24 Tc^r Mc^r strains and the Tc^r Mc^s strain, BM4212, hybridized with *tet*(L) only (data not shown). None of the probes hybridized with the susceptible strain *L. monocytogenes* L017RF. The presence of DNA on the membranes was assessed by using as a probe the 3.3-kb *Hin*cII fragment containing the gene encoding InlA, a protein involved in the entry of *L. monocytogenes* into epithelial cells (8).

Genetic support of Tc^r in *L. monocytogenes*. The transferability of Tc^r Mc^r from 12 strains of *L. monocytogenes* containing *tet*(M) was tested by filter mating. Seven strains transferred the resistance determinant to plasmid-free strains *L. monocytogenes* L017RF and *E. faecalis* BM4110 at frequencies of 10^{-5} and 10^{-6}, respectively. *E. faecalis* BM4110 transconjugants corresponding to the seven donors were able to retransfer Tc^r Mc^r to *L. monocytogenes* L017RF at frequencies ranging from 10^{-6} to 10^{-7}. Total DNA extracted from *L. monocytogenes* and *E. faecalis* transconjugants hybridized with *tet*(M) and *int-Tn* and no plasmid DNA was detected in these strains by ultracentrifugation in cesium chloride-ethidium bromide and by agarose gel electrophoresis of crude bacterial lysates (data not shown).

L. monocytogenes BM4212 containing *tet*(L) transferred tetracycline resistance by conjugation to *L. monocytogenes* L017RF at a frequency of 1.6×10^{-8} but not to *E. faecalis* BM4110 ($<10^{-9}$). A transconjugant, BM4213, was selected for further studies. Retransfer of Tc^r from BM4213 to *L. monocytogenes* EGDSmR and to *E. faecalis* BM4110 was not detected ($<10^{-9}$). Plasmid DNA of strains BM4212 and BM4213 was purified by ultracentrifugation in cesium chloride-ethidium bromide and electrophoresed after digestion with *Eco*RI and *Hin*dIII endonucleases (Fig. 1). Comparative analysis revealed that two plasmids designated pIP812

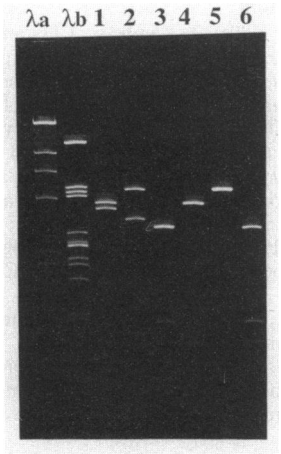

FIG. 1. Analysis of plasmid DNA from *L. monocytogenes* BM4212 (lanes 1 to 3) and BM4213 (lanes 4 to 6) by agarose (0.8%) gel electrophoresis. Plasmid DNA (2 to 3 μg) was digested with *Eco*RI (lanes 1 and 4), *Hin*dIII (lanes 2 and 5), or *Eco*RI plus *Hin*dIII (lanes 3 and 6). Bacteriophage λ DNA digested by *Hin*dIII (λa) and *Pst*I (λb) was used as a molecular size standard.

and pIP813 were present in *L. monocytogenes* BM4212, whereas pIP813 was detected only in BM4213. The molecular sizes of pIP812 and pIP813 were estimated to be 4 and 5 kb, respectively. Nucleotide sequences homologous to *tet*(L) were detected in pIP813 DNA by dot blot hybridization (data not shown), whereas pIP812 remained cryptic. Plasmid pIP813 was introduced by electrotransformation in *E. faecalis* BM4110 and by transformation in *E. coli* JM83, where it was able to replicate and confer resistance to tetracycline. Taken together, these results indicate that the *tet*(L) gene of BM4212 is carried by pIP813. The failure to transfer this plasmid by mating from *L. monocytogenes* BM4213 to *L. monocytogenes* EGDSmR indicate that it is not self-transferable. Therefore, it is likely that transfer of pIP813 from the wild-type strain *L. monocytogenes* BM4212 to *L. monocytogenes* L017RF occurred by mobilization, although we did not detect any large, potentially conjugative, plasmid in the donor.

DISCUSSION

We have studied the genetic basis of tetracycline resistance in 25 strains of *L. monocytogenes* isolated in the United Kingdom from human listeriosis between 1967 and 1990 (18, 19). Among these strains, 24 were Tc^r Mc^r and contained sequences related to *tet*(M). The remaining strain, BM4212, was Tc^r Mc^s and harbored *tet*(L).

The *tet*(M) gene is common in enterococci and streptococci (39) and has also been found in *Staphylococcus* spp. (2), *Clostridium difficile* (12, 23), cell wall-less bacteria (32), and gram-negative pathogens (33). Dissemination of this resistance gene may reflect the fact that it is carried by broad-host-range conjugative transposons such as Tn*1545* and Tn*916*. Our results suggest that the presence of *tet*(M) in *L. monocytogenes* is secondary to acquisition of Tn*1545*-like elements. This proposal is based on (i) the association, in all the Tc^r Mc^r strains, of *tet*(M) with *int-Tn*, the gene encoding the protein required for the movements of this class of transposons, and (ii) the cotransfer of *tet*(M) and *int-Tn* among *L. monocytogenes* cells and from *L. monocytogenes* to *E. faecalis* at frequencies similar to those of the prototype element Tn*1545* (5; also unpublished results). The failure to transfer *tet*(M) in 5 of the 12 strains studied may result from rearrangements in genes or *cis*-active sequences essential for conjugal transposition. Derivatives of Tn*916* defective for conjugation have been found in *Gardnerella vaginalis* and in commensal *Neisseria* spp. (33). Since these transposons are rare in staphylococci (29) but are widespread in enterococci and streptococci (39), it is therefore likely that their presence in *L. monocytogenes* is due to acquisition of genetic information originating in enterococci and streptococci. It has been shown that the transfer frequency of Tn*1545* from *E. faecalis* to *L. monocytogenes* can be significantly enhanced, both in vitro and in vivo, by subinhibitory concentrations of tetracycline in the mating medium (7). It is therefore tempting to speculate that the therapeutic use of tetracycline has favored the dissemination of this class of transposons and hence of Tc^r in and among *L. monocytogenes* strains.

In *L. monocytogenes* BM4212, *tet*(L) is carried by a 5-kb plasmid designated pIP813. In gram-positive bacteria, this determinant is frequently borne by small (<10 kb) and interrelated plasmids that replicate by using a single-stranded DNA intermediate (11). These plasmids generally possess a broad host range of replication which includes gram-positive and gram-negative bacteria. The fact that pIP813 can replicate in *L. monocytogenes*, *E. faecalis*, and *E. coli* suggests that it belongs to this plasmid family. As a consequence of their mode of replication, these replicons are highly recombinogenic and this might favor their intra- and intergeneric dissemination (11). The presence of *tet*(L) on this type of plasmids among evolutionarily distant gram-positive bacteria such as *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Bacillus*, and *Listeria* spp. is consistent with this notion. *tet*(L) is the second most prevalent Tc^r gene in enterococci and streptococci but is rarely detected in staphylococci (39). It is therefore likely that pIP813 also originated in *Enterococcus* and *Streptococcus* species.

The results presented here, combined with a previous report (27), indicate that two types of movable genetic elements (plasmids and transposons) in enterococci and streptococci are responsible for the emergence of drug resistance in *L. monocytogenes*. *L. monocytogenes* is a frequent inhabitant of the gastrointestinal tracts of humans and animals (14) where various *Enterococcus* and *Streptococcus* species harboring conjugative resistance plasmids or transposons are also common. The demonstration that direct exchange of genetic information from *E. faecalis* to *L. monocytogenes* can occur in the digestive tracts of animals (7) is additional support for the notion that the intestinal ecosystem is the most probable site for intergeneric transfer between these two bacterial genera. The treatment of choice for listeriosis is benzylpenicillin or ampicillin combined with gentamicin. *E. faecalis* strains that harbor conjugative plasmids or transposons encoding β-lactamases and enzymes conferring high-level resistance to gentamicin have been described (25, 31). Acquisition by *L. monocytogenes* of resistance to these antibiotics can thus be predicted.

REFERENCES

1. **Abraham, L. J., D. I. Berryman, and J. I. Rood.** 1988. Hybridization analysis of the class P tetracycline resistance determinant from the *Clostridium perfringens* R-plasmid, pCW3. Plasmid **19**:113–120.
2. **Bismuth, R., R. Zilhao, H. Sakamoto, J. L. Guesdon, and P. Courvalin.** 1990. Gene heterogeneity for tetracycline resistance in *Staphylococcus* spp. Antimicrob. Agents Chemother. **34**: 1611–1614.
3. **Clewell, D. B.** 1990. Movable genetic elements and antibiotic

resistance in enterococci. Eur. J. Clin. Microbiol. Infect. Dis. 9:90–102.
4. **Clewell, D. B., S. E. Flannagan, L. A. Zitow, Y. A. Su, P. He, E. Senghas, and K. Weaver.** 1991. Properties of conjugative transposon Tn*916*, p. 39–44. *In* G. M. Dunny, P. P. Cleary, and L. L. McKay (ed.), Genetics and molecular biology of streptococci, lactococci, and enterococci. American Society for Microbiology, Washington, D.C.
5. **Courvalin, P., and C. Carlier.** 1986. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae*. Mol. Gen. Genet. **205**:291–297.
6. **Cruz-Rodz, A. L., and M. S. Gilmore.** 1990. High efficiency introduction of plasmid DNA into glycine treated *Enterococcus faecalis* by electroporation. Mol. Gen. Genet. **224**:152–154.
7. **Doucet-Populaire, F., P. Trieu-Cuot, I. Dosbaa, A. Andremont, and P. Courvalin.** 1991. Inducible transfer of conjugative transposon Tn*1545* from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts of gnotobiotic mice. Antimicrob. Agents Chemother. **35**:185–187.
8. **Gaillard, J. L., P. Berche, C. Frehel, E. Gouin, and P. Cossart.** 1991. Entry of *L. monocytogenes* into cells is mediated by a repeat protein analogous to surface antigens from Gram-positive, extracellular pathogens. Cell **65**:1–20.
9. **Gaillard, J. L., P. Berche, and P. Sansonetti.** 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. Infect. Immun. **52**:50–55.
10. **Gellin, B. G., and C. V. Broome.** 1989. Listeriosis. JAMA **261**:1313–1320.
11. **Gruss, A., and S. D. Ehrlich.** 1989. The family of highly interrelated single-stranded deoxyribonucleic acid plasmids. Microbiol. Rev. **53**:231–241.
12. **Hächler, H., F. H. Kayser, and B. Berger-Bächi.** 1987. Homology of a tetracycline resistance determinant of *Clostridium difficile* with *Streptococcus* (*Enterococcus*) *faecalis* transposon Tn*916*. Antimicrob. Agents Chemother. **31**:1033–1038.
13. **Hoshino, T., T. Ikeda, N. Tomizuka, and K. Furukawa.** 1985. Nucleotide sequence of the tetracycline resistance gene of pTH15, a thermophilic *Bacillus* plasmid: comparison with staphylococcal Tc^r controls. Gene **37**:131–138.
14. **Kampelmacher, E. H., and L. M. Van Noorle Jansen.** 1969. Isolation of *Listeria monocytogenes* from faeces of clinically healthy humans and animals. Zentralbl. Bakteriol. I. Abt. Orig. **211**:353–359.
15. **Khan, S. A., and R. P. Novick.** 1983. Complete nucleotide sequence of pT181: a tetracycline resistance plasmid from *Staphylococcus aureus*. Plasmid **10**:251–259.
16. **Kvenberg, J. E.** 1988. Outbreaks of listeriosis/*Listeria*-contaminated foods. Microbiol. Sci. **5**:355–358.
17. **Levy, S. B.** 1988. Tetracycline resistance determinants are widespread. ASM News **54**:418–421.
18. **MacGowan, A. P., H. A. Holt, M. J. Bywater, and D. S. Reeves.** 1990. *In vitro* antibiotic susceptibility of *Listeria monocytogenes* isolated in the UK and other *Listeria* species. Eur. J. Clin. Microbiol. Infect. Dis. **9**:767–770.
19. **MacGowan, A., D. S. Reeves, and J. McLauchlin.** 1990. Antibiotic resistance of *Listeria monocytogenes*. Lancet **336**:513–514.
20. **Martin, P., P. Trieu-Cuot, and P. Courvalin.** 1986. Nucleotide sequence of the *tetM* tetracycline determinant of the streptococcal conjugative shuttle transposon Tn*1545*. Nucleic Acids Res. **14**:7047–7058.
21. **McLauchlin, J.** 1990. Distribution of serovars of *Listeria monocytogenes* isolated from different categories of patients with listeriosis. Eur. J. Clin. Microbiol. Infect. Dis. **9**:210–213.
22. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
23. **Mullany, P., M. Wilks, I. Lamb, C. Clayton, B. Wren, and S. Tabaqchali.** 1990. Genetic analysis of a tetracycline resistance element from *Clostridium difficile* and its conjugal transfer to and from *Bacillus subtilis*. J. Gen. Microbiol. **136**:1343–1349.
24. **National Committee for Clinical Laboratory Standards.** 1984. Approved standards: performance standards for antimicrobial disk susceptibility tests (M2-A3), 3rd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
25. **Patterson, J. E., A. Wanger, K. K. Zscheck, M. J. Zervos, and B. E. Murray.** 1990. Molecular epidemiology of β-lactamase-producing enterococci. Antimicrob. Agents Chemother. **34**:302–305.
26. **Pérez-Diaz, J. C., M. F. Vicente, and F. Baquero.** 1982. Plasmids in *Listeria*. Plasmid **8**:112–118.
27. **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.
28. **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 Tn*1545*: homologies with other site-specific recombinases. EMBO J. **8**:2425–2433.
29. **Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, and P. Courvalin.** 1991. Nucleotide sequences specific for Tn*1545*-like conjugative transposons in pneumococci and staphylococci resistant to tetracycline. Antimicrob. Agents Chemother. **35**:1657–1660.
30. **Quentin, C., M. C. Thibaut, J. Horovitz, and C. Bébéar.** 1990. Multiresistant strain of *Listeria monocytogenes* in septic abortion. Lancet **336**:375.
31. **Rice, L. B., G. M. Eliopoulos, C. Wennersten, D. Goldmann, G. A. Jacoby, and R. C. Moellering.** 1991. Chromosomally mediated β-lactamase production and gentamicin resistance in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **35**:272–276.
32. **Roberts, M. C.** 1990. Characterization of the Tet M determinants in urogenital and respiratory bacteria. Antimicrob. Agents Chemother. **34**:476–478.
33. **Roberts, M. C., S. L. Hillier, J. Hale, K. K. Holmes, and G. Kenny.** 1986. Tetracycline resistance and *tetM* in pathogenic urogenital bacteria. Antimicrob. Agents Chemother. **30**:810–812.
34. **Sambrook, J., F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Sougakoff, W., B. Papadopoulou, P. Nordmann, and P. Courvalin.** 1987. Nucleotide sequence and distribution of the gene *tetO* encoding tetracycline resistance in *Campylobacter coli*. FEMS Microbiol. Lett. **44**:153–159.
36. **Steers, E., E. L. Foltz, B. S. Graves, and J. Riden.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. (Basel) **9**:307–311.
37. **Storrs, M. J., C. Poyart-Salmeron, P. Trieu-Cuot, and P. Courvalin.** 1991. Conjugative transposition of Tn*916* requires the excisive and integrative activities of the transposon-encoded integrase. J. Bacteriol. **173**:4347–4352.
38. **Trieu-Cuot, P., C. Carlier, and P. Courvalin.** 1988. Conjugative plasmid transfer from *Enterococcus faecalis* to *Escherichia coli*. J. Bacteriol. **170**:4388–4391.
39. **Zilhao, R., B. Papadopoulou, and P. Courvalin.** 1988. Occurrence of the *Campylobacter* resistance gene *tetO* in *Enterococcus* and *Streptococcus* spp. Antimicrob. Agents Chemother. **32**:1793–1796.