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Transferable Erythromycin Resistance in *Listeria* spp. Isolated from Food

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An erythromycin-resistant (Em^r) *Listeria innocua* and an Em^r *Listeria monocytogenes* isolate both carried *ermC* genes, which code for rRNA methylases. The *ermC* genes were transferable by conjugation to recipient *L. monocytogenes*, *Listeria ivanovii*, and *Enterococcus faecalis* but did not appear to be associated with conjugative plasmids.

Listeria species are widespread in the environment and the intestines of humans and animals and can also be found in food (1, 4, 6, 11). Until recently, the genus was thought to be uniformly susceptible to antibiotics active against gram-positive bacteria. Penicillin is normally the drug of choice for treatment, but both erythromycin and tetracycline are alternatives for patients who are allergic to penicillin (8). Now both singly and multiply antibiotic-resistant listerias have been described (3, 4, 6, 7, 9, 11, 13). We (7) as well as others (4, 9, 11) have found tetracycline resistance due to the Tet M determinant in *Listeria innocua*, *Listeria monocytogenes*, and, more recently, *Listeria welshimeri* from food, the environment, and human disease. The Tet K and L determinants have been found in a few *L. innocua* isolates from food (7), while the Tet S determinant has been found in *L. monocytogenes*, *L. innocua*, and *L. welshimeri* (3, 4). Multiantibiotic resistance plasmids encoding chloramphenicol (*cat*221/*cat*223), macrolide/lincosamide/streptogramin (MLS) (*ermB*), and tetracycline resistance (*tetM*) or chloramphenicol, MLS, streptomycin, and tetracycline resistance have been found in *L. monocytogenes* from both France and Switzerland (9). Recently, trimethoprim and streptomycin resistance has also been found in the genus (4). One streptomycin-resistant *L. innocua* strain carried a streptomycin nucleotidyltransferase related to the *aad6* gene (4). These reports illustrate that more listerias are becoming antibiotic resistant by the acquisition of known gram-positive antibiotic resistance genes.

We have been examining antibiotic resistance genes in listerias isolated from food (6, 7). Previously, we found that 11 of 12 *L. innocua* isolates from chicken or turkey frankfurters and mozzarella cheese were resistant to tetracycline and carried the *tetM* gene (6, 7). In the same studies, we identified one *L. innocua* and one *L. monocytogenes* strain which were resistant to erythromycin (MIC, >256 μg/ml). Neither was tetracycline resistant. The *L. monocytogenes* isolate carried two small plasmids of roughly 3 and 7 kb. In this study on erythromycin determinants, Southern blots of purified total cell DNA (7, 10) were hybridized under stringent conditions with each of the following intragenic Erm probes: Erm A, pEM9592, 0.7-kb *Ssp*I; Erm B, pJIR229, 0.8-kb *Pst*I-*Eco*RI; Erm C, pBR328:33RV, 0.9-kb *Hpa*I (15–17). We found that both isolates hybridized with the Erm C determinant but not Erm A or Erm B. The hybridization was with the chromosomal band and not with the small *L. monocytogenes* plasmids (data not shown). The Erm C determinant has not previously been reported in listerias but is common in other gram-positive species and some gram-negative species (14–17). The presence of the *ermC* gene was confirmed by a PCR assay as previously described (2) followed by hybridization of the PCR product with labeled DNA carrying the *ermC* gene (15). Figure 1 shows a representative agarose gel with PCR products (Fig. 1A) and hybridization of these PCR products with the Erm C probe (Fig. 1B). The PCR products hybridized with the Erm C probe but not with Erm A or Erm B probes.

The *ermC* gene has been shown to be associated with both mobile plasmids and transposons and has been transferred between a variety of species (14, 16). Multidrug-resistant listerial plasmids carrying the *ermB* gene and the conjugative transposon Tn*1545* carrying the *ermB* gene have been transferred into and out of *L. monocytogenes* isolates (5, 12). Therefore, it was of interest to determine if these *ermC* genes from *L. innocua* 38p and *L. monocytogenes* 119A were transferable by conjugation. In our study, we used the two Em^r donors and the following recipients: *L. monocytogenes* ATCC 984, *L. ivanovii* CIP 7842, and *Enterococcus faecalis* JH2-2 (7, 15, 16) (Table 1). All three recipients were susceptible to erythromycin and resistant to rifampin (10 μg/ml) and fusidic acid (10 μg/ml), as previously described (7, 15), and did not hybridize with the *ermC* gene. We did matings on plates and selected for transfer of the erythromycin-resistant (Em^r) phenotype as previously described (7, 15). However, the two donors were not first grown in subinhibitory concentrations of erythromycin, as was needed when tetracycline resistance was transferred (7). After overnight incubation, the mating mixtures were plated onto agar plates supplemented with erythromycin (10 μg/ml) and fusidic acid (10 μg/ml) or with erythromycin (10 μg/ml) and rifampin (10 μg/ml). Transconjugants were verified by determining if they were resistant to rifampin, fusidic acid, and erythromycin as previously described (7, 15, 16). The *E. faecalis* transconjugants were confirmed with a chromosomal DNA probe (15, 16).

No plasmids were seen in the transconjugants. The frequency of transfer, expressed as the number of transconjugants per recipient, was low when the JH2-2 recipient was used (10^{-8} to 10^{-9} per recipient) but was similar to what we found with

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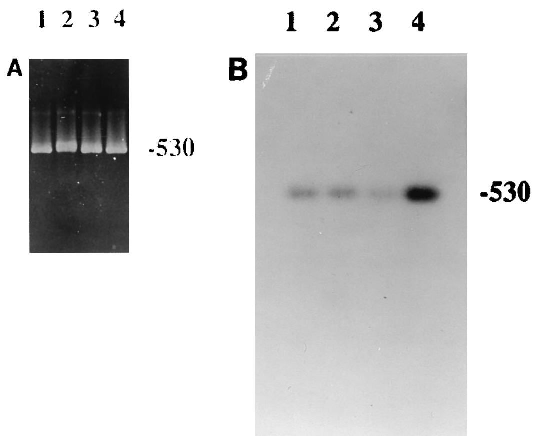

FIG. 1. (A) PCR products run on a 1.5% agarose gel visualized with ethidium bromide. (B) Autoradiogram of the gel in panel A hybridized with labeled plasmid pBR328:33RV. Lane 1, *L. innocua* 38p; lane 2, *L. monocytogenes* 119A; lane 3, *E. faecalis* transconjugant from mating between *E. faecalis* and *L. innocua* 38p donor; lane 4, plasmid pBR328:22RV carrying the *ermC* gene. The size is indicated in base pairs.

transfer of *tetM* from *L. innocua* to JH2-2 (7). With the two *Listeria* recipients (*L. monocytogenes* and *L. ivanovii*), the frequency of transfer was 10- to 100-fold higher (10^{-6} to 10^{-8} per recipient). All the transconjugants were resistant to erythromycin (MIC, $\geq$256 μg/ml) and hybridized with the *ermC* gene by Southern blot hybridization of the chromosomal band with total whole-cell transconjugant DNA (Table 1 and Fig. 1). We confirmed the presence of *ermC* by PCR and by hybridization of the PCR products (Fig. 1). No plasmids were seen in the transconjugants, suggesting a chromosomal location of the *ermC* gene.

Recently, one other Em^r *L. innocua* and two Em^r *L. monocytogenes* isolates have been identified and shown to transfer their *ermC* genes at frequencies similar to those of the first two isolates described to the same three recipients without pregrowth in subinhibitory concentrations of erythromycin. Now a total of five *Listeria* strains with the *ermC* gene isolated from food have been identified. Each has been able to act as a donor of the *ermC* gene in mating experiments with both listerial and enterococcal recipients. This differs from what we found with the *tetM* gene from this group of listerias (7); only 1 of 10 *L. innocua* isolates examined could transfer the *tetM* gene, and that was only after the donor had been grown in low doses of tetracycline. We have determined that this isolate carries the complete Tet M transposon, while the other nine isolates have incomplete Tet M transposons and lacked both ends of the transposon, which are needed for conjugation (unpublished observation). Similar findings of incomplete transposons, defined as no hybridization with the *int-Tn* probe, have been reported for *L. innocua* and *L. monocytogenes* isolates from both food and the environment (4).

TABLE 1. Distribution of Erm determinants in *L. innocua* and *L. monocytogenes* and their transconjugants

Donor	Recipient	Donor determinant	Transconjugant determinant
L. innocua 38p	*E. faecalis* JH2-2	Erm C	Erm C
	L. monocytogenes ATCC 984	Erm C	Erm C
	L. ivanovii CIP 7842	Erm C	Erm C
L. monocytogenes 119A	*E. faecalis* JH2-2	Erm C	Erm C
	L. monocytogenes ATCC 984	Erm C	Erm C
	L. ivanovii CIP 7842	Erm C	Erm C

Listerias are a common food contaminant and have clearly begun to acquire a number of different antibiotic resistance genes, many of which are associated with conjugative elements. Erythromycin resistance due to the presence of the *ermB* gene on multiresistant conjugative plasmids has been described for *L. monocytogenes* (4, 9). In this report, we found food isolates of both *L. monocytogenes* and *L. innocua* with conjugative *ermC* genes which do not appear to be associated with plasmids, making this the second mobile rRNA methylase gene to be described in listerias. As antibiotic-resistant listerias in food become more common, it is increasingly important that listerial isolates from food be monitored for antibiotic resistance and for the appearance of new antibiotic resistance phenotypes.

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