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Distribution of Erythromycin Esterase and rRNA Methylase Genes in Members of the Family *Enterobacteriaceae* Highly Resistant to Erythromycin

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The distribution of nucleotide sequences related to *ereA*, *ereB*, and *ermAM* was studied by colony hybridization in 112 strains of members of the family *Enterobacteriaceae* that are highly resistant to erythromycin. The *ereA* and *ereB* genes encoding erythromycin esterases type I and II, respectively, were detected in strains inactivating the 14-membered macrolides erythromycin and oleandomycin. Because all 52 strains resisting these antibiotics by inactivation were detected by *ereA* ($n = 23$), *ereB* ($n = 23$), or both probes ($n = 6$), only two classes of genes accounted for this resistance phenotype. The *ermAM* gene encoding a streptococcal rRNA methylase was detected in 21 strains of *Escherichia coli* and two strains of *Klebsiella* spp. Determination of the MICs of macrolide, lincosamide, and streptogramin (MLS) antibiotics demonstrated a correlation between hybridization with *ermAM* and the so-called MLS resistance phenotype. The presence of 11 strains coresistant to MLS antibiotics that did not hybridize to the *ermAM* probe suggests that, as in gram-positive organisms, MLS resistance in members of the family *Enterobacteriaceae* involves more than one class of rRNA methylase. Numerous strains ($n = 18$) were found to produce both an erythromycin esterase type II and an rRNA methylase. Physical linkage between *ereB* and *ermAM* may be responsible for the codissemination of the genes. Despite their exogenous origin, *ereB* and *ermAM* are already disseminated in various genera of the *Enterobacteriaceae*.

Macrolide, lincosamide, and streptogramin (MLS) antibiotics are mainly active against gram-positive bacteria. Acquired resistance to MLS antibiotics has been extensively studied in these bacteria and is generally due to N^6-dimethylation of a specific adenine residue in 23S rRNA (28). The modified ribosome binds the MLS antibiotics less efficiently, thus producing coresistance to these chemically dissimilar 50S subunit inhibitors (for a review, see reference 30). Members of the family *Enterobacteriaceae*, like most gram-negative organisms, are intrinsically resistant to low levels of erythromycin ($2 \leq$ MIC ≤ 250 μg/ml), probably by impermeability (23, 27). Higher local antibiotic concentrations (0.5 to 6 mg/g of feces), however, are obtained in the lumen of the intestinal tract after oral absorption of usually recommended therapeutic doses (3). The subsequent reduction of the aerobic gram-negative flora of the intestinal tract has recently found therapeutic applications (2, 3). *Enterobacteriaceae* that are highly resistant to erythromycin (MIC, ≥500 μg/ml) can be isolated, usually after prior intake of the drug (2). *Escherichia coli* BM2195 (1) is resistant to high levels of erythromycin (MIC, 4,000 μg/ml) by synthesis of a plasmid-mediated erythromycin esterase type I which hydrolyzes the lactone ring of the 14-membered macrolides erythromycin and oleandomycin (7). A preliminary study of the distribution of the *ereA* gene encoding erythromycin esterase type I (22) in *Enterobacteriaceae* indicated polymorphism of the genes specifying an erythromycin modifying activity (4). We recently described *E. coli* BM2570, the first gram-negative clinical isolate that has been found to be resistant to high levels of MLS antibiotics (6). Two genes, *ereB* and *erxA* of plasmid pIP1527, contribute cooperatively to erythromycin resistance of BM2570 by two different mechanisms. The gene *ereB* encodes erythromycin esterase type II (5). Comparison of the amino acid sequences of erythromycin esterases types I and II deduced from the nucleotide sequences of *ereA* and *ereB* indicated the absence of statistically significant homology between these two isozymes. Analysis of the nucleotide sequence of *ereB* suggested that, as opposed to *ereA*, this gene should be exogenous to *E. coli* (5). The nucleotide sequence of *erxA*, which confers resistance to MLS antibiotics in BM2570, is nearly identical (homology greater than 98%) to the sequence of *ermAM*, which encodes an rRNA methylase in gram-positive cocci (P. Trieu-Cuot, M. Arthur, and P. Courvalin, *in* R. Curtiss III and J. J. Ferretti, ed., *Genetics of Streptococci*, in press; A. Brisson-Noël, M. Arthur, and P. Courvalin, manuscript in preparation). Gene *erxA* therefore encodes an rRNA methylase, belongs to the *ermAM* class of hybridization, and is designated *ermAM*. Preferential codon usage in the *ermAM* class of genes displays a strong bias in favor of codons that are rich in adenine and thymine (A+T). This codon usage differs from that of *E. coli* genes and is characteristic of microorganisms with an A+T-rich genome, such as gram-positive cocci. Because *ermAM* is widely spread in gram-positive cocci, *E. coli* has probably acquired this gene from *Streptococcus* spp. or *Staphylococcus* spp. (Trieu-Cuot et al., in press; Brisson-Noël et al., in preparation). Although *ereB* has not yet been detected in gram-positive organisms, physical linkage of *ereB* and *ermAM* on pIP1527 (6) and similarities of codon usage in both genes (5; Trieu-Cuot et al., in press; Brisson-Noël et al., in preparation) suggest that both genes originated in gram-positive bacteria. It was the purpose of this investiga-

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TABLE 1. Properties of the *Enterobacteriaceae* that are highly resistant to erythromycin

Species	No. of strains (%)[a]:				
	Inactivating erythromycin	Hybridizing with the following probes:			Total
		ereA	*ereB*	*ermAM*	
Escherichia coli	45[b] (40)	26 (23)	25 (22)	21 (19)	69 (62)
Klebsiella spp.	4 (4)	1 (1)	3 (3)	2 (2)	17 (15)
Citrobacter spp.	1 (1)	1 (1)	0 (0)	0 (0)	9 (8)
Proteus spp.	1 (1)	0 (0)	1 (1)	0 (0)	9 (8)
Enterobacter spp.	1 (1)	1 (1)	0 (0)	0 (0)	8 (7)
Total	52 (46)	29 (26)	29 (26)	23 (21)	112 (100)

[a] Numbers in parentheses are the percentage of the total number (112) of isolates.

[b] Among these 45 strains, 20 hybridized with *ereA*, 19 with *ereB*, and 6 with *ereA* and *ereB*.

tion to study the distribution of *ereA*, *ereB*, and *ermAM* in *Enterobacteriaceae* that are highly resistant to erythromycin.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. The properties of the strains and plasmids used in this study are listed in Tables 1 and 2, respectively. Strains of members of the family *Enterobacteriaceae* that are highly resistant to erythromycin (Table 1) were isolated from the feces of hospitalized patients receiving erythromycin by plating 0.1 ml of a 10^{-2} dilution of the sample on Drigalski agar (Diagnostics Pasteur) supplemented with 400 μg of erythromycin per ml. Bacteria were identified by API 20E galleries (API System; La Balme-Les Grottes, France) Ten strains from our laboratory collection belonging to the species represented in this study were used as MLS-susceptible control strains. Strains BM2195, which produces an erythromycin esterase type I (1), and BM2570, which produces an erythromycin esterase type II and an rRNA methylase (6), were included. Recombinant plasmids were introduced into *E. coli* BM694 (16) by transformation. Bacteriophage M13mp10 (19) and its hybrid derivative containing the *ermAM* probe were transfected in *E. coli* JM101 (18).

Media. Brain heart infusion broth (Difco Laboratories, Detroit, Mich.) was used. Disk agar diffusion tests and inactivation of macrolides were done on Mueller-Hinton agar (Diagnostics Pasteur). All incubations were at 37°C.

Determination of MICs. The method described by Steers et al. (26) was used to determine the MICs of the antibiotics.

Inactivation of macrolides. Inactivation of MLS antibiotics was screened by the test described by Gots (13) and modified as described previously (1). The products of detoxification of erythromycin and oleandomycin by resting cells were separated by thin-layer chromatography on precoated silica gel plates (60 F 254; E. Merck AG, Darmstadt, Federal Republic of Germany) in three different solvent systems and were revealed by spraying ethanolic H_2SO_4, as described previously (6). Inactivation of 14-membered macrolides by strains of *Proteus* was tested by thin-layer chromatography only, because the microbiological technique is not suitable for mobile microorganisms.

Preparation of DNA and agarose gel electrophoresis. Purification of plasmid (12) and of M13mp10 and derivative replicative-form (RF) phag DNA (31) was as described previously. The DNA restriction fragments were separated by electrophoresis in horizontal slab gels (20 by 20 by 0.7 cm) containing 0.8% agarose type II or type VII (Sigma Chemical Co., St. Louis, Mo.). Fragments were extracted from low-temperature-gelling agarose type VII, as described previously (17).

Probes and their construction. The presence of *ereA* was tested with the 716-base-pair (bp) insert of plasmid pAT66, which is included in the open reading frame (ORF) for the erythromycin esterase type I (4).

Plasmid pAT72 (6) consists of pUC8 plus a 1,906-bp pIP1527 DNA fragment which contains *ereB*, the structural gene for the erythromycin esterase type II. The ORF for the esterase (1,257 bp) extends from the ATG codon at position 383 to the TAA codon at position 1640 (5). The recognition sites for the endonucleases *Eco*RI at position 530 and *Pst*I at position 1368 delimit a 838-bp DNA fragment which was selected to construct a probe specific for *ereB*. The 838-bp *Eco*RI-*Pst*I fragment of pAT72 was purified and cloned in pUC8, which was digested with the same endonucleases and dephosphorylated. The plasmid content of transformants resistant only to ampicillin was analyzed by agarose gel electrophoresis of crude bacterial lysates (8). DNA from a hybrid plasmid, pAT76, with the expected size (3.5 kilobases [kb]) was purified and compared with that of pAT72 and pUC8 after digestion with *Eco*RI-*Pst*I. Plasmid pAT76 consists of pUC8 (2.7 kb) and the 838-bp fragment.

Plasmid pAT69 is pUC8 plus a 1.8-kb pIP1527 DNA fragment containing *ermAM*, the structural gene for the rRNA methylase (6; Trieu-Cuot et al., in press). To determine the nucleotide sequence of the insert of pAT69, we subcloned various portions of this DNA fragment in the *Sma*I site of M13mp10 (Brisson-Noël et al., in preparation). One of these hybrid bacteriophages contained the 349-bp *Rsa*I DNA fragment which is included in the ORF for the methylase. The RF DNA of the phage was digested by *Bam*HI-*Sst*I, and the smaller of the two DNA fragments was purified and used as a specific probe for *ermAM*. It consisted

TABLE 2. Plasmids and their origins

Plasmid	Relevant characteristics[a]	Origin	Reference or source
pIP1100	Tra^+ IncX *ereA* Ap Gm Sm	Natural plasmid	Andremont et al. (1)
pIP1527	Tra^+ *ereB ermAM* Cm Tc	Natural plasmid	Arthur and Courvalin (6)
pBR329	Tra^- Mob^- Ap Cm Tc	In vitro construction	Covarrubias and Bolivar (11)
pUC8	Tra^- Mob^- Ap	In vitro construction	Vieira and Messing (29)
pAT63	Tra^- Mob^- Ap *ereA*	pBR322Ω[pIP1100 partial *Sau*3A(*ereA*)-1.66 kb]	Ounissi and Courvalin (22)
pAT66	Tra^- Mob^- Tc	pBR329Ω[pIP1100*Eco*RI-*Pst*I-716 bp]	Arthur et al. (4)
pAT69	Tra^- Mob^- Ap *ermAM*	pUC8Ω[pIP1527 *Hin*dIII-*Pst*I(*ermAM*)-1.8 kb]	Arthur and Courvalin (6)
pAT72	Tra^- Mob^- Ap *ereB*	pUC8Ω[pIP1527 partial *Sau*3A(*ereB*)-1,906 bp]	Arthur and Courvalin (6)
pAT76	Tra^- Mob^- Ap	pUC8Ω[pIP1527 *Eco*RI-*Pst*I-838 bp]	This study

[a] Genetic symbols are from Arthur and Courvalin (6), Horinouchi et al. (14), and Ounissi and Courvalin (22); and phenotypic characters are from Novick et al. (20).

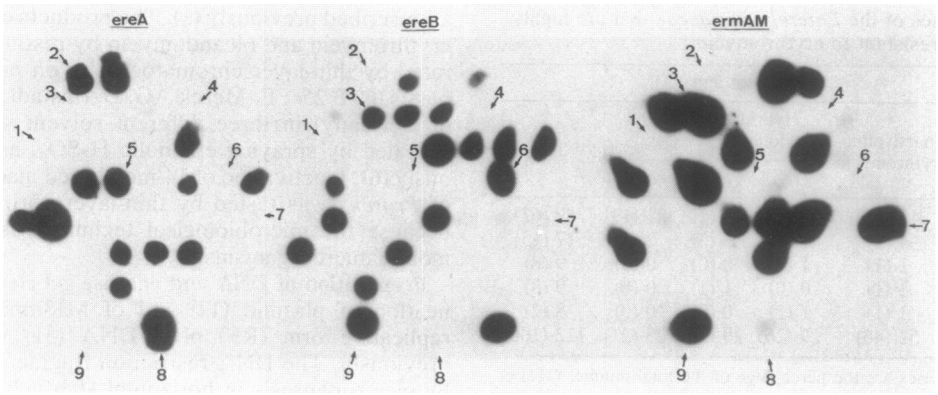

FIG. 1. Analysis of DNA by colony hybridization. Total DNA was transferred to nitrocellulose sheets and hybridized to the probes indicated at the top. 1, BM694; 2, BM694(pIP1100) (*ereA*); 3, BM694(pIP1527) (*ereB ermAM*); 4, BM694(pBR329); 5, BM694(pAT63) (*ereA*); 6, BM694(pAT72) (*ereB*); 7, BM694(pAT69) (*ermAM*); 8, BM2195 (*ereA*); 9, BM2570 (*ereB ermAM*). The absence of hybridization with pBR329 and pUC8 (data not shown) indicates that the esterase probes that also did not cross-hybridize were not contaminated with vector DNA. The specificity of the *ermAM* probe was tested by dot blot hybridization, with DNA of pAT69, M13mp10, and the probe used as targets (data not shown). Unidentified spots represent clinical isolates that were tested.

of the 349-bp derived from pAT69 plus 7 and 6 bp of M13mp10 at the 5′ and 3′ ends, respectively.

Colony hybridization. Nick translation of the purified restriction DNA fragments and hybridization in 50% formamide at 42°C were as described previously (17). UV-sterilized nitrocellulose filters (BA-85; Schleicher & Schuell, Inc.) were inoculated with a Steers inoculator (26). To ensure that negative hybridization did not result from slow bacterial growth, three series of filters were incubated on Mueller-Hinton agar for 3, 5, and 7 h. Bacterial lysis and binding of purified DNA to the filters (dot blot) were as described previously (17).

Enzymes and reagents. Restriction endonucleases *Bam*HI, *Eco*RI, *Pst*I, and *Sst*I; DNA polymerase I; T4 DNA ligase; and calf alkaline phosphatase (Boehringer GmbH, Mannheim, Federal Republic of Germany) were used according to the recommendations of the manufacturer. [α-^{32}P]dATP, triethylammonium salt (specific activity, 400 Ci/mmol), was obtained from the Radiochemical Centre, Amersham, England. The antibiotics were provided by the following laboratories: spiramycin and pristinamycins I and II, Rhône-Poulenc; clindamycin and lincomycin, The Upjohn Co., Kalamazoo, Mich.; erythromycin, Roussel-Uclaf; josamycin, Spret-Mauchant; midecamycin, Clin-Midy; oleandomycin, Pfizer, Inc., New York, N.Y.

RESULTS

Properties of the *Enterobacteriaceae* studied. The 112 strains of *Enterobacteriaceae* that are highly resistant to erythromycin fell into 5 genera and 10 species (Table 1). Inactivation of erythromycin and oleandomycin was detected by a microbiological technique (1, 13) in 52 of 112 strains (46%). Inactivation was more frequent in *E. coli* (45 of 69 strains [65%]) than in other species, (7 of 43 strains [16%]). Three *E. coli* strains were found to inactivate pristinamycin factor I, but we did not detect any inactivation of pristinamycin factor II, spiramycin, josamycin, midecamycin, lincomycin, or clindamycin. The 10 control strains that were susceptible to MLS did not inactivate the antibiotics. Modification of erythromycin and oleandomycin by 10 strains that were randomly selected was further studied by thin-layer chromatography (6). The RF values of the modified antibiotics were indistinguishable from those obtained with reference strains BM2195 and BM2570, which produce erythromycin esterase type I and II, respectively. By this technique, we did not detect modification of the antibiotics in five strains that gave negative results with the microbiological test.

TABLE 3. Hybridization classes of the *Enterobacteriaceae*

Class	No. of strains (%)	Inactivation of erythromycin	Hybridization with the following probes:		
			ereA	*ereB*	*ermAM*
I	56 (50)	−	−	−	−
II	22 (20)	+	+	−	−
III	14 (13)	+	−	+	+
IV	8 (7)	+	−	+	−
V	5 (5)	−	−	−	+
VI	4 (4)	+	+	+	+
VII	3 (3)	+	+	+	−

Hybridization. (i) Colony hybridization. We tested the presence of nucleotide sequences that were structurally related to *ereA*, *ereB*, and *ermAM* in the strains of *Enterobacteriaceae* by colony hybridization (Fig. 1 and Table 1). Homology with the *ereA* probe was detected in 26 strains of *E. coli* and in 1 strain each from the genera *Klebsiella*, *Citrobacter*, and *Enterobacter*. Total DNA of 25 *E. coli*, 3 *Klebsiella*, and 1 *Proteus* isolates hybridized with the *ereB* probe. Six *E. coli* isolates shared homology with both probes. All these strains were found to inactivate erythromycin and oleandomycin. Conversely, all the strains that inactivated the two antibiotics were detected with *ereA*, *ereB*, or both probes. Nucleotide sequences homologous to *ermAM* were detected in 21 strains of *E. coli* and in 1 strain each of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. Seven of the eight predictable hybridization classes were found (Table 3). Among the strains which hybridized to *ereB* and *ermAM*, the majority, 62% and 78% respectively, hybridized to both probes. By contrast, we did not find any strain hybridizing with *ereA* and *ermAM* but not with the *ereB* probe. Comparison of the resistance phenotypes toward sixteen antibiotics indicated that none of the classes was due to a strain epidemic (data not shown). The control susceptible strains did not hybridize with the probes.

TABLE 4. Inhibitory activities of MLS antibiotics against the *Enterobacteriaceae*

Hybridization class (no. of strains)[a]	Antibiotic[b]	MIC (μg/ml)[c]			No. of highly resistant strains (%)[d]
		Range	50%	90%	
Susceptible control strains (10)	Ery	32–256	128	256	0 (0)
	Spi	128–4,000	512	2,000	0 (0)
	Cli	16–2,000	256	1,000	0 (0)
	Lin	512–8,000	2,000	8,000	0 (0)
	P-II	256–>512	512	>512	NA[e]
	Pri	32–256	128	256	NA
II + IV + VII; strains hybridizing with esterase probes (33)	Ery	1,000–>8,000	2,000	4,000	33 (100)
	Spi	128–>4,000	1,000	>4,000	6 (18)
	Cli	16–4,000	512	1,000	1 (3)
	Lin	1,000–>8,000	2,000	8,000	3 (9)
	P-II	64–>512	512	>512	NA
	Pri	32–>512	128	256	NA
V; strains hybridizing with *ermAM* (5)	Ery	4,000–>8,000	>8,000	>8,000	5 (100)
	Spi	>4,000	>4,000	>4,000	5 (100)
	Cli	4,000–8,000	4,000	8,000	5 (100)
	Lin	>8,000	>8,000	>8,000	5 (100)
	P-II	128–512	256	512	NA
	Pri	32–512	128	512	NA
III + VI; strains hybridizing with *ermAM* and type II or both esterase probes (18)	Ery	>8,000	>8,000	>8,000	18 (100)
	Spi	>4,000	>4,000	>4,000	18 (100)
	Cli	4,000–8,000	4,000	8,000	18 (100)
	Lin	>8,000	>8,000	>8,000	18 (100)
	P-II	128–512	512	512	NA
	Pri	32–512	128	128	NA
I; strains which did not hybridize (56)	Ery	512–8,000	2,000	8,000	56 (100)
	Spi	256–>4,000	2,000	>4,000	23 (41)
	Cli	128–>8,000	512	8,000	10 (18)
	Lin	256–>8,000	4,000	>8,000	13 (23)
	P-II	64–>512	512	>512	NA
	Pri	16–>512	256	>512	NA

[a] Classes of hybridization are as defined in Table 3.
[b] Abbreviations: Ery, erythromycin; Spi, spiramycin; Cli, clindamycin; Lin, lincomycin; P-II, pristinamycin factor II; Pri, pristinamycin.
[c] 50% and 90%, MIC for 50 and 90% of strains, respectively.
[d] High level resistance (MIC, in micrograms per milliliter) is defined as follows: erythromycin, >256; spiramycin, >4,000; clindamycin, >2,000; lincomycin, >8,000.
[e] NA, Not applicable.

(ii) Correlation between the hybridization classes and the MLS resistance phenotypes. The MICs of nine commercially available MLS antibiotics against the 112 strains of *Enterobacteriaceae* that are highly resistant to erythromycin and the 10 susceptible control strains were determined (Table 4). High-level resistance to erythromycin was always associated with high-level resistance to oleandomycin (up to the solubility limit of the drug; MIC, >1,000 μg/ml). The MICs of MLS antibiotics against classes II, IV, and VII (defined in Table 3) which correspond to bacteria that are detected by either or both esterase probes were similar. This group and strains belonging to class I (absence of hybridization) were less resistant to erythromycin (MIC for 50% of strains tested, 2,000 μg/ml) than strains which hybridized with the *ermAM* probe (MIC for 50% of strains tested, >8,000 μg/ml).

To test if hybridization with *ermAM* correlated with a MLS resistance phenotype, we defined a high level of resistance to spiramycin (MIC, >4,000 μg/ml), clindamycin (MIC, >2,000 μg/ml), and lincomycin (MIC, >8,000 μg/ml). These breakpoints correspond to the lowest concentrations that inhibit 100% of the control susceptible strains (Table 4). All the strains detected by the *ermAM* probe were resistant to high levels of the three antibiotics, while coresistance was observed for only 10 strains of class I (no hybridization) and 1 strain of class II (hybridization with the *ereA* probe). We were unable to perform a similar analysis for midecamycin and josamycin because the MICs for 50% of the control strains were equal to the solubility limit of the two drugs (1,000 μg/ml). None of the strains detected by the *ermAM* probe, however, was inhibited at this concentration (data not shown).

Resistance to pristinamycin factor I could not be detected because this antibiotic is not inhibitory for the *Enterobacteriaceae*, including the susceptible control strains, up to the solubility limit of the drug (500 μg/ml). Pristinamycin factor II was more active (MIC, ≥64). MICs of pristinamycin were, in most cases, lower than those of pristinamycin factors I and II, indicating synergy between the two components, as has been described against gram-positive microorganisms (10). The MICs of pristinamycin and pristinamycin factor II against the control strains and strains belonging to different hybridization classes were similar.

DISCUSSION

The study of the distribution of *ereA* and *ereB* by colony hybridization indicated the spread of genes encoding erythromycin esterases types I and II in various species of

Enterobacteriaceae that are highly resistant to erythromycin (Table 1 and Fig. 1). All the strains detected by either or both probes were found to inactivate erythromycin and oleandomycin. Analysis by thin-layer chromatography confirmed the presence of an erythromycin esterase in the strains that were studied. It therefore appears that the probes that were constructed are specific for genes that express either type of erythromycin esterase. Conversely, all the strains that inactivated erythromycin and oleandomycin were found to possess *ereA*, *ereB*, or both. Thus, it seems that there are only two classes of genes that specify detoxification of these antibiotics in this collection. The incidence of type I and II enzymes was similar (26%), but resistance by inactivation was more frequently encountered in *E. coli* (65%) than in other species (16%).

Nucleotide sequences that were structurally related to *ermAM* were detected in 21 *E. coli* strains and in 2 strains of *Klebsiella* (Table 1 and Fig. 1). These strains probably produced an rRNA methylase because they were coresistant to high levels of erythromycin, spiramycin, and lincosamides but not to pristinamycin factor II (Table 4). Because of the intrinsic resistance of *Enterobacteriaceae* and the low solubility of the drugs, we were unable to define high-level resistance to josamycin, midecamycin, and pristinamycin factor I. Cellular impermeability masked the full phenotypic expression of MLS resistance which was observed by cloning *ermAM* of pIP1527 in an *E. coli* mutant that was susceptible to MLS antibiotics (6).

Seven classes of hybridization were defined on the basis of the results obtained with the three probes (Table 3). Analysis of the resistance phenotypes of the strains to 16 antibiotics indicated that none of these classes resulted from a strain epidemic (data not shown). The erythromycin esterase type II and the methylase genes were found more frequently in association than separately. By contrast, most of the strains producing an erythromycin esterase type I did not hybridize to *ereB* or *ermAM*. Because the genes for the esterase type II and the methylase are separated by 3.6 kb on plasmid pIP1527 (6), it is likely that physical linkage is responsible for codissemination. In addition, the presence of the two genes confers a selective advantage to the host because they contribute cooperatively to high-level erythromycin resistance (6). In certain clinical isolates, however, *ermAM* alone was sufficient to confer high-level resistance to this antibiotic (Table 4).

Fifty-six strains (50%) did not hybridize to any of the probes (Table 3, class I). A minority of these (18%) was highly resistant to macrolides and lincosamides (Table 4), which implies heterogeneity of the genes that confer this type of resistance. Strains of *E. coli* that are coresistant to MLS antibiotics have been obtained by in vitro mutagenesis (25). Analysis of the mutants indicated that MLS resistance was due to a single type of transversion in the 23S ribosomal gene. It is doubtful, however, that such a mutation could be selected in vivo because resistance to high levels of MLS antibiotics was only observed when the mutated 23S rRNA gene was present on a high-copy-number plasmid, thus outnumbering the chromosomic copies of the wild-type rRNA genes. In class I, coresistance to the chemically distinct MLS antibiotics is therefore likely to be due to the presence of other class(es) of methylase. In gram-positive bacteria rRNA methylases are encoded by several classes of genes that are derived from a common ancestor but which do not cross-hybridize under usual stringency conditions (21). This heterogeneity may also be present in *Enterobacteriaceae*.

The presence of *ermAM* and probably of *ereB* on pIP1527 seems to be due to recent direct transfer of genetic information from gram-positive bacteria to gram-negative organisms under natural conditions (5; Trieu-Cuot et al., in press; Brisson-Noël et al., in preparation). Our results indicate that, despite their exogenous origin, these two genes are already disseminated in various genera of the family *Enterobacteriaceae*. We postulated that acquisition by *Enterobacteriaceae* of genes of gram-positive origin occurred by a transformationlike process, followed by integration of the foreign DNA into indigenous replicons by illegitimate recombination (Trieu-Cuot et al., in press). In fact, *ermAM* genes are part of transposons Tn*917* and Tn*1545* from *Enterococcus faecalis* and *Streptococcus pneumoniae*, respectively (9, 24). Both transposons are active in *E. coli* (9, 15; P. Courvalin and C. Carlier, Mol. Gen. Genet., in press). Because the probability of occurrence of transformation followed by transposition is probably extremely low, the dissemination of *ermAM* in *Enterobacteriaceae* is more likely due to its presence on self-transferable plasmids than to multiple and independent transfers between gram-positive and -negative bacteria.

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