ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1987, p. 735–743 Vol. 31, No. 5
0066-4804/87/050735-09$02.00/0

Molecular Epidemiology of Macrolides-Lincosamides-Streptogramin B Resistance in *Staphylococcus aureus* and Coagulase-Negative Staphylococci

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Received 12 September 1986/Accepted 6 February 1987

Macrolides-lincosamides-streptogramin B (MLS) resistance is commonly found in *Staphylococcus aureus* and coagulase-negative staphylococci (22 and 45%, respectively, among isolates from three New Jersey hospitals). We have examined representative subsets of 107 MLS-resistant isolates for the molecular nature of the resistance determinant, the *erm* gene, by dot blot and Southern hybridization analysis. All of 35 *S. aureus* isolates examined and 39 of 42 coagulase-negative isolates examined were found to harbor the *ermA* or *ermC* evolutionary variant. Genes of the *ermC* class occurred exclusively on a small plasmid similar to or indistinguishable from one (pNE131) previously described in *S. epidermidis*. Genes of the *ermA* class occurred exclusively in the chromosome, and restriction patterns indicated that they were part of a transposon, Tn*554*, characteristic of the classical *S. aureus ermA* strain. Unlike *S. aureus ermA* strains examined previously, which harbor Tn*554* at a single specific (primary) site, four of our *S. aureus* isolates had second inserts at different chromosomal sites. The majority of our coagulase-negative isolates had two or more inserts, neither of which occurred at the classical primary site and many of which differed from one another in location (as inferred from restriction patterns). Coagulase-negative staphylococci constitute a large reservoir of the *ermA* and *ermC* class of determinants, with clear potential for interspecies spread.

The MLS phenotype refers to cross-resistance to three groups of antibiotics: the macrolides (exemplified by erythromycin), the lincosamides (exemplified by clindamycin), and the streptogramins B (exemplified by vernamycin Bα). The classical phenotype was described for four *Staphylococcus aureus* isolates, in which resistance to the macrolide spiramycin was shown to be inducible by low levels of erythromycin (2). The phenotype has since been shown to occur in a variety of bacterial genera (5), but the *S. aureus* system has been subjected to the most intensive scrutiny and is understood in greatest detail (see references 26 and 36 for summaries). Neither lincosamides, streptogramins, nor most macrolides are effective inducers. The induction process entails activation of an mRNA that encodes a 23S RNA methylase, the *erm* methylase, which in turn renders newly synthesized ribosomes resistant to the MLS agents by methylating a specific adenosine residue (equivalent to *Escherichia coli* 23S RNA A2058 [33]) of the rRNA component of the peptidyl transferase center. The activation involves alteration in the secondary structure of the mRNA, resulting from a stall of an erythromycin-ribosome complex on the leader region(s) encoding short polypeptides. Constitutive variants readily arise via mutational changes in these leader regions.

Three distinguishable classes of *erm* gene have been described for *S. aureus* and are designated *ermA*, *ermB*, and *ermC*. On the basis of direct sequencing for the prototypes of *ermA* (20) and *ermC* (9), or, for *ermB*, close similarity to a sequenced streptococcal *erm* gene, *ermAM* (8, 29, 38), it can be concluded that *ermA*, *ermB*, and *ermC* are only about 60% homologous and do not cross-hybridize under moderately stringent conditions (38). *ermA* was initially detected in an inducible strain, 1206, isolated in Wisconsin (37). It occurs on a transposon, Tn*554*, that at least in laboratory strains has a pronounced tendency to insert into a single specific site in the *S. aureus* chromosome, the primary insertion site (12, 23, 25, 26). *ermB* was discovered in Japan (17); it typically occurs on a transposon, Tn*551*, as part of a 28-kilobase (kb) plasmid, pI258 (27). *ermC* was discovered in Bucharest as part of a 3.7-kb plasmid, pE194 (9, 10). The MLS phenotype has also been detected in *S. epidermidis* (28), and at least some isolates harbor an *erm* gene (*M*) very similar to *ermC* (15).

Despite the continuing high prevalence (20 to 45%) of MLS strains among staphylococci isolated from hospitals (5; W. D. Jenssen, S. Thakker-Varia, D. T. Dubin, and M. P. Weinstein, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, A8, p. 2), there is no information on the relative frequencies of the three prototypic *S. aureus erm* gene classes, or on the possible occurrence of yet other classes, in clinical isolates. In this paper, we present data addressing this question, obtained from a study of a series of 107 MLS clinical isolates of *S. aureus*, *S. epidermidis*, and other coagulase-negative staphylococci. We also present a molecular characterization of the most common staphylococcal *erm* gene classes in our region, *ermA* and *ermC*.

MATERIALS AND METHODS

Aside from two MLS-resistant strains described previously (33), the strains examined in the present work were from a series of 332 consecutive staphylococcal isolates collected at the clinical microbiology laboratories of three central New Jersey hospitals in 1984 and 1985. Initial identification as *S. aureus* or coagulase-negative staphylococci was based on colony and microscopic morphology and coagulase testing. Coagulase-negative isolates were identi-

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TABLE 1. Standard strains

Strain	Plasmid	Description and use	Source or reference
E. coli HB101	pKH80	Chimeric plasmid used as source of pE194 probe and target	K. Hardy (6)
S. aureus RN4932	pEM715	Tn*554* inserted into primary target in plasmid pT181; similar to pEM9717 (21); used as source of *ermA* probe and as standard target	E. Murphy
S. aureus RN2466	pRN3173	Deletion of pI258; used as source of *ermB* probe and as standard target	E. Murphy (27)
S. aureus RN11	pI258	Used as standard target	E. Murphy
S. aureus RN2442	pE194	Used as standard target	E. Murphy
S. aureus RN2863		Chromosomal Tn*554*; used as standard for single Tn*554* inserted into primary target site	E. Murphy
S. aureus 1206		Original *ermA* isolate used as source of standard target	B. Weisblum (37)
S. epidermidis	pNE131	Used as source of probe and target	J. Parisi (28)
Streptococcus faecalis JH2-2	pAMβ1	Used as standard target in dot blots	D. Clewell (3)

fied to the species level by using API Staph-Ident strips (Analytab Products, Plainview, N.Y.) and by novobiocin susceptibility testing. All isolates were characterized as to the MLS phenotype by agar diffusion (37) with disks containing erythromycin (15 μg), clindamycin (2 μg), or vernamycin Bα (25 μg). Strains showing cross-resistance to these agents, either constitutive or inducible, were scored as MLS resistant and saved for further study. Spectinomycin susceptibility was tested by plating the isolates onto medium containing 300 μg of drug per ml. Clinical isolates, as well as standard strains obtained from other laboratories (Table 1), were repurified from single colonies, and the MLS phenotypes of cultures grown from these colonies were rechecked just prior to dot-blot analysis or DNA preparation. Strains were stored in CY broth (24) plus 30% glycerol at −70°C.

Erythromycin was purchased from Sigma Chemical Co., St. Louis, Mo., clindamycin and spectinomycin were gifts from The Upjohn Co., Kalamazoo, Mich., and vernamycin Bα was a gift from the Squibb Institute for Medical Research.

For dot-blot analysis, cells were pelleted from a 1-ml overnight culture in CY broth, washed with 10 mM EDTA (pH 7), suspended in 50 μl of 10 mM Tris hydrochloride (pH 8.0) containing 1 mg each of lysostaphin and lysozyme per ml, and incubated at 37°C for 1 h. An equal volume of 0.5 M NaOH containing 0.2% Triton X-100 was added, and the incubation was continued for 20 min at room temperature. The solution was neutralized with 200 μl of 3 M sodium acetate (pH 6.0). The samples were frozen and thawed, and 10-μl aliquots, made up to 100 μl with 12× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) were applied to a nitrocellulose membrane by using a Minifold apparatus (Schleicher & Schuell, Inc., Keene, N.H.) under gentle suction. The filters were baked and hybridized as described below for Southern transfers. They were then washed three times with 6× SSC and three times with 1× SSC for 30 min each at room temperature and exposed to Kodak XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) for preliminary screening. For more stringent conditions, the filters were washed again three times with 0.1× SSC at 55°C. Quantitation of these and other autoradiograms was obtained by densitometric scanning with a Kontes 800 fiber optic scanner.

Total cellular DNA was prepared by the method of Mekalanos (16). Cells from a 6-ml culture, grown and washed as above, were suspended in 2 ml of 10 mM EDTA, and lysozyme and lysostaphin were added to concentrations of 50 μg/ml each. After incubation for 1 h at 37°C, sodium dodecyl sulfate and pronase were added to 0.5% and 100 μg/ml, respectively, and the preparation was held at 37°C for a further 2 h. The viscous lysate was then extracted with phenol followed by chloroform-isoamyl alcohol (24:1, vol/vol). The DNA was precipitated with 2.5 volumes of ethanol in the presence of 0.3 M sodium acetate. The DNA was then held at 65°C for 3 h in TE (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA) to bring the bulk of it into solution.

Plasmid DNA was prepared from staphylococci by a modification of the procedure of Novick et al. (27) and from *E. coli* by the procedure of Holmes and Quigley (7).

DNA for use as probes was purified, after restriction of plasmid preparations (see Fig. 1), by electrophoresis through 0.7% agarose gels. Appropriate fragments were extracted by a version of the freeze-squeeze method (34), adsorbed to an Elutip column (Schleicher & Schuell), and eluted with 1.0 M NaCl containing 20 mM Tris hydrochloride (pH 7.5) and 1 mM EDTA.

For restriction analysis, DNA (generally 3 to 6 μg) was digested for 3 h with 20 to 60 U of enzyme in a reaction volume of 30 μl under the conditions specified by the supplier (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), except that the *Hae*III reactions were conducted at 45°C. Aliquots of digests (containing ca. 1 μg of DNA) were fractionated by electrophoresis in 0.7% agarose gels in Tris-borate buffer (pH 8.0) at 100 V/14 cm. Fragments were transferred to nitrocellulose sheets (type BA-85; Schleicher & Schuell) by the method of Southern (32). The sheets were baked at 78°C for 1 h and prehybridized in 50% formamide containing 5× SSC, 0.04% bovine serum albumin, 0.04% polyvinylpyrrolidone, 0.04% Ficoll (Pharmacia Fine Chemicals, Piscataway, N.J.), and heat-denatured salmon sperm DNA (100 μg/ml) for 1 h at 37°C. Approximately 10^6 cpm of denatured, nick-translated probe DNA (specific activity, 10^7 to 10^8 cpm/μg [31]) was then added, and the incubation was continued for 17 h at 37°C. The filters were washed once in 6× SSC, twice in 2× SSC, and four times in 1× SSC, each for 30 min at room temperature, air dried, and autoradiographed on Kodak XAR-5 film. Sizes of fragments were estimated by comparison with unlabeled markers from *Hin*dIII digests of bacteriophage lambda and *Hae*III digests of the replicative form of bacteriophage φX174, as detected by ethidium-induced fluorescence prior to the Southern transfer.

RESULTS

Dot blots. Dot-blot hybridizations were performed on 35 MLS *S. aureus* isolates and 42 MLS coagulase-negative

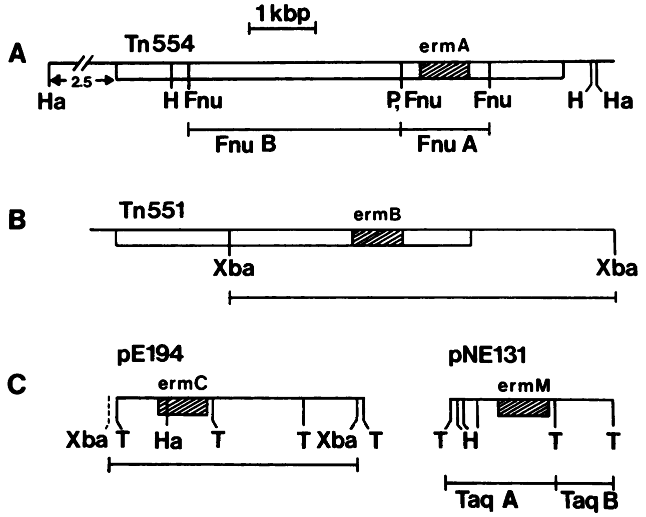

FIG. 1. Diagrams of *erm* regions. (A) Tn*554* in its primary chromosomal location, with pertinent restriction sites as given by Krolewski et al. (12), and updated by subsequent studies (21; Murphy, personal communication). (B) Tn*551* in plasmid pRN3173 with approximate locations of *ermB* and of *Xba*I sites (26, 27). (C) *ermC* gene in plasmid pE194, and its close homolog, the *ermM* gene in plasmid pNE131. Both plasmids are arbitrarily linearized at *Taq*I sites upstream of the *erm* genes. For pE194, the *Xba*I sites shown correspond either to the single site of the intact circular molecule or to the two *Xba*I sites bounding the plasmid as inserted into *E. coli* plasmid pKH80 (6), the source of our probe. Symbols: ▭, transposons; ▨, *erm* genes. Restriction sites: Ha, *Hae*III; H, *Hin*dIII; Fnu, *Fnu*4HI; P, *Pst*I; *Xba*I; T or Taq, *Taq*I. The extents of the various probes used for hybridization are shown below each diagram.

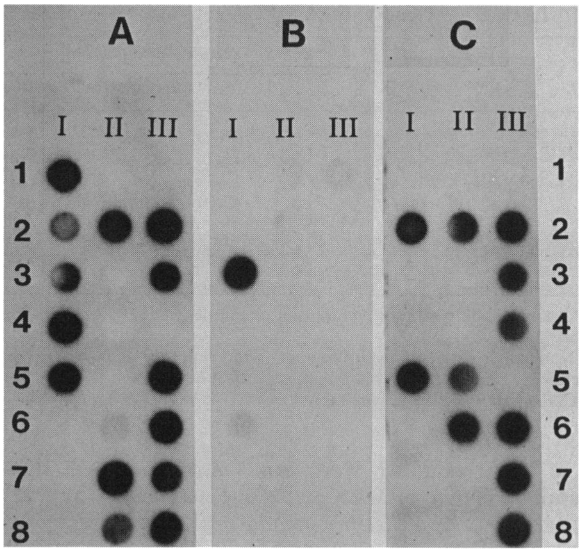

FIG. 2. Dot-blot hybridizations. Extracts were processed as described in Materials and Methods to generate triplicate target filters according to the key in Table 2. Each filter was hybridized with an *erm* probe corresponding to the heading of each panel. The *ermA* probe was the *Fnu*4HI A segment of Tn*554*; the *ermB* probe was the *Xba*I fragment of pRN3173; and the *ermC* probe was an *Xba*I fragment corresponding to linearized pE194, all as shown in Fig. 1. We show the results obtained after the second, more stringent, wash.

staphylococcal isolates (Jenssen et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986). Replicate samples of total-cell extract were processed as described in Materials and Methods and hybridized with ^{32}P-labeled probes containing *ermA*, *ermB*, or *ermC* (Fig. 1). Representative results are illustrated in Fig. 2 and scored in Table 2. Interpretation was somewhat complicated by the fact that some strains (14 *S. aureus* and 3 coagulase-negative staphylococci) hybridized about equally well with *ermA* and *ermC* probes. Eight of these strains were subjected to Southern hybridization of purified DNA as described below. All yielded typical strong *ermC* patterns, and none yielded prominent discrete bands hybridizing with *ermA* or *ermB* probes. We infer that our *ermA* probe cross-hybridizes with various (non-*erm*) staphylococcal sequences, as indeed can be seen by the degree to which the probe hybridized to the *ermB* and *ermC* standard strains (Fig. 2; target spots I2 and I3) and an MLS-susceptible *S. aureus* strain (target spot II8). This cross-hybridization was only marginally diminished when the stringency of the wash was increased (as described in Materials and Methods).

On the other hand, of the strains subsequently found to yield *ermA* patterns on Southern analysis (see below), all registered positive *ermA* blots and negative *ermB* and *ermC* blots. We infer that our *ermB* and *ermC* probes were quite specific for the respective *erm* gene classes, despite the relatively large complement of non-*erm* sequence in these probes. Again, the results for standard *S. aureus* strains (Fig. 2B and C) supported this inference. The lower specificity of our *ermA* probe relative to those of the *ermB* and *ermC* probes is unexplained. Nevertheless, it seems safe to score, as we did, isolates yielding positive *ermC*, or *ermC* and *ermA*, blots as *ermC*-class strains, and isolates yielding only *ermA* blots as *ermA*-class strains. (None of our isolates yielded positive *ermB* blots.)

A total of 31% of *S. aureus* and 19% of coagulase-negative staphylococcal MLS isolates were *ermA* strains and virtually

TABLE 2. Scoring of dot blots shown in Fig. 2[a]

Row no.	Column		
	I	II	III
1	RN4932; *ermA* standard	*S. saprophyticus*; *erm* zero	*S. hominis*; *erm* zero
2	RN2442; *ermC* standard	*ermC*	*ermC*
3	RN2446; *ermB* standard	*S. saprophyticus*; *erm* zero	*S. epidermidis*; *ermC*
4	1206; *ermA* standard	Enterococcus; *erm* zero	*S. epidermidis*; ermC
5	*ermC*	*S. epidermidis*; *ermC*	*ermA*
6	*S. faecalis* JH2-2 (*ermB*-like)	*S. epidermidis*; *ermC*	*ermC*
7	*B. subtilis*; MLS susceptible	*S. epidermidis*; *ermA*	*ermC*
8	*B. subtilis*; MLS susceptible	*S. aureus*; MLS susceptible	*ermC*

[a] Unless otherwise noted, strains are MLS *S. aureus*.

TABLE 3. Genotypes of MLS staphylococcal isolates

Strains	No. of strains with:		
	ermA	*ermC*	*erm* zero[a]
S. aureus			
MLS inducible	7 (5[b])	8	0
MLS constitutive	4 (2[b])	16	0
CNS[c]			
MLS inducible	3 (3[b])	11	1
MLS constitutive	5 (5[b])	19	2

[a] Strains yielding no positive dot blot reactions with *ermA*, *ermB*, or *ermC* probe.

[b] Number of strains resistant to spectinomycin (only one *ermC* strain was spectinomycin resistant).

[c] CNS, Coagulase-negative staphylococci.

all of the remainder were *ermC* strains (Table 3). (The overall incidence of MLS resistance was found to be 22% in *S. aureus* and 45% in coagulase-negative staphylococci [Jenssen et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986].) Of the coagulase-negative isolates scored as *ermC* strains by dot blot, 17 were *S. epidermidis*, 10 were *S. haemolyticus*, and 3 were *S. hominis*. The coagulase-negative *ermA*-class isolates were distributed among four species (see the legend to Fig. 6). Of three MLS staphylococcal isolates yielding no positive blots with our probes (*erm* zero in Table 3), two were *S. saprophyticus* and one was *S. hominis*.

Southern blot characterization of *ermC* strains. We expected the *ermC*-class of determinants to reside on a small plasmid resembling the classical plasmid pE194. Accordingly, an initial series of Southern hybridizations was performed on a subset of the *ermC* strains listed in Table 3, with a linearized form of pE194 as probe (Fig. 1). Plasmid and or total cellular DNA preparations from the clinical isolates were fractionated by agarose gel electrophoresis, with or without prior digestion by restriction endonucleases; the two types of DNA preparations yielded concordant results. In all cases, unrestricted samples yielded two major, discrete hybridizing bands that appeared to correspond to supercoiled and nicked circular forms of a small plasmid. (Such a pattern from total-DNA preparations demonstrates that the hybridizing determinant is on a plasmid.) The size of the plasmid, as estimated from migration of a minor band considered to be the linearized form, was about 2.4 kb. This is significantly smaller than pE194 (3.7 kb), but resembles the size obtained for an *erm*-containing plasmid originally recovered from *S. epidermidis*, plasmid pNE131, that also hybridized with pE194 (28). Accordingly, in subsequent studies, we used pNE131 as probe or (Fig. 3) as target. Lanes designated U in Fig. 3 illustrate the findings cited above for unrestricted samples. Figure 3 also compares DNA from a typical clinical isolate (an inducible *S. aureus* strain) with DNA from pE194 and pNE131 with regard to patterns generated by three restriction endonucleases, still with pE194 as probe. *Taq*I yielded two hybridizing bands of ca. 1.4 and 0.9 kb for pE194 and single bands of 1.5 to 1.6 kb for pNE131 and the clinical isolate. *Hin*dIII did not attack pE194 but yielded single major bands of ca. 2.1 kb for both pNE131 and the clinical isolate; and *Hae*III linearized pE194 but did not attack the clinical isolate or pNE131. The results for pE194 and pNE131 are as expected from their restriction maps (the 1.4-kb *Taq*I band from pE194 representing two unresolved fragments of 1.35 and 1.44 kb) (Fig. 1), if one assumes no significant homology between pE194 and the *Taq*I B fragment of pNE131. Parisi et al. (28) did report slight hybridization between this latter fragment and pE194. However, recent sequence analysis (15) has shown that the homology is limited to a stretch of 18 bases, which is presumably too short to register under our conditions. The results for our clinical *S. aureus* isolate support the view that its plasmid is closely related to pNE131.

More definitive evidence on the *ermC* replicons of our isolates was obtained in experiments with *Taq*I fragments of pNE131 as probes. As shown for a representative strain in

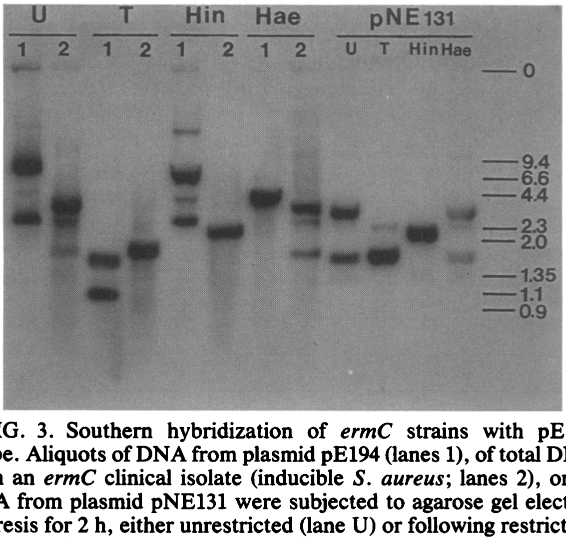

FIG. 3. Southern hybridization of *ermC* strains with pE194 probe. Aliquots of DNA from plasmid pE194 (lanes 1), of total DNA from an *ermC* clinical isolate (inducible *S. aureus*; lanes 2), or of DNA from plasmid pNE131 were subjected to agarose gel electrophoresis for 2 h, either unrestricted (lane U) or following restriction with *Taq*I (lane T), *Hin*dIII (lane Hin), or *Hae*III (lane Hae). After Southern transfer, hybridization was performed with ^{32}P-labeled linearized pE194. The origin (O) and position of pertinent size markers run in parallel are indicated.

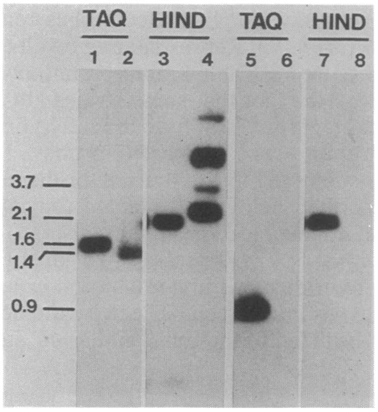

FIG. 4. Southern hybridization of *ermC* strains with pNE131 probes. Duplicate blots were prepared from agarose gels for hybridization to the *Taq*I A fragment of pNE131 (lanes 1 to 4) or the *Taq*I B fragment (lanes 5 to 8). In each gel were run *Taq*I (TAQ) and *Hin*dIII (HIND) digests of DNA from each of five inducible *ermC* isolates (three *S. aureus*, two *S. epidermidis*) and from the standard pE194-containing strain, RN2442. The patterns from our clinical isolates were all the same; lanes from an *S. epidermidis* preparation (lanes 1, 3, 5, and 7) together with neighboring RN2442 lanes (lanes 2, 4, 6, and 8) are shown. Approximate sizes (in kilobases) of pertinent bands, as compared with DNA markers run in parallel, are indicated: 3.7 for linear pE194; 2.1 for the large *Hin*dIII fragment of the clinical strains; 1.6 and 0.9 for the two *Taq*I fragments of these strains; and 1.4 for the *erm* gene-containing *Taq*I fragment of pE194.

TABLE 4. Restriction analysis of *ermA* staphylococcal isolates[a]

Restriction enzymes	*S. aureus* fragments (kb)			Coagulase-negative staphylococcal fragments (kb)				
	Standard primary insert	Extra insert		*S. simulans*[b]	*S. haemolyticus*	*S. epidermidis, S. hominis*[c]		
		WJ8, WJ45, WJ137	DR3					
Hind-Pst vector[d]	3.5	3.5	3.4	3.5	3.5	3.5	3.5	3.5
Hind-Pst gene[d]	2.8	3.5	3.2	5.4	3.3	4.2	10.0	2.8
Hind-Hind	6.5	7.0	6.7	9.0	12.0	7.5	14.0	6.5
Hae-Pst vector[d]	7.0	10.0	4.3	5.5	7.0	4.6	5.5	5.6
Hae-Pst gene[d]	2.9	3.1	8.5	2.9	2.9	4.1	3.1	5.5
Hae-Hae	10.0	13.0	12.0	9.0	12.0	9.0	11.0	11.0

[a] Sizes of fragments, in kilobases, were estimated by comparison with DNA markers (Fig. 3).

[b] Two *S. simulans* strains had inserts corresponding to column 4; one yielded, in addition, fragments corresponding to column 6.

[c] Three *S. epidermidis* and the *S. hominis* strain examined shared the fragments in columns 6 and 7. The *S. hominis* had a third insert, corresponding to column 8.

[d] Vector and gene moieties indicate preferential hybridization with the *Fnu*4HI B or A fragment, respectively (Fig. 1).

Fig. 4, all *ermC*-class isolates subjected to Southern transfer examination (eight *S. aureus*, eight *S. epidermidis*, and two *S. haemolyticus*) yielded *Taq*I fragments of 1.5 to 1.6 and 0.9 kb that hybridized with pNE131 *Taq*I fragments A and B, respectively. The larger *Taq*I fragment, as well as the *Hin*dIII fragment and the undigested plasmid, of inducible strains ran marginally more slowly than corresponding preparations from pNE131 and from most constitutive isolates (compare Fig. 3, lanes 8 and 9). This is in accord with the finding (14) that the constitutive pNE131 lacks a short stretch of gene leader sequence present in the inducible *ermC* prototype.

In the study illustrated in Fig. 4, we also ran a *Taq*I digest of pE194, and the results confirm the extensive homology between the *erm* genes of pE194 and pNE131 and the absence of detectable homology between pE194 and the non-*erm* gene-containing *Taq*I fragment (B) of pNE131 (Fig. 4, lanes 6 and 8).

One form of restriction site heterogeneity emerged from our studies on *ermC*-class strains. Plasmids from two inducible *S. aureus* isolates were linearized by restriction with *Hae*III (data not shown), as is the case with pE194 but not pNE131 (Fig. 1). We believe that this reflects retention of the pE194 *Hae*III site that occurs within the *ermC* gene at residue 2738 (9). In the sequence of the corresponding region of the pNE131 *erm* gene, the GGCC of the *Hae*III site is altered to GGGC, which does not change the encoded amino acid (the in-frame GGC or GGG of the tetranucleotide, encoding glycine in both cases) (14).

Southern blot characterization of *ermA S. aureus* strains. The *S. aureus ermA*-class isolates (Table 3) were characterized by Southern blot analysis of total cellular DNA, since we expected the relevant determinants to be chromosomal. Initial screening was performed by using the *Fnu*4HI A segment (Fig. 1) as probe and undigested, *Hin*dIII-digested, or *Hae*III-digested, samples as target. In no case did unrestricted DNA yield a discrete hybridizing band, indicating that the *ermA* gene was indeed chromosomal. Isolates with the classical primary insert are expected to yield single bands of ca. 6.5 or 10 kb with this probe for *Hin*dIII and *Hae*III digests, respectively; since Tn*554* lacks a *Hae*III site, the *Hae*III bands would be expected to contain the intact transposon (Fig. 1). Within the limits of precision of the measurements involved, seven isolates did yield *Hin*dIII and *Hae*III bands as expected; these strains yielded patterns indistinguishable from each other and from a prototype laboratory strain RN2863 (Table 1) with a single insert in the primary target site. This pattern is referred to in Table 4 as that of the standard primary insert. However, the remaining four *S. aureus* strains yielded two major bands with both enzymes, one similar to the expected band and one running slightly behind it.

To characterize our isolates further, we performed experiments in which samples were digested with *Hin*dIII plus *Pst*I or *Hae*III plus *Pst*I (the latter corresponding to a site overlapping an *Fnu*4HI site within Tn*554*), as well as with *Hin*dIII or *Hae*III alone; and blots were probed with *Fnu*4HI fragment A, to detect the *erm* gene-containing portion of the transposon, or *Fnu*4HI fragment B, to detect only the non-*erm* gene-containing (vector) portion (Fig. 1). Hybridization was done in parallel on duplicate filters or serially on a single filter; the results were concordant. Findings for serially hybridized samples from a typical single-insert strain (WJ139) and a double-insert strain (WJ137), both constitutive MLS isolates from the same hospital, are shown in Fig. 5A and B. The *Hae*III pattern for WJ137 (lanes 1) shows the extra major band running more slowly than the standard *Hae*III band (lanes 2); in Fig. 5B, lane 5, the extra *Hin*dIII band can be clearly seen. The extra *Hae*III band is due to a vector *Hae*III-*Pst*I fragment ca. 3 kb longer than the standard one (Fig. 5B, lanes 3 and 4). The extra *Hin*dIII band is due to an *erm*-gene *Hin*dIII-*Pst*I fragment ca. 0.7 kb longer than its corresponding standard fragment, which causes it to run with the vector *Hin*dIII-*Pst*I fragments common to all Tn*554* inserts (Fig. 5, lanes 7 and 8). Patterns indistinguishable from those of WJ137 were obtained for two of the other *S. aureus* isolates with double inserts (summarized in Table 4). We obtained a somewhat different pattern for the fourth such *S. aureus* strain, DR3 (which was isolated earlier than the others and was the subject of our initial rRNA studies [33]). The *Hae*III-*Pst*I results were especially striking and are shown in Fig. 5C, together with patterns from two single-insert strains, including the classical strain 1206, processed in parallel. The second insert of DR3 yielded two clearly resolved extra *Hae*III-*Pst*I bands, the larger hybridizing preferentially with the *erm* gene and the smaller hybridizing preferentially with the vector probe. These results, and those from restriction with other enzymes, are included in Table 4.

Although the numbers involved are small, there was a suggestion of a correlation between single inserts on the one hand, and inducibility and spectinomycin susceptibility on the other (Tn*554* normally contains a spectinomycin resistance [Sp^r] determinant). Five of the seven single-insert strains were inducible, compared with two of four double-insert strains; and four of the seven single-insert strains, but

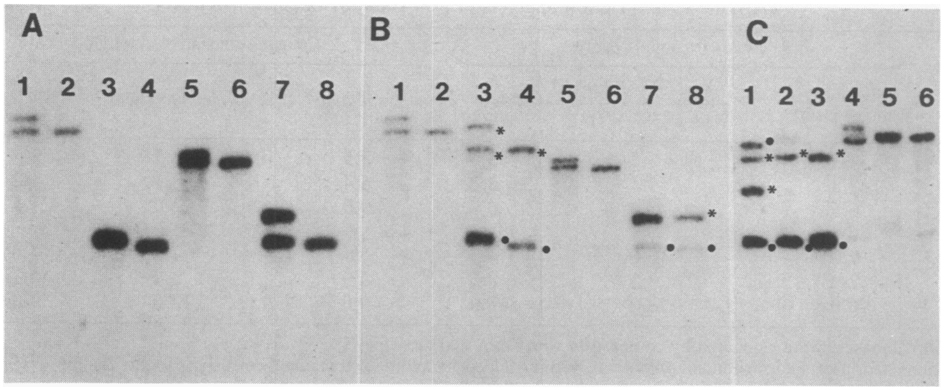

FIG. 5. Southern hybridization of *S. aureus ermA* strains. (A) Patterns after hybridization with the *Fnu*4HI A segment; (B) patterns after rehybridization with the B segment. Lanes 1, 3, 5, and 7 were generated by the double-insert isolate, WJ137, and lanes 2, 4, 6, and 8 were generated by a typical single-insert isolate, WJ139. Restriction digestion was as follows: lanes 1 and 2, *Hae*III; lanes 3 and 4, *Hae*III plus *Pst*I; lanes 5 and 6, *Hin*dIII; lanes 7 and 8, *Hin*dIII plus *Pst*I. Lanes 5 to 8 in panel B represent an exposure without intensifying screens to enhance resolution; other exposures were with screens. (C) Patterns from the final exposure of a similar study on *S. aureus* DR3 (lanes 1 and 4); *S. aureus* 1206 (lanes 2 and 5); and another typical single-insert clinical isolate, *S. aureus* WJ6 (lanes 3 and 6). Lanes 1 to 3 represent *Hae*III-*Pst*I digests, and lanes 4 to 6 represent *Hae*III digests. Total cellular DNA was processed as for Fig. 3, except that the electrophoresis time was 3.5 h. In panels B and C, bands that hybridized preferentially with gene (●) or vector (*) probes are indicated (note that the darker band of panel B, lane 7, hybridized with both probes). Sizes of bands (see Table 4) were estimated by markers as for Fig. 3 (not shown); the origins correspond to the top of the photograph.

none of the double-insert strains, were spectinomycin susceptible.

Southern blot characterization of *ermA* coagulase-negative staphylococci. The coagulase-negative *ermA* strains (Table 3) were screened by Southern hybridization, as for the *S. aureus* isolates. Again, results for unrestricted total cellular DNA indicated that *ermA* was invariably integrated into the chromosome. Results for *Hin*dIII and *Hae*III digests probed with the *Fnu*4HI A fragment (data not shown; summarized in Table 4) were generally also similar to the *S. aureus* results. Three of the isolates yielded single bands after *Hin*dIII or *Hae*III digestion, and five yielded two major bands after digestion with at least one of the enzymes; in addition, the bands were in the same range as those for *S. aureus*, 6 to 14 kb. However, close comparison revealed five distinct patterns, differing from each other and from any of the *S. aureus* patterns. A striking illustration of the multiplicity of patterns was provided by Southern analysis of double digests with vector and gene Tn*554* probes (Fig. 6), as had been applied to *S. aureus*. In Table 4, columns 4 to 8, we summarize these results, giving approximate sizes for the various Tn*554*-related restriction fragments released. We have sorted the fragments into five sets, corresponding to five classes of putative Tn*554* inserts, in a manner that is somewhat arbitrary for strains with apparent extra inserts. Our approach assumes (i) that Tn*554* in coagulase-negative staphylococci resembles its *S. aureus* counterpart in lacking a *Hae*III site and hence that the *Hae*III-*Hae*III fragments include the entire transposon; and (ii) that the extra fragments in the *S. hominis* patterns (compared with the *S. epidermidis* pattern) and in one *S. simulans* pattern (compared with the other *S. simulans* patterns) correspond to sets derived from discrete extra inserts. We have also sorted with a view to optimizing agreement between sums of subfrag-

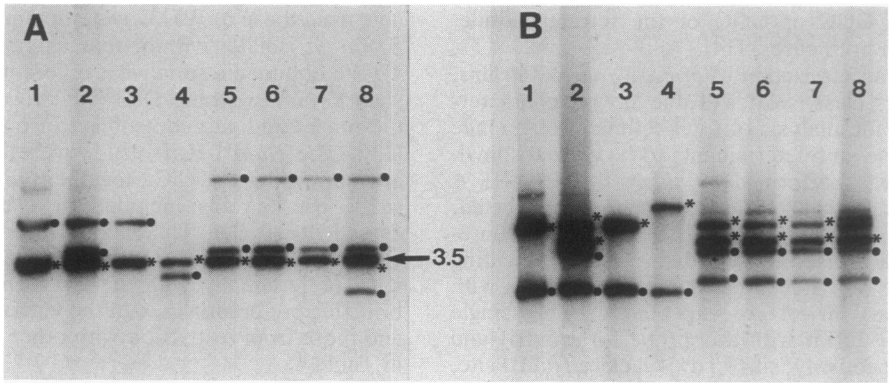

FIG. 6. Southern hybridization of coagulase-negative staphylococcal *ermA* strains: *Hin*dIII-*Pst*I and *Hae*III-*Pst*I digests. Total cellular DNA was processed as for Fig. 5, but only the patterns obtained after the second hybridization are shown; preferential hybridization with vector or gene probes, as inferred from comparison with patterns from the initial hybridization, is indicated as for Fig. 5. (A) *Hin*dIII-*Pst*I digests; (B) *Hae*III-*Pst*I digests. The lanes represent DNA from *S. simulans* isolates (lanes 1 to 3); an *S. haemolyticus* isolate (lane 4); *S. epidermidis* isolates (lanes 5 to 7), and an *S. hominis* isolate (lane 8). The arrow designates the 3.5-kb *Hin*dIII-*Pst*I vector bands common to all staphylococcal *ermA* isolates. The apparent doublet in panel B, lane 8, running about as the neighboring 5.5 kb vector bands yielded signals with both probes and probably contains a mixture of three fragments (Table 4).

ments and their putative singly digested parent fragments (as applied especially to the *Hin*dIII-*Pst*I results in columns 6 and 7). This agreement proved fairly close in most cases, the most flagrant exception being the *Hin*dIII-*Pst*I analysis of the *S. haemolyticus* insert; there apparently are additional *Pst*I sites within, or near, this insert.

DISCUSSION

Perhaps our most important conclusion is that the vast majority of MLS staphylococcal isolates from local hospitals, both coagulase-positive and coagulase-negative species, are accounted for by *erm* genes that are, or are very closely related to, classical *ermA* and *ermC* genes. However, there is considerable variation from the classical prototypes in the nature of the associated replicon for the *ermC* class and in the characteristics of chromosomal insertions for the *ermA* class.

***ermC* isolates.** The original *S. aureus* plasmid harboring *ermC*, pE194, was conspicuously absent from our isolates. Rather, our *ermC*-class genes occurred in plasmids resembling, or the same as, the constitutive MLS plasmid, pNE131, initially recovered from *S. epidermidis* (28). The *ermM* gene of pNE131 is 99% homologous to *ermC* over its coding region; its leader region has a 107-base deletion relative to *ermC*, which could account for its constitutivity (14). Lampson and Parisi (Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, H164, p. 118) have also reported slightly larger inducible variants of pNE131, and, as noted, our results are compatible with the presence of an extra 0.1 kb in the gene portions of our inducible *ermC* strains. For the purpose of the present discussion, we combine inducible and constitutive variants of pNE131 and consider *ermM* to be equivalent to *ermC*.

Earlier work characterizing known or presumed *ermC* plasmids from series of clinical isolates is sparse. Lacey (13) reported 36 inducible MLS *S. aureus* strains that were collected from London hospitals and whose resistance phenotype was associated with the presence of small plasmids. Of these, 33 were estimated to be 1.8×10^6 daltons in size (about 2.9 kb) and may have been similar to those of our clinical isolates. Dunny et al. (4) recovered eight MLS staphylococcal strains form animals in this country and found that the isolates contained plasmids resembling pE194; however, three strains that were isolated from humans and examined concurrently yielded plasmids in the size range of pNE131. Individual *S. aureus* strains isolated from humans in Brazil (1) and Moscow (18) similarly yielded plasmids in this latter size range. Lampson and Parisi (14) estimated that 75% of a series of constitutive MLS *S. epidermidis* isolates from a Missouri hospital harbored pNE131.

The present work is the only systematic molecular-epidemiological analysis of *ermC* replicons in *S. aureus* and coagulase-negative staphylococci and clearly shows the predominance of pNE131 in both categories of staphylococci. pNE131 is also homologous, over *erm* gene and putative vector moieties, to a recently sequenced *Bacillus subtilis* plasmid (18). The reason for the apparent evolutionary fitness of the pNE131 class as vector for the directionality of the extensive interspecific spread that has apparently occurred remain to be determined. It is clear, however, that coagulase-negative staphylococci constitute a large clinical reservoir of pNE131 for potential spread to the more virulent *S. aureus*.

The restriction site polymorphism we noted in *ermC*, the presence or absence of a *Hae*III site, corresponds to one of only seven nucleotide differences between the *ermM* and *ermC* coding regions. The 2 strains containing plasmids with a *Hae*III site were in a group of 4 inducible *S. aureus ermC* strains examined for the site, whereas plasmids from all other *ermC* strains characterized in this regard (4 constitutive *S. aureus* and 10 coagulase-negative isolates) lacked the site. This implies some linkage, albeit weak, between the *Hae*III site and the classical *S. aureus* inducibility features described for *ermC* in pE194 (9). More strains and further sequence analysis are required to lend substance to this idea.

***ermA* isolates of *S. aureus*.** *ermA* strains constitute a major fraction of our MLS isolates, especially for *S. aureus*. The transposon that characteristically harbors *ermA*, Tn*554*, has unusual properties, one of which is the aforementioned avidity for a unique chromosomal primary target site. Thus, the apparent presence of a copy of Tn*554* in this site in all of our *ermA S. aureus* isolates is not unexpected. However, the occurrence of extra chromosomal inserts in 4 of 11 *S. aureus* (and, as discussed below, 5 of 8 coagulase-negative staphylococcal) *ermA* isolates is most surprising. Rare insertions of Tn*554* into a secondary site, different in sequence from the primary target site, have been achieved in the laboratory only when the secondary site is on a plasmid and the primary chromosomal site is occupied (22, 23) or when the primary target site has been deleted (E. Murphy, personal communication).

The spectinomycin data may help explain the occurrence of extra inserts in some of our *S. aureus* isolates. The *Spc* gene of Tn*554* apparently has undergone mutational drift toward inactivation in the past, as evidenced by the spectinomycin susceptibility of most of our single-insert *S. aureus* strains. Perhaps the increased use of spectinomycin associated with the appearance of penicillin-resistant *Neisseria gonorrhoeae* has created increased evolutionary pressure for spectinomycin resistance in *S. aureus*, and the response of the organism has been to acquire a second chromosomal copy of Tn*554*, with an active *Spc* gene. Why this might occur in nature, but not in the laboratory, is not known.

Transduction is likely to be involved in the spread of Tn*554* among staphylococcal strains, in view of the general importance of this mechanism in the evolution of the staphylococcus (25). Whatever the mechanism of cell-to-cell transfer, insertion into the recipient chromosome of an extra copy of the transposon could then arise by either of two distinct molecular mechanisms. (i) Transposition into a duplicated chromosomal primary target site might occur; this would lead to new bands on Southern hybridization if the hypothetical duplication did not extend to the flanking restriction sites examined or if mutation leading to restriction site polymorphism postdates the duplication. (ii) The transposon may insert into secondary targets, as can be forced to occur with plasmid recipients (22, 23). Experiments to distinguish between these two mechanisms are in progress.

Extra insertions would presumably have to circumvent the *trans* inhibition of transposition exerted by established Tn*554* (19, 30). At least for a multicopy plasmid with Tn*554* in a secondary site, the inhibition can be explained by titration of required factors (such as transposase[s]) by a short (<90-base-pair) locus, TnpI, at an end of the transposon (19). Whether in nature *trans* inhibition is overcome by a specific property of extra transposons (such as enhanced affinity for transposase or high template activity for transposase mRNA) or simply represents selection over long periods for very low frequency events remains to be determined.

***ermA* coagulase-negative isolates.** This is the first description, to our knowledge, of *ermA* determinants in coagulase-negative staphylococci. Although the coagulase-negative *ermA*-containing MLS isolates constituted a smaller fraction of total MLS isolates than found for *S. aureus*, *ermA* was about equally prevalent in coagulase-negative isolates, in view of the higher overall incidence of MLS resistance among these isolates in our study (45 versus 22% for *S. aureus*). The variation of the restriction patterns found for the *ermA* coagulase-negative staphylococcal species, both relative to *S. aureus* and among each other, is perhaps to be expected in view of the substantial DNA sequence differences among the species involved (11). Nevertheless, that the *ermA* gene occurs in Tn*554* or a closely related element in all cases is indicated by the conservation of the 3.5-kb vector fragment released by *Hin*dIII-*Pst*I digestion (arrow in Fig. 6) and the absence of any other vector fragment in such digests (Fig. 6; Table 4). A second feature characteristic of Tn*554*, spectinomycin resistance, is also present. However, the apparent correlation between spectinomycin susceptibility and single inserts, as seen in *S. aureus*, does not hold for the coagulase-negative strains examined; thus the high frequency of extra inserts in these strains cannot be plausibly attributed to selection by spectinomycin usage. Speculation as to mechanisms of extra insertions are as for *S. aureus*; we presume that all the species have the same primary target sequence, despite the apparent variation in flanking sequences, but this must of course be verified experimentally.

The variety and complexity of restriction patterns generated by mixed digestion (Fig. 6) suggest that such patterns might be useful for epidemiologic studies of *ermA* coagulase-negative staphylococci, as recently described for random chromosomal probes in *Salmonella* spp. (35). Many more *ermA* isolates are required for this idea to be evaluated. In any event, our results suggest that, as for *ermC*, coagulase-negative staphylococci can serve as a major reservoir of *ermA* determinants for interspecies spread.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-14957 from the National Institute for General Medical Sciences, a General Research Support Grant awarded to the University of Medicine and Dentistry of New Jersey-Rutgers Medical School (now known as Robert Wood Johnson Medical School), and grant 86-40 from the American Heart Association, New Jersey Affiliate. S.T-V. received a stipend from Public Health Service grant CA-09069 from the National Cancer Institute, and W.D.J. received a stipend from the Foundation of the University of Medicine and Dentistry of New Jersey.

We thank J. Parisi, D. Clewell, B. Weisblum, and E. Murphy for sending strains, J. Parisi and E. Murphy for communicating results prior to publication, and E. Murphy for valuable discussions.

LITERATURE CITED

1. **Bastos, M. C. F., M. D. Bonaldo, and E. G. C. Penido.** 1980. Constitutive erythromycin resistance plasmid in *Staphylococcus aureus*. J. Gen. Microbiol. **121**:513–516.
2. **Chabbert, Y.** 1956. Antagonisme *in vitro* entre l'erythromycine et la spiramycine. Ann. Inst. Pasteur (Paris) **90**:787–790.
3. **Clewell, D. B., Y. Yagi, G. M. Dunny, and S. K. Schultz.** 1974. Characterization of three plasmid deoxyribonucleic acid molecules in a strain of *Streptococcus faecalis*: identification of a plasmid determining erythromycin resistance. J. Bacteriol. **117**:283–289.
4. **Dunny, G. M., P. J. Christie, J. C. Adsit, E. S. Baron, and R. P. Novick.** 1981. Effects of antibiotics in animal feed on the antibiotic resistance of the gram positive bacterial flora of animals and man, p. 557–565. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity and ecology of bacterial plasmids. Plenum Publishing Corp., New York.
5. **Duval, J.** 1985. Evolution and epidemiology of MLS resistance. J. Antimicrob. Chemother. **16**(Suppl. A):137–149.
6. **Hardy, K., and C. Haefeli.** 1982. Expression in *Escherichia coli* of a staphylococcal gene for resistance to macrolide, lincosamide, and streptogramin type B antibiotics. J. Bacteriol. **152**:524–526.
7. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **14**:193–197.
8. **Horinouchi, S., W. H. Byeon, and B. Weisblum.** 1983. A complex attenuator regulates resistance to macrolides, lincosamides, and streptogramin type B antibiotics in *Streptococcus sanguis*. J. Bacteriol. **154**:1252–1262.
9. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. J. Bacteriol. **150**:804–814.
10. **Iordanescu, S.** 1976. Three distinct plasmids originating in the same *Staphylococcus aureus* strain. Arch. Roum. Pathol. Exp. Microbiol. **35**:111–118.
11. **Kloos, N. E.** 1980. Natural populations of the genus staphylococcus. Annu. Rev. Microbiol. **34**:559–592.
12. **Krolewski, J. J., E. Murphy, R. P. Novick, and M. G. Rush.** 1981. Site specificity of the chromosomal insertion of *Staphylococcus aureus* transposon Tn554. J. Mol. Biol. **152**:19–33.
13. **Lacey, R. W.** 1984. Antibiotic resistance in *Staphylococcus aureus* and streptococci. Br. Med. Bull. **40**:77–83.
14. **Lampson, B. C., and J. T. Parisi.** 1986. Naturally occurring *Staphylococcus epidermidis* plasmid expressing constitutive macrolide-lincosamide-streptogramin B resistance contains a deleted attenuator. J. Bacteriol. **166**:479–483.
15. **Lampson, B. C., and J. T. Parisi.** 1986. Nucleotide sequence of the constitutive macrolide-lincosamide-streptogramin B resistance plasmid pNE131 from *Staphylococcus epidermidis* and homologies with *Staphylococcus aureus* plasmids pE194 and pSN2. J. Bacteriol. **167**:888–892.
16. **Mekalanos, J. J.** 1983. Duplication and amplification of toxin genes in *Vibrio cholerae*. Cell **35**:253–263.
17. **Mitsuhashi, S., H. Hashimoto, M. Kono, and M. Morimura.** 1965. Drug resistance of staphylococci. II. Joint elimination and joint transduction of the determinants of penicillinase production and resistance to macrolide antibiotics. J. Bacteriol. **89**:988–992.
18. **Monod, M., C. Denoya, and D. Dubnau.** 1986. Sequence and properties of pIM13, a macrolide-lincosamide-streptogramin B resistance plasmid from *Bacillus subtilis*. J. Bacteriol. **167**:138–147.
19. **Murphy, E.** 1983. Inhibition of Tn*554* transposition: deletion analysis. Plasmid **10**:260–269.
20. **Murphy, E.** 1985. Nucleotide sequence of *ermA*, a macrolide-lincosamide-streptogramin B determinant in *Staphylococcus aureus*. J. Bacteriol. **162**:633–640.
21. **Murphy, E., L. Huwyler, and M. C. Bastos.** 1985. Transposon Tn*554*: complete nucleotide sequence and isolation of transposition-defective and antibiotic-sensitive mutants. EMBO J. **4**:3357–3365.
22. **Murphy, E., and S. Lofdahl.** 1984. Transposition of Tn*554* does not generate a target duplication. Nature (London) **307**:292–294.
23. **Murphy, E., S. Phillips, I. Edelman, and R. P. Novick.** 1981. Tn*554*: isolation and characterization of plasmid insertions. Plasmid **5**:292–305.
24. **Novick, R. P., and R. Brodsky.** 1972. Studies on plasmid replication. I. Plasmid incompatibility and establishment in *Staphylococcus aureus*. J. Mol. Biol. **68**:285–302.
25. **Novick, R. P., S. A. Khan, E. Murphy, S. Iordanescu, I. Edelman, J. Krolewski, and M. Rush.** 1980. Hitchhiking transposons and other mobile genetic elements and site-specific

recombination systems in staphylococci. Cold Spring Harbor Symp. Quant. Biol. **45:**67–76.

26. **Novick, R. P., and E. Murphy.** 1985. MLS-resistance determinants in *Staphylococcus aureus* and their molecular evolution. J. Antimicrob. Chemother. **16**(Suppl. A)**:**101–110.
27. **Novick, R. P., E. Murphy, T. J. Gryczan, E. Baron, and I. Edelman.** 1979. Penicillinase plasmids of *Staphylococcus aureus*: restriction-deletion maps. Plasmid **2:**109–129.
28. **Parisi, J. T., J. Robbins, B. C. Lampson, and D. W. Hecht.** 1981. Characterization of a macrolide, lincosamide, and streptogramin resistance plasmid in *Staphylococcus epidermidis*. J. Bacteriol. **148:**559–564.
29. **Perkins, J. B., and P. J. Youngman.** 1984. A physical and functional analysis of Tn*917*, a *Streptococcus* transposon in the Tn*3* family that functions in *Bacillus*. Plasmid **12:**119–138.
30. **Phillips, S., and R. P. Novick.** 1979. Tn*554*—a site-specific repressor-controlled transposon in *Staphylococcus aureus*. Nature (London) **278:**476–478.
31. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling of deoxyribonucleic acid to high specific activity *in vitro* by nick translation. J. Mol. Biol. **113:**237–251.
32. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
33. **Thakker-Varia, S., A. R. Ranzini, and D. T. Dubin.** 1985. Mechanism of erythromycin-resistance in *Staphylococcus aureus* and *E. coli*. The "MLS" oligonucleotide of 23S RNA. Plasmid **14:**152–161.
34. **Thuring, R. W. J., J. P. M. Sanders, and P. Borst.** 1975. A freeze-squeeze method for recovering long DNA from agarose gels. Anal. Biochem. **66:**213–220.
35. **Tompkins, L. S., N. Troup, A. Labigne-Roussel, and M. L. Cohen.** 1986. Cloned random chromosomal sequences as probes to identify *Salmonella* species. J. Infect. Dis. **154:**156–162.
36. **Weisblum, B.** 1985. Inducible resistance to macrolides, lincosamides and streptogramin type B antibiotics: the resistance phenotype, its biological diversity, and structural elements that regulate expression—a review. J. Antimicrob. Chemother. **16**(Suppl. A)**:**63–90.
37. **Weisblum, B., and V. Demohn.** 1969. Erythromycin-inducible resistance in *Staphylococcus aureus*: survey of antibiotic classes involved. J. Bacteriol. **98:**447–452.
38. **Weisblum, B., S. R. Holder, and S. M. Halling.** 1979. Deoxyribonucleic acid sequence common to staphylococcal and streptococcal plasmids which specify erythromycin resistance. J. Bacteriol. **138:**990–998.