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Resistance to Mercury and to Cadmium in Chromosomally Resistant *Staphylococcus aureus*

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Apparently chromosomally located mercury resistance determinants in five methicillin-resistant *Staphylococcus aureus* strains of different geographical origin were structurally homologous to plasmid-located mercury resistance determinants in *S. aureus*. These were all located on a 6.3-kilobase (kb) *BglII* fragment, as evident from Southern hybridization experiments with the 6.3-kb *BglII* fragment of plasmid pI258 as the probe. These methicillin-resistant *S. aureus* strains exhibited similar phage susceptibility patterns and biochemical reactions. They differed, however, in the DNA location of the mercury resistance determinants, as evidenced by neighboring cleavage sites for restriction endonucleases *EcoRI*, *HindIII*, and *PstI*. In an environmental (nonhospital) strain in which mercury resistance was also apparently chromosomally conferred, these determinants were also homologous to pI258 DNA, but they were located on a 6.6-kb *BglII* fragment. Cadmium resistance determinants in the five methicillin-resistant *S. aureus* strains and the environmental *S. aureus* strain were not similar to the known plasmid-located determinants *cadA* and *cadB*. Cd^{2+} resistance was based on an efflux mechanism for Cd^{2+}. However, no parallel resistance to zinc was conferred. The 3.2-kb *XbaI-BglII* fragment obtained from plasmid pI258 and used as a *cadA*-specific probe did not hybridize to total DNA digests of the strains with apparently chromosomally determined cadmium resistance.

Resistance to mercury has been found in a number of *Staphylococcus aureus* strains, especially in those connected with outbreaks of infections in hospitals (6, 30). In the strains investigated until now, mercury resistance has been plasmid encoded: on plasmid pI258 the *merA* gene codes for the mercuric reductase enzyme, and the *merB* gene codes for an organomercurial lyase enzyme (25). Both determinants are located on a 6.3-kilobase (kb) fragment obtained after digestion with restriction endonuclease *BglII*. This fragment is present on other plasmids that confer mercury resistance (15, 22). Resistance to cadmium is widespread, occurring in a variety of different *S. aureus* strains (13, 15). It is conferred by two separate genes, *cadA* and *cadB* (14, 17, 26). While *cadA* codes for an energy-dependent efflux mechanism (23), the *cadB* gene product may bind Cd^{2+} (17). These determinants are located on plasmids (14, 26). However, there also has been preliminary evidence for chromosomal genes conferring cadmium resistance in *S. aureus* (R. P. Novick, personal communication; unpublished results), but no data on the mechanism of resistance or a relation to *cadA* or *cadB* have been reported.

Multiple-antibiotic-resistant *S. aureus* strains with resistance to methicillin have been described in different countries (3, 5, 18, 19). Methicillin-resistant *S. aureus* strains isolated in the German Democratic Republic exhibit a rather unusual phenotype with regard to their phage susceptibility patterns and biochemical characteristics. In addition to chloramphenicol resistance (plasmid conferred, if present), resistance to other antibiotics, as well as to mercury and to cadmium, is apparently conferred by chromosomally located determinants in these strains (W. Witte, Nguyen Van Dip, and D. Dünnhaupt, J. Hyg. Epidemiol. Microbiol. Immunol. [Prague], in press).

The mechanisms of mercury and cadmium resistance in these apparently plasmidless strains, as well as the DNA homology relationship between the chromosomally located and the known plasmid-located determinants for mercury and cadmium resistance, are of interest from the evolutionary and epidemiological points of view. Our study of mercury and cadmium resistance includes methicillin-resistant *S. aureus* strains from the German Democratic Republic and from Vietnam, Austria, and the Soviet Union, as well as a mercury- and cadmium-resistant environmental strain. The data presented in this paper show that the mechanism of mercury resistance in methicillin-resistant *S. aureus* is volatilization and that the genetic determinants are structurally homologous to known plasmid-located ones. The mechanism of cadmium resistance in methicillin-resistant *S. aureus* is efflux. However, the determinant is not homologous to *cadA*.

MATERIALS AND METHODS

Bacterial strains and media. Wild-type *S. aureus* strains and their characteristics are listed in Table 1. *S. aureus* 8325-4 (strain 8325 cured of a temperate phage; R. P. Novick, personal communication) and 8325-4 with plasmids pI258, pI524, and pII147 (14, 15, 25, 26) were provided by R. P. Novick.

For growth in liquid medium, 2×NY broth (25 g of casein hydrolysate [Sigma Chemical Co.], 10 g of yeast extract [Difco Laboratories], and 2.5 g of NaCl per liter of deionized water), nutrient broth (Difco), and tryptone broth (16 g of tryptone [Difco] and 10 g of NaCl per liter) were used. For growth on solid medium, nutrient agar (Difco) was used.

Susceptibility tests. The agar diffusion test with disks loaded with mercuric chloride and phenylmercury acetate was described previously (25). Growth inhibition by Cd^{2+}

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TABLE 1. Phenotypes of wild-type *S. aureus*

Strain[a]	MIC (μg/ml) of[b]:											Phage pattern[c]	Biochemical characteristics[d]				
	Pn	Cm	Tc	Mn	Em	Lm	Sm	Gm	Ox	Cd	Hg		C(HP)	C(BP)	CV	HT	Fib
108	0.25[e]	64	128	64	16	1	128	16	128	128	16	A994, RTD	+	−	C	A	+
1172	0.25[e]	64	128	64	32	2	128	8	64	128	16	A994, RTD	+	−	C	B	+
1309	0.5[e]	0.5	128	64	32	1	128	0.25	128	128	16	A994, RTD	+	−	C	B	+
1599	0.25[e]	64	128	64	64	1	128	16	64	128	16	A994, RTD	+	−	C	B	+
1725	0.0025[f]	0.125	128	64	32	2	128	8	64	128	16	77, A994, RTD	+	−	C	B	+
1791	0.0125[f]	0.25	0.025	0.025	0.025	0.5	0.5	0.25	0.5	128	16	NT	+	−	C	A	+

[a] The first five strains were new hospital isolates of methicillin-resistant *S. aureus* from hospitals in the German Democratic Republic, the Soviet Union, Austria, and Vietnam (Witte et al., in press). Strain 1791 is an environmental isolate from the nose of a worker exposed to mercury-containing dust.

[b] Abbreviations: Pn, benzylpenicillin; Cm, chloramphenicol; Tc, oxytetracycline; Mn, minocycline; Em, erythromycin; Lm, lincomycin; Sm, streptomycin; Gm, gentamicin; Ox, oxacillin; Cd, cadmium nitrate; and Hg, mercury chloride.

[c] Determined as described previously (12,28,29). RTD, Routine test dilution; NT, not typable at 100 times the routine test dilution.

[d] The conditions of these tests were as described by Meyer et al. (12). Abbreviations: C(HP), coagulase, human plasma; C(BP), coagulase, bovine plasma; CV, crystal violet type; HT, hemolysin type; Fib, fibrinolysin formation. The crystal violet test involves the color of colonies on crystal-violet-containing agar. Type C colonies stain violet with an orange tone. Type A, B, D, and E colonies stain yellow, yellow with a violet edge, blue, and white, respectively. The hemolysin types refer to lysis patterns on agar containing sheep erthyrocytes (12).

[e] β-Lactamase formation, as determined by the microiodometric test.

[f] Lack of β-lactamase formation, as determined by the microiodometric test.

and Zn^{2+} was determined in nutrient broth cultures as described previously (17). MICs were determined by the agar dilution test as described previously (29).

Characterization of *S. aureus* strains. Phage typing and biochemical typing were performed as described by Meyer et al. (12) (see Table 1, footnotes *c* and *d*).

Mercury volatilization assays. Mercury volatilization assays were run with broth cultures and were performed as described previously (20, 25).

Cadmium efflux. $^{109}Cd^{2+}$ was used to determine cadmium efflux. The procedure described by Tynecka et al. (23) was followed.

Preparation of cellular DNA from *S. aureus*. The cells were grown overnight at 37°C in 5 ml of 2×NY broth with shaking. Fresh 2×NY broth (50 ml) was inoculated with 1 ml of the overnight culture and shaken for 7 h at 37°C. The cells were centrifuged and washed with 20 ml of TES buffer (0.05 M Tris hydrochloride, 0.05 M disodium EDTA, 0.025 M NaCl, pH 7.3). After being washed, the cells were resuspended in 3.6 ml of TES buffer, and 0.2 ml of lysostaphin solution (1 mg/ml in TES, pH 7.3) and 0.2 ml of lysozyme solution (10 mg/ml in TES) were added. After incubation at 37°C for 30 min, 8 ml of lysis buffer (1% Brij 58 in TES buffer) was added. The resulting lysate was cooled on ice and cleared by centrifugation at 4°C for 30 min at 40,000 × *g*. Immediately after being cleared, the lysate was extracted two times with an equal volume of phenol-chloroform (1:2, vol/vol). After this treatment, the lysate was subjected to extraction twice with the same volume of ethyl ether. To the aqueous phase, sodium acetate was added to give a final concentration of 0.3 M. The DNA was precipitated by the addition of twice the volume of absolute ethanol (−20°C). After at least 2 h at −20°C, the precipitate was collected by centrifugation, dried under vacuum, and suspended in 250 μl of DNA buffer (10 mM Tris hydrochloride, pH 7.9, 10 mM NaCl, 0.1 mM EDTA).

DNA-DNA hybridization studies. For agarose gel electrophoresis and digestion with restriction endonucleases, we used previously published procedures (2, 11). Methods for transfer to nitrocellulose filters (Southern blotting), nick translation, and hybridization with radioactive probes were described previously (4, 8). Hybridization was done under stringent conditions at 65°C for 16 to 20 h in a buffer consisting of 0.5 M NaCl, 0.1 M Na_2HPO_4, 5 mM EDTA, and 1% Sarkosyl (8), with an initial rinse at 25°C with 1 mM Tris hydrochloride (pH 8)–1% Sarkosyl followed by four 5-min washes at 25°C with 1 mM Tris hydrochloride, pH 8.

Probe DNA. To obtain probe DNA fragments specifically carrying the mercury resistance determinant and the *cadA* gene, we cloned the 12.5-kb *Xba*I fragment of plasmid pI258 into plasmid pUC12 (24). The restriction site map of plasmid pUC12 with the integrated 12.5-kb fragment of plasmid pI258 and the fragments used as probes is shown in Fig. 1. After restriction endonuclease treatment and electrophoresis, the fragments of interest were isolated from agarose by the glass powder method (4).

RESULTS

Lack of plasmids in the investigated methicillin-resistant *S. aureus* strains and in strain 1791. When the six test strains were lysed by lysostaphin and screened for the occurrence of plasmids, no plasmid-specific DNA band could be detected in agarose gels (data not shown). However, the method used (9) is rather mild and allowed the demonstration of rather large bacterial plasmids. The 28-kb plasmid pI258 was readily visualized in control lysates. The possibility remains, of course, that the hospital isolates contain large or difficult-to-isolate plasmids or both that we could not detect with the procedures used.

Mercury resistance. The five methicillin-resistant *S. aureus* strains and strain 1791 were resistant to mercuric ions and to phenylmercury acetate; control strain 8325-4 was susceptible (data not shown). The presence of an inducible mercuric reductase was shown by appropriate volatilization experiments (Fig. 2).

Whether the mercury resistance determinants in the methicillin-resistant *S. aureus* strains were homologous at the DNA sequence level to those carried on plasmids in *S. aureus* was tested by Southern blotting hybridization experiments. In these experiments, the 6.3-kb *Bgl*II fragment of plasmid pI258 served as a probe (Fig. 1). This fragment carries the *merA* and *merB* determinants (14). After nick translation, this probe was hybridized to *Bgl*II digests of total cellular DNA isolated from the five methicillin-resistant *S. aureus* strains and strain 1791. The probe was also hybridized to *Bgl*II digests of whole cellular DNA isolated from derivatives of strain 8325-4 carrying plasmids pI258, pI524, and pII147 as representatives of different well-

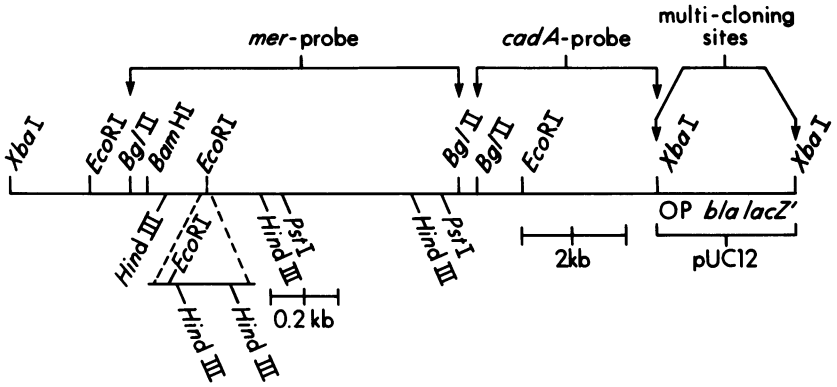

FIG. 1. Restriction endonuclease map of plasmid pUC12 with the integrated 12.5-kb *Xba*I fragment of plasmid pI258. The fragments used as DNA probes for the mercury resistance determinant (*mer*-probe) and the *cadA* gene (*cadA*-probe) are indicated. The restriction endonuclease sites above the line were determined by cleavage and agarose gel electrophoresis and confirmed by direct DNA sequencing (Laddaga et al., in preparation); those below the line (for the *mer*-probe only) were determined from the DNA sequence. Note the expanded scale near the *Eco*RI site.

characterized *S. aureus* plasmids that confer mercury resistance (22) and pP1408, a plasmid from a recent wild-type isolate (27) (Fig. 3). The 6.3-kb probe hybridized to the corresponding fragments from the *S. aureus* plasmids and also to the corresponding fragments of total cellular DNA from the five methicillin-resistant *S. aureus* strains. The *Bgl*II fragment from the chromosomal DNA of strain 1791 to which the 6.3-kb probe hybridized was slightly larger (6.6 kb). The 6.3-kb probe DNA did not hybridize to chromosomal *Bgl*II digests of strain 8325-4 (Fig. 3, lane A) or to *Bgl*II and *Eco*RI digests of cellular DNA from a spontaneously occurring mercury-susceptible variant of strain 1172 (Fig. 4, lanes G and H). On the basis of the results of phage typing, the methicillin-resistant *S. aureus* strains included in these experiments seem to be very similar (Table 1). The question arose as to whether these strains are representatives of a single "clone" with a uniform location of the mercury resistance determinant apparently on the chromosome. We checked the location of the mercury-resistance-specific *Bgl*II fragment in relation to the neighboring restriction endonuclease cleavage sites by using the 6.3-kb *Bgl*II fragment (Fig. 1) as a probe for hybridization to fragments of total cellular DNA obtained after separate digestions with *Eco*RI, *Hin*dIII, and *Pst*I. The 6.3-kb *Bgl*II fragment of pI258 has one internal site for *Eco*RI (14) (now known from DNA sequencing analysis to occur 1,516 base pairs from the left end of the *Bgl*II fragment, as shown in Fig. 1; R. A. Laddaga et al., manuscript in preparation). Assuming complete homology of this sequence to the sequence of the chromosomal mercury resistance determinants, then hybridization of the probe to chromosomal *Eco*RI digests should give two bands. If there are two copies of this sequence in the cell at different locations, four different bands should appear. Figure 4 shows the results of this hybridization

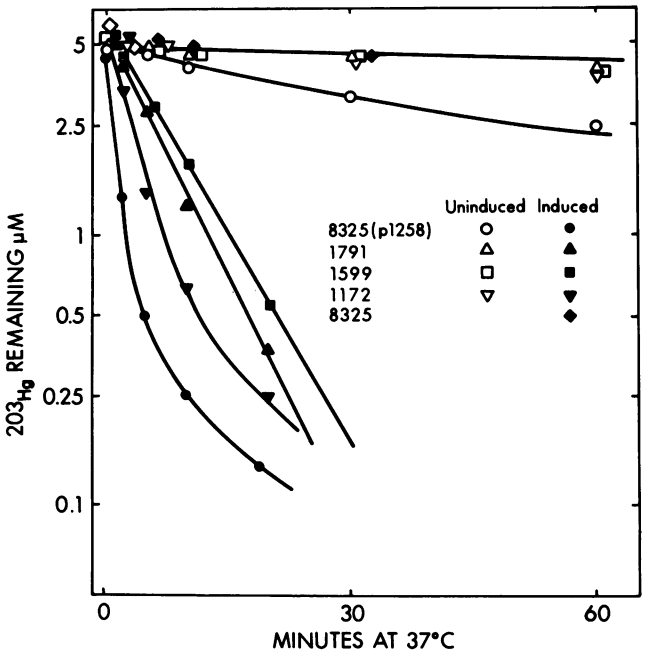

FIG. 2. Volatilization of ^{203}Hg from $^{203}Hg^{2+}$. *S. aureus* 1791, 1599, 1172, 8325-4, and 8325-4(pI258) were grown in nutrient broth. Mercury volatilization activity was induced by the addition of 5 μM Hg^{2+} and growth for 1 h. The cells were harvested by centrifugation, and the rate of volatilization of mercury from 5 μM $^{203}Hg^{2+}$ was determined as previously described (25).

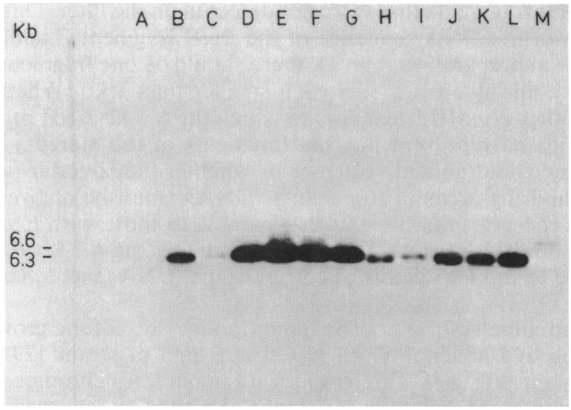

FIG. 3. Hybridization of the ^{32}P-labeled mercury resistance probe (6.3-kb *Bgl*II fragment of plasmid pI258) to *Bgl*II digests of whole cellular DNA. Lanes: A, strain 8325-4, control; B, 6.3-kb *Bgl*II probe DNA, 1 μg; C, probe DNA, 0.1 μg; D, 8325-4(pI258); E, 8325-4(pI524); F, 8325-4(pII147); G, 8325-4(p1408); H through M, strains 108, 1309, 1172, 1599, 1725, and 1791, respectively.

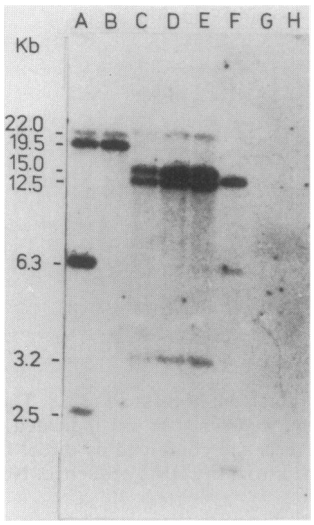

FIG. 4. Hybridization of the ^{32}P-labeled mercury resistance probe (6.3-kb *Bgl*II fragment of plasmid pI258) to *Eco*RI digests of whole cellular DNA. Lanes: A through F, strains 108, 1309, 1172, 1599, 1725, and 1791, respectively; G and H, *Bgl*II and *Eco*RI digests, respectively, of whole cellular DNA from the mercury-susceptible variant of strain 1172.

experiment. The mercury resistance determinants were uniformly located in strains 1172, 1599, and 1725. The location was different between the above-mentioned strains and strains 108, 1309, and 1791. The four bands of hybridization with DNA from strains 108, 1172, 1599, and 1725 could be interpreted as indicating two different locations. However, incomplete digestion of the cellular DNA or the occurrence of common DNA sequences in the mercury resistance probe and in other genes might also account for these patterns.

When the five hospital *S. aureus* strains and strain 1791 were compared, hybridization of the 6.3-kb *Bgl*II probe to *Hin*dIII and *Pst*I digests of total cellular DNA (Fig. 5) was similar to that described for the *Eco*RI digests. The radioactive bands detected after *Hin*dIII digestion and hybridization of the 6.3-kb *Bgl*II probe summed up to a lower molecular mass than that of the probe DNA (Fig. 5, lanes D to J). Digestion of the probe DNA with *Hin*dIII produced a fragment of approximately 3.3 kb (Fig. 5, lanes B and C) and smaller fragments that were not detected on the filter. On the basis of the DNA sequence of the *Bgl*II fragment (Laddaga et al., in preparation; Fig. 1), there should be one fragment of 2.9 kb and five fragments each smaller than 1 kb. Whether the other *Hin*dIII fragments to which the 6.3-kb *Bgl*II probe hybridized represent internal fragments of the mercury resistance determinant sequence or whether they overlap with neighboring areas of the cellular DNA cannot be answered from these results. Results comparable to those with *Eco*RI and *Hin*dIII were obtained by hybridizing the 6.3-kb *Bgl*II probe to the *Pst*I digest of chromosomal DNA (Fig. 5, lanes K to O).

Cadmium resistance. The inhibition by Cd^{2+} of the growth of the five hospital *S. aureus* strains and of strain 1791 is shown in Fig. 6A. The strains with apparently chromosomally conferred cadmium resistance exhibited an intermediate level of cadmium resistance, in between susceptible strain 8325-4 and plasmid-conferred (*cadA*) resistance levels. This behavior is similar to that of plasmid-conferred *cadB* resistance (17). However, both *cadA* and *cadB* confer parallel resistance to Zn^{2+}, which was not found in the five hospital methicillin-resistant *S. aureus* strains or in strain 1791 (Fig. 6B). These wild-type strains were as susceptible to Zn^{2+} as the susceptible reference strain 8325-4. This result suggests that the cadmium resistance determinant in these strains is neither *cadA* nor *cadB*. Because of these findings, we checked whether the mechanism of cadmium resistance in these strains was an efflux process, as described by Tynecka et al. (23). Cells were loaded with $^{109}Cd^{2+}$, and the efflux of $^{109}Cd^{2+}$ was determined (Fig. 7). No efflux was observed in strain 8325-4, in contrast to strain 8325-4(pI258) and hospital strain *S. aureus* 108. Data obtained for strains 1309, 1172, 1599, 1725, and 1791 were similar to those obtained for strain 108 and are therefore not shown. This efflux could be nearly completely inhibited by incubation of the cells at 4°C and was slightly inhibited by the presence of carbonyl cyanide *m*-chlorophenylhydrazone (an uncoupler of respiratory electron transport), suggesting an energy-dependent efflux mechanism, as reported earlier (23). Since the mechanism of cadmium resistance in the methicillin-resistant *S. aureus* strains and in strain 1791 was efflux, as is the case with *cadA* but not with *cadB*, and since parallel resistance to Zn^{2+} was lacking, it was important to check whether there was DNA sequence homology between the *cadA* gene and the apparently chromosomally located cadmium resistance determinants in these strains. A *cadA*-specific DNA probe was hybridized to restriction endonuclease digests of total cellular DNA. The *cadA*-specific probe was the 3.2-kb fragment obtained after *Xba*I-*Bgl*II double digestion of the 12.5-kb *Xba*I fragment of plasmid pI258 cloned into plasmid pUC12 (Fig. 1). The *Xba*I-*Bgl*II *cadA* probe hybridized to *Xba*I-*Bgl*II double digests and to *Eco*RI digests of the reference plasmids pI258 and pII147 exactly as predicted from the published maps of plasmids pI258 and pII147 (14) (Fig. 8). The probe hybridized to a 3.2-kb *Xba*I-*Bgl*II fragment of pI258 and to a 2.8-kb *Xba*I-*Bgl*II fragment of pII147. The

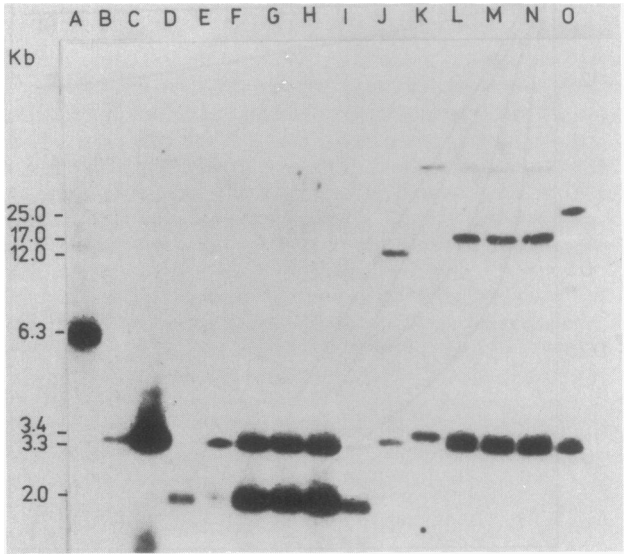

FIG. 5. Hybridization of the ^{32}P-labeled mercury resistance probe (6.3-kb *Bgl*II fragment of plasmid pI258) to *Hin*dIII and *Pst*I digests of whole cellular DNA. Lanes: A, 6.3-kb *Bgl*II probe fragment; B, 6.3-kb *Bgl*II probe fragment digested with *Hin*dIII, 0.1 μg; C, plasmid pI258 digested with *Hin*dIII, 1 μg; D through J, *Hin*dIII-digested total DNA from strains 108, 1309, 1172, 1599, 1725, and 1791, respectively; K through O, *Pst*I-digested total DNA from strains 108, 1309, 1172, 1599, 1725, and 1791, respectively.

weaker hybridization to a 6.3-kb fragment of the *Xba*I-*Bgl*II digest of pII147 was unexpected and cannot be currently explained.

The *cadA*-specific probe DNA did not hybridize to *Xba*I-*Bgl*II digests of the cellular DNA from the five hospital *S. aureus* strains and strain 1791 (Fig. 8), indicating an absence of a significant structural relationship under these high stringency conditions between the cadmium resistance determinants in these strains and the *cadA* gene.

DISCUSSION

The results described show that the apparently chromosomally located mercury resistance determinants in methicillin-resistant *S. aureus* have DNA sequences homologous to those on the penicillinase plasmids of *S. aureus*. This result suggests that they may have originated from corresponding plasmids. In *S. aureus*, the integration of the entire plasmid pI258 into the chromosome (21) and the integration of clearly translocatable resistance determinants (Tn*551* for resistance to erythromycin and other macrolide antibiotics [16]; Tn*4001* for aminoglycoside resistance [10]) have been described in connection with laboratory experiments. Also, naturally occurring strains possess such translocatable elements integrated into their chromosomes (β-lactamase in strain PS*80* [1]; macrolide, lincosamide, and streptogramin B resistance in discrete multiple-antibiotic-resistant hospital strains [27]; aminoglycoside resistance in different multiple-antibiotic-resistant strains [28]). From previous studies of different penicillinase plasmids of *S. aureus* (22), it was concluded that the mercury resistance determinants on the 6.3-kb *Bgl*II fragment might be a part of a translocatable element which became integrated into different plasmids. The question of whether methicillin-

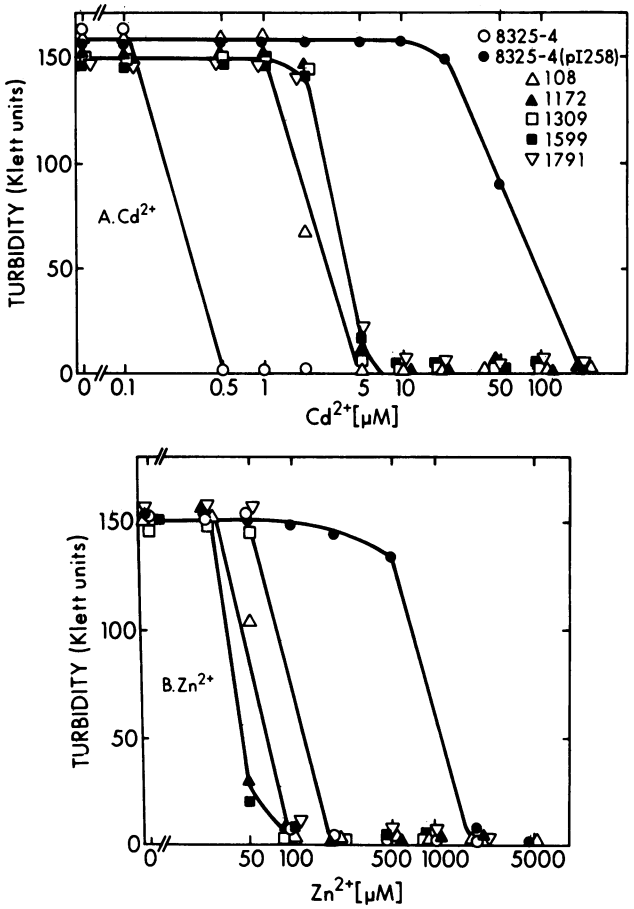

FIG. 6. Growth inhibition by cadmium and by zinc of methicillin-resistant *S. aureus* 108, 1172, 1309, and 1599, environmental strain 1791, and controls without and with *cadA*-mediated resistance. Overnight cultures in nutrient broth were diluted 1:200 either into nutrient broth containing various concentrations of $CdCl_2$ (A) or into nutrient broth containing 50 mM potassium phosphate (pH 6.8) and various concentrations of $ZnSO_4$ (B). Culture turbidities were measured after 7 h of growth at 37°C.

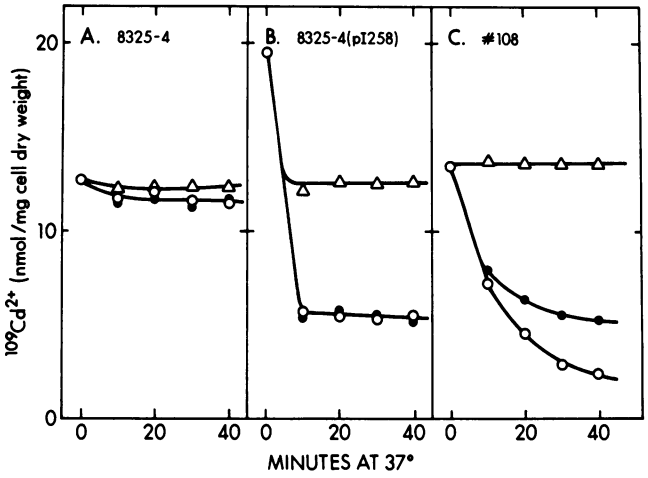

FIG. 7. Efflux of $^{109}Cd^{2+}$ from *S. aureus* without cadmium resistance (strain 8325-4) (A), with cadmium resistance conferred by *cadA* [strain 8325-4(pI258)] (B), and with cadmium resistance apparently conferred by chromosomal genes (strain 108) (C). Cells were grown in tryptone broth for 20 min in the presence of 20 μM $^{109}Cd^{2+}$ (strain 8325-4) or 100 μM $^{109}Cd^{2+}$ [strains 8325-4(pI258) and 108]. Dilution-induced efflux was assayed at 37°C (○), at 37°C in the presence of 100 μM carbonyl cyanide *m*-chlorophenylhydrazone (●), and at 4°C (△) as described previously (23, 26).

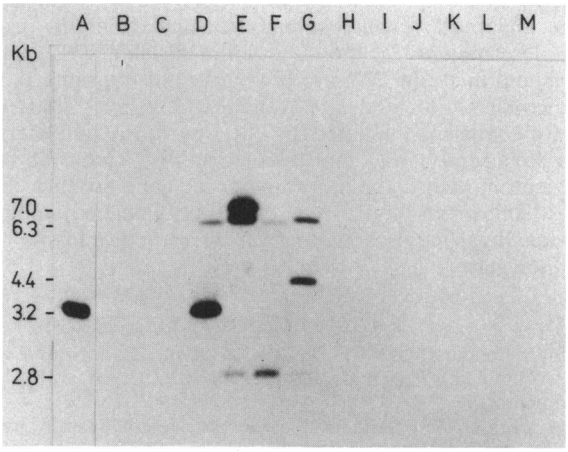

FIG. 8. Hybridization of the *cadA*-specific probe (3.2-kb *Xba*I-*Bgl*II fragment of plasmid pI258) to digests of whole cellular DNA. Lanes: A, 3.2-kb DNA probe, 1.0 μg, control; B, DNA probe, 0.1 μg; C, 8325-4 cellular DNA, *Xba*I-*Bgl*II double digest; D, 8325-4(pI258) cellular DNA, *Xba*I-*Bgl*II double digest; E, 8325-4(pI258), *Eco*RI digest; F, 8325-4(pII147) cellular DNA, *Xba*I-*Bgl*II double digest; G, 8325-4(pII147), *Eco*RI digest; H through M, *Xba*I-*Bgl*II digests of total cellular DNA from strains 108, 1309, 1172, 1599, 1725, and 1791, respectively.

resistant *S. aureus* strains originally had an entire plasmid incorporated into the chromosome or whether a mercury-resistance-conferring translocatable element was incorporated into the chromosome remains open. As evident from the mercury-susceptible spontaneous variant of strain 1172, the 6.3-kb *Bgl*II fragment can be lost from the chromosome, as was previously described in cases of the instability of mercury resistance carried on plasmids (22).

Clearly, the 6.3-kb *Bgl*II fragment can occupy different locations on the cellular DNA of methicillin-resistant *S. aureus*, as shown by the hybridization to total DNA digests obtained with different restriction endonucleases. The possibility of restriction endonuclease site polymorphism (the existence of different restriction endonuclease site patterns within the mercury resistance regions [7]) as an alternative explanation is rather unlikely, since the similarities (strains 1172, 1599, and 1725) or differences (strains 108 and 1309) between the strains were shown when digests obtained with different restriction endonucleases were used. The methicillin-resistant *S. aureus* strains showed common characteristics when tested by conventional phage typing and biotyping methods (Table 1). However, differentiation between the strains was possible with the location of the mercury resistance determinants by use of a specific probe and Southern DNA-DNA hybridization analysis. The use of this molecularly based method for differentiation between strains or clones is a very valuable addition to more conventional methods for describing clones in bacterial epidemiology (27). Detailed analysis in a similar sense has been described for the location of insertion element IS*5* on the chromosome of natural *Escherichia coli* isolates (4).

The mercury resistance determinant of environmental strain 1791 was homologous to that from plasmid pI258. However, it was located on a slightly larger *Bgl*II fragment (6.6 kb; Fig. 1). This result points to a change between the part of the DNA carrying the mercury resistance determinant in this strain and the corresponding DNA region in the hospital methicillin-resistant *S. aureus* strains, although the loss of a single *Bgl*II site due to a single nucleotide change could account for this (see the relative positions of the *Bgl*II sites in Fig. 1).

The apparently chromosomally located cadmium resistance determinant in the methicillin-resistant *S. aureus* strains and in strain 1791 are obviously not the same as the known plasmid-located *cadA* and *cadB* genes. The Cd^{2+} efflux mechanism mediated by the chromosomal determinants apparently does not recognize Zn^{2+} ions. It may represent another cadmium-transporting gene product. This seems likely from the absence of DNA sequence homology between these new cadmium resistance determinants and the *cadA* gene.

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ADDENDUM IN PROOF

The probe DNA carrying the mercury resistance determinant used in our studies had recently been sequenced (R. A. Laddaga et al., manuscript in preparation). This *Bgl*II fragment, which was referred to as 6.3 kb from its mobility on agarose gel electrophoresis, was shown to be 6,404 base pairs in length.

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