Penicillin and Tetracycline Resistance Plasmids in *Staphylococcus epidermidis*

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The genetic nature of penicillin (Pc) and tetracycline (Tc) resistance plasmids in *Staphylococcus epidermidis* were studied and compared with those in *S. aureus*. Of 10 *S. epidermidis* strains transduced for penicillin resistance, we could isolate Pc plasmids from only 3. One of these plasmids also encoded for cadmium resistance and another encoded for resistance to ethidium bromide, traits also associated with *S. aureus* Pc plasmids. Endonuclease fingerprinting of the Pc plasmids from the two species revealed extensive heterogeneity. Two *S. epidermidis* strains were also transduced for tetracycline resistance. Both harbored plasmids indistinguishable from *S. aureus* Tc plasmids as judged by endonuclease fingerprinting. These data suggest that genetic exchange between *S. aureus* and *S. epidermidis* occurs in vivo.

Although the penicillin (Pc) and tetracycline (Tc) resistance plasmids of *Staphylococcus aureus* have been well characterized (8), little is known about these resistance determinants in *S. epidermidis*. Only recently has a Pc^r plasmid been identified in *S. epidermidis* (18). Before this time, the only evidence for *S. epidermidis* Pc^r plasmids was cotransduction or loss of penicillin resistance with other traits (14, 19, 21, 22). The Tc^r plasmids of *S. epidermidis* have been studied in greater detail and are the same size as those of *S. aureus* (12, 20). Recently the Tc^r plasmids of *S. epidermidis* and *S. aureus* were compared by deoxyribonucleic acid (DNA) homology and restriction enzyme analysis and appeared to be similar but not identical (5).

Antibiotic resistances in *S. epidermidis* are clinically important for two reasons: (i) *S. epidermidis* has been increasingly implicated as an opportunistic pathogen (9, 10, 25), and the spread of antibiotic resistance markers within this species could seriously complicate chemotherapy; and (ii) if staphylococci exchange plasmids in vivo, antibiotic resistances harbored by *S. epidermidis* may spread to coresident *S. aureus* strains. The importance of this interspecific exchange is evident when one considers that more strains of *S. epidermidis* are antibiotic resistant than are strains of *S. aureus* isolated under comparable conditions (1, 10).

To determine whether *S. epidermidis* and *S. aureus* strains exchange plasmid DNA in vivo, we characterized the Tc^r and Pc^r plasmids of

these two species. Similarities of phenotypic and physical characteristics were demonstrated, and the significance of these findings was examined.

MATERIALS AND METHODS

Media. Yeast extract broth (YETS) contained a 0.3% yeast extract (Difco Laboratories) and 3% Trypticase soy broth (BBL Microbiology Systems). All agar media used contained 1.5% agar (Difco).

Bacteria and bacteriophages. *S. epidermidis* typing phages and propagating strains of *S. epidermidis* have been described (29) and were kindly supplied by J. T. Parisi. Table 1 lists strains of *S. epidermidis* and their sources. *S. aureus* strains were taken from the stock culture collection of J. N. Baldwin. Staphylococci were identified by the Gram stain reaction, catalase production, and the ability to produce acid from glucose anaerobically after incubation at 37°C for 7 days (27). The coagulase test was used to identify the species of the isolates. Spectinomycin-resistant strains were isolated on YETS plates containing 200 µg of spectinomycin per ml (Trobicin; The Upjohn Co.).

Transduction. Transduction was accomplished as described by Olson et al. (18), using transducing phages 29, 108, and 113. Transductants were selected on plates containing 0.16 µg of penicillin G or 10 µg of tetracycline per ml and were confirmed by their spectinomycin resistance and cryptic plasmid content as detected by the Brij minilysis technique (33).

Phenotypic testing. Antibiotic susceptibility was determined by using a modification of the standardized disc diffusion test (11). YETS was used in place of Mueller-Hinton agar as the test medium. Penicillin- and tetracycline-resistant colonies were scored after growth for 48 h on YETS plates containing 10 µg of tetracycline or 0.16 µg of penicillin per ml. Penicillinase production was detected by the starch iodine overlay technique (1). Aerobic production of acid from man-

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TABLE 1. Characteristics and source of strains of *S. epidermidis* used in transduction experiments^a

Strain	Phenotypic response ^a								Source
	Cd	Asa	Hg	PMA	EB	Pc	Tc	Bla	
Recipient									
JB216	R	NT	R	R	S	S	S	—	UGa
JB248	S	S	S	S	S	S	NT	—	JTP
Donor									
PS29	S	S	S	S	R	R	R	+	JTP
PS108	R	S	S	S	S	R	S	+	JTP
12228	S	S	S	S	S	R	R	+	ATCC
JB117	R	S	R	R	S	R	R	+	UGa
JB20	S	S	R	R	S	R	S	+	UGa
JB41	R	S	R	R	S	R	R	+	UGa
JB44	R	S	R	R	S	R	S	+	UGa
JB36	S	R	S	S	S	R	NT	+	UGa
JB49	S	R	S	S	S	R	NT	+	UGa
JB104	S	R	S	S	S	R	NT	+	UGa

^a Cd, Cadmium; Asa, arsenate; Hg, mercury; PMA, phenylmercuric acetate; EB, ethidium bromide; Pc, penicillin; Tc, tetracycline; Bla, beta-lactamase production; R, resistant; S, susceptible; +, positive test; —, negative test; UGa, from nasal cultures of University of Georgia students; JTP, J. T. Parisi; ATCC, American Type Culture Collection.

nitro and ribose was determined by the method of Schaeffer (22). Deoxyribonuclease test agar and Christianson urea agar were obtained from Difco and were used according to the manufacturer's directions. Ethidium bromide (EB) resistance was scored on YETS plates containing 19 μ g of EB.

Heavy-metal resistances were screened by the well diffusion method of Summers and Jacoby (28) with the following solutions of heavy metals: 0.1 M CdCl₂, 0.1 M HgCl₂, 5 μ M phenylmercury acetate, and a saturated solution of sodium arsenate. Mercury and cadmium resistances were further tested by the procedures published by Weiss et al. (31) and Novick and Roth (17). Zones of inhibition were compared with those of *S. aureus* strains 8325-4 and 8325-4 (p1258), the susceptible and resistant controls.

Isolation and analysis of plasmid DNA. The Brij minilysis procedure (33) was used for screening strains for plasmid content. For isolation of plasmid DNA, the lysostaphin-cleared lysis procedure (26) followed by CsCl-EB centrifugation was used with the following modifications. The medium used for overnight growth of penicillin-resistant strains contained 5 μ g of methicillin per ml and 0.5% glycine. The concentrations of lysostaphin used were 15 μ g/ml for *S. aureus* and 30 μ g/ml for *S. epidermidis*. These increased concentrations of lysostaphin and glycine gave better lysis of *S. epidermidis* strains. Plasmid DNA isolated from CsCl-EB gradients was exhaustively dialyzed against TE buffer [10 mM tris(hydroxymethyl)aminomethane-1 mM ethylenediaminetetraacetic acid, pH 7.5] and was subsequently concentrated threefold by evaporation. For electron microscopy, DNA was further purified by chloroform-isomyl alcohol (24:1, vol/vol) extraction followed by ethanol precipitation and resuspension in TE buffer.

Agarose gel electrophoresis. The procedure of Wilson and Baldwin (32) was used. Gel concentrations varied from 0.7 to 1.0%, depending on the experiment.

Electron microscopy. Open circular plasmids

were obtained by limited digestion with deoxyribonuclease (30) and were prepared for electron microscopy by the technique of Kleinschmidt (7). Contour lengths of 20 to 25 molecules of each plasmid were determined along with an internal standard, bacteriophage PM2 (6.4 megadaltons [Mdal] [4]), kindly donated by R. Wilson.

Compatibility. Compatibility of phenotypically different plasmids was determined by the method of Cabello et al. (2). Transductants were selected on media containing 19 μ g of EB per ml or 60 mM CdCl₂. After two serial single-colony isolations on the appropriate selective media, transductants were analyzed for plasmid content by agarose gel electrophoresis. Displacement of the resident plasmid by the selected transduced plasmid was the behavior expected for the incompatible plasmids.

Restriction enzyme analysis. *EcoRI*, *HaeIII*, *HindIII*, and *HpaII* were obtained from New England Biolabs (Beverly, Mass.), and plasmids were digested as published (32). Standards used to determine molecular weights of restriction fragments were lambda and PM2, both cut with *HindIII* as published (32).

RESULTS

Transduction and phenotypic testing. Table 1 summarizes the phenotypic properties of *S. epidermidis* donors and recipients used in the transduction experiments. All donor strains could transduce both recipients. Strain JB248 was susceptible to heavy metals, and cotransduction of these traits with penicillin resistance could be tested in this recipient. However, JB248 harbored multiple plasmids. Therefore, the restriction analysis and electron microscopy were carried out with transductants of the other recipient, JB216.

Strains were tested for cotransduction of penicillin resistance with erythromycin (Em) and heavy-metal resistance traits associated with *S. aureus* Pc^r plasmids. The four donor strains resistant to Hg²⁺ and phenylmercury acetate did not cotransduce these traits with penicillin resistance. Of the four Cd donor strains, only one (PS108) cotransduced this trait with penicillin resistance. All donor strains were erythromycin susceptible. Therefore, Em^r genes were not present on these Pc^r plasmids as previously reported for some *S. aureus* strains (8). Cotransduction of EB with penicillin occurred with PS29, the only EB-resistant strain tested. All Pc^r transductants produced penicillinase.

Cotransduction of Pc^r with other traits reported to be associated with *S. epidermidis* Pc^r plasmids could not be tested with our strains. Both recipient strains were urea and ribose positive; therefore, cotransduction of these traits with penicillin resistance could not be tested. Because all Pc^r donors did not ferment mannitol or produce nuclease, these traits were not linked with penicillin resistance in these strains.

Tc^r transductants were obtained by using JB216 as the recipient and strains JB12228 and PS29 as the donors. No other donor strains were tested for transduction of tetracycline resistance.

Plasmid content of transductants. When CsCl-EB-purified plasmid DNA was electrophoresed on agarose gels, the Pc^r transductants from donor strains PS29, PS108, and 12228 showed additional plasmid bands; the other seven Pc^r transductants did not. The results of one transduction experiment (Fig. 1) show that a plasmid (pAJ1003) contained by the donor, 12228 (slot 4), was also harbored by the transductant (slots 2 and 3). The recipient, JB216 (slot 1), did not contain a plasmid of this size. Plasmids were also found in Pc^r transductants when either JB216 or JB248 was used as the recipient. The other seven strains served as donors of penicillin resistance, but no new plasmids were detected in the recipients either by the Brij minilysis technique or by lysostaphin lysis followed by CsCl-EB centrifugation. Analysis of the Tc^r transductants revealed plasmids (pAJ1004 and pA51005) which migrated similarly to the *S. aureus* Tc^r plasmid (pCW6).

Electron microscopy. Molecular masses of Pc^r plasmid DNA isolated from the transductants JB217, JB218, and JB219 and five strains of *S. aureus* were determined by electron microscopy (Table 2). *S. epidermidis* Tc^r plasmids

were not sized by electron microscopy, since their migration on agarose gels was similar to that of the 2.9-Mdal *S. aureus* Tc^r plasmid.

Compatibility testing. Plasmid pAJ1001 was transduced into strain JB218 (harboring pAJ1002) by selecting for EB resistance. Both plasmids appeared on electrophoresis of the transductant and were therefore compatible. Similarly, plasmid pAJ101 was transduced into strain JB219 (harboring pAJ1003) but, due to the similar size, the presence of both plasmids could not be resolved electrophoretically. However, *Hae*III digests of the transductant revealed fragments of both plasmids, indicating that these plasmids were compatible. Compatibility testing between plasmids pAJ1002 and pAJ1003 gave uninterpretable results.

Restriction endonuclease digest patterns. Selected Pc^r plasmids of *S. aureus* after digestion with restriction enzymes were compared with digests of Pc^r plasmids from *S. epidermidis* transductants JB217, JB218, and JB219. JB216 served as a control to position the digest products of the cryptic plasmid present in the transductants. Figure 2 shows comparisons of *S. aureus* and *S. epidermidis* plasmids digested with *Eco*RI. Slot 9 contained the cryptic plasmid control. Although plasmids of *S. aureus* (slots 1 through 5) and *S. epidermidis* (slots 6 through 8) appeared quite heterogeneous, a few exceptions were noted. Slots 2 and 5 contained digests which appeared to have three bands in common. Three other digests (slot 4, 5, and 8) had 1.5- and 0.74-Mdal bands in common. It is also interesting to note that digests of four of the five *S. aureus* plasmids tested (slots 2 through 5) had 1.4-Mdal fragments. This region of the gel was masked by the cryptic plasmid in strains of *S. epidermidis*. However, since the molecular size totals of the fragments produced from pAJ1001, pAJ1002, and pAJ1003 were >13.7, 10.0, and 16.8 Mdal, respectively, and the molecular sizes calculated by electron microscopy were 15.5, 9.9, and 17.2 Mdal, a 1.4-Mdal band was probably not present in digests of the latter two plasmids.

*Hae*III digestion patterns of *S. aureus* and *S. epidermidis* Pc^r plasmids were also compared (Fig. 3). The cryptic plasmid present in the *S. epidermidis* strains was not digested by *Hae*III; only the covalently closed and open circular forms were present on gels. The position of these bands relative to linear digest products of the Pc^r plasmids could be changed by altering the agarose concentrations (compare Fig. 3A and 3B), allowing us to detect bands previously masked. Although the overall digestion patterns of the Pc^r plasmids were quite different, many of the plasmids appeared to have bands in com-

FIG. 1. Agarose gel electrophoresis of Brij minilysates obtained from transduction with JB216 as recipient and Pc^r the marker transduced. (1) JB216 (recipient), (2 and 3) JB219 (transductant), and (4) JB12228 (donor). P, Pc^r plasmid (pAJ1003); LF, linear fragments (chromosome); C, cryptic plasmid.

TABLE 2. Characteristics of plasmids used in endonuclease studies^a

Plasmid	Resistance	Molecular mass (Mdal)	Donor strain	Transductant	Source
<i>S. epidermidis</i>					
pAJ1001	Pc ^r , EB ^r	15.5 ± 0.4	PS29	JB217	
pAJ1002	Pc ^r , Cd ^r	9.9 ± 0.3	PS108	JB218	
pAJ1003	Pc ^r	17.2 ± 0.5	12228	JB219	
pAJ1004	Tc ^r	2.9	PS29	JB227	
pAJ1005	Tc ^r	2.9	12228	JB228	
Not detected	Pc ^r		7 strains: JB117, JB20, JB41, JB44, JB36, JB49, JB104	7 strains	
<i>S. aureus</i>					
pAJ1	Pc ^r , Asa ^r , Hg ^r	21.2			Wild-type strain
pAJ2	Pc ^r , Em ^r	26.1			
pAJ3	Pc ^r , Cd ^r , Asa ^r , Em ^r	10.6			Wild-type strain
pAJ5	Pc ^r , Cd ^r , Asa ^r , Hg ^r	19.5			Trans-formant of 8325-4
pI258	Pc ^r , Cd ^r , Asa ^r , Hg ^r , Em ^r	18.6			Trans-formant of 8325-4
pCW6	Tc ^r	2.9			Trans-formant of 8325-4

^a Molecular masses were determined on transductants of JB216. Heavy-metal resistances were determined on transductants of JB248. Tc plasmid molecular masses were determined by endonuclease fragment sizes on agarose gels. Em, Erythromycin.

FIG. 2. *EcoRI* digests of plasmid DNA from strains of *S. aureus* and *S. epidermidis*. Digests of *S. aureus* Pc^r plasmids are in slots 1 through 5: (1) pAJ3, (2) pI258, (3) pAJ1, (4) pAJ2, and (5) pAJ5. Digests of *S. epidermidis* Pc^r plasmids are in slots 6 through 8: (6) pAJ1001, (7) pAJ1002, and (8) pAJ1003. These plasmids were isolated from transductants of JB216, and the digests therefore include the 1.6-Mdal cryptic plasmid (slot 9). Molecular masses (in megadaltons) of significant fragments are indicated on the left.

mon. For instance, a 1.9-Mdal band was present in digests of all the *S. epidermidis* and *S. aureus* Pc^r plasmids tested.

Unlike *HaeIII* digestion, plasmids of *S. epidermidis* and *S. aureus* digested with *HindIII* and electrophoresed on agarose gels appeared to contain very few fragments in common (data not shown).

Tc^r plasmids of *S. epidermidis* and *S. aureus* were also compared (Fig. 4). Again, JB216 served to position the fragments generated by enzyme digestion of the cryptic plasmid. *HindIII* (Fig. 4A) and *HpaII* (Fig. 4B) digestion products of pCW6 (from *S. aureus*, slot 3) and pJB1005 (from *S. epidermidis*, slot 1) were the same size. Because the intact plasmids migrated to similar positions on agarose gels (not shown), we assumed that the molecular weights of these plasmids were very similar. Therefore, a fragment of the size that would be masked by the cryptic plasmid could not be present in the enzyme digests.

DISCUSSION

In this study, Pc^r plasmids were found in three strains of *S. epidermidis*. These plasmids were identified by transduction into penicillin-susceptible recipients and had molecular sizes of 15.5, 9.9, and 17.2 Mdal. No Pc^r plasmids could be

FIG. 3. *Hae*III digests of *Pc'* plasmids. (A) Digests were electrophoresed on 1% agarose for 11 h at 32 V. The molecular masses of the control (PM2 cut with *Hind*III, slot 2) are listed (in megadaltons) on the left. Plasmid DNA of *S. epidermidis* digested with *Hae*III is in slots 3 through 6. The positions of the covalently closed circular (CCC) and open circular (OC) forms of cryptic plasmid (slot 3) are indicated by arrows. Slots containing *Pc'* plasmids in addition to the cryptic plasmid were: (4) pAJ1003, (5) pAJ1002, and (6) pAJ1001. Digests of the *Pc'* plasmids of *S. aureus* are in the following slots: (1 and 10) pAJ3, (7) pAJ5, (8) pAJ1, and (9) pI258. (B) *Hae*III digests electrophoresed under conditions (0.8% agarose, 15 h, 25 V) different from those of (A) in order to expose linear fragments masked by cryptic plasmid DNA. *S. aureus* *Pc'* plasmid digests are: (1) pI258, (2) pAJ1, and (3) pAJ3. The position of the cryptic plasmid of *S. epidermidis* can be seen in slot 7 and is indicated. *S. epidermidis* plasmid digests were (4) pAJ1001, (5) pAJ1002, and (6) pAJ1003. A 1.9-Mdal fragment which was masked in Fig. 5A can now be seen in slots 4, 5, and 6.

FIG. 4. *S. aureus* and *S. epidermidis* *Tc'* plasmids digested with *Hind*III (A) and *Hpa*II (B). Slots in both sections contain digests of: (1) pAJ1005, the *S. epidermidis* *Tc'* plasmid and the resident cryptic plasmid, (2) the cryptic plasmid of the *S. epidermidis* recipient, and (3) pCW6, the *S. aureus* *Tc'* plasmid.

demonstrated in transductants by using seven other donor strains and the technique of lyso-staphin-mediated Brij lysis followed by agarose gel electrophoresis. This procedure should detect plasmid DNA similar to the DNA of the *Pc'* plasmids of *S. aureus* or of *Pc'* plasmids identified in the other strains of *S. epidermidis*. Per-

haps the *Pc'* genes in these strains are on plasmids which were not isolated by our technique because they were present in only a few copies per cell or were extremely labile. It is also possible that a large plasmid in the donor could be fragmented when transduced and incorporated into the recipient chromosome. Although chromosomal *Pc'* genes are rare in *S. aureus* (8), their presence in these seven strains of *S. epidermidis* would also explain the results.

Donor strains and transductants were also tested for properties reportedly associated with penicillin resistance in *S. epidermidis*. These include aerobic acid production from ribose and mannitol, urease production, and nuclease production (14, 22). No association between those traits and penicillin resistance was found in our strains. The *S. epidermidis* *Pc'* plasmids were also compared by compatibility testing and were found to be in at least two compatibility groups, indicating possible differences in replication control (2) or membrane attachment sites (6) for these molecules.

The *Pc'* plasmids of *S. epidermidis* were similar to those found in *S. aureus* in several respects. One *S. epidermidis* plasmid encoded for EB resistance and another encoded for resistance to cadmium, traits also associated *S. aureus* *Pc'* plasmids. The plasmids of these two species

were in the same size range (see Table 2). Although these staphylococcal plasmids appeared heterogeneous by endonuclease digestion, they shared several fragments of the same size after digestion with *EcoRI*. A 1.9-Mdal fragment generated by *HaeIII* digestion (see Fig. 3) was shared by all staphylococcal *Pc^r* plasmids and could possibly indicate a conserved region in these plasmids. Recently, Novick et al. also reported the physical heterogeneity of *S. aureus* *Pc^r* plasmids (16). These differences could be partially attributed to transposons and several transpositional "hot spots" identified on these plasmids (13, 15). Possible conserved regions such as the 1.9-Mdal *HaeIII* fragments could represent a plasmid core onto which transposons have inserted. The Southern blotting technique (24) could be used to further examine the areas of possible homology in the plasmids used in our study.

The two *Tc^r* *S. epidermidis* plasmids in our study had indistinguishable patterns after digestion with *HindIII* and *HpaII*, indicating that these two species may harbor the same plasmid. Surprisingly, as judged by the same criteria, the *Tc^r* plasmids of *S. aureus* are similar in several other strains tested (23, 32). The reason for the conservation of the sequences of the *Tc^r* plasmids opposed to the heterogeneity of the *Pc^r* plasmids of *S. aureus* and *S. epidermidis* is not known. Recently Groves (5) found extensive homology but slightly different restriction enzyme patterns between the *Tc^r* plasmids of *S. aureus* and *S. epidermidis*. Although these plasmids may have sequence differences which would explain the divergent restriction enzyme fingerprints, possible restriction-modification differences between the *S. aureus* and *S. epidermidis* hosts would also explain these results.

Lacey (8) has postulated that *S. epidermidis* may act as a reservoir of resistance determinants for the more clinically significant *S. aureus*. In vitro studies have shown that staphylococci can exchange DNA by transduction or protoplast fusion (34; M. L. Stahl and P. A. Pattee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, H32, p. 113). In this study, we have shown that plasmid DNA may be exchanged between these two staphylococcal species in vivo based on the similarity of their *Tc^r* plasmids. However, some *S. epidermidis* strains also harbor resistance determinants not frequently found in *S. aureus*. For instance, 7 of the 10 strains of *S. epidermidis* in our study did not transduce *Pc^r* plasmids physically similar to those found in *S. aureus*. Also, Wilson et al. (33) surveyed tetracycline-resistant strains of *S. epidermidis* and found that 2.9-Mdal plasmids were missing in many of them. Perhaps transfer of resistance determinants in vivo is confined mainly to plasmid species, and

therefore chromosomal *Pc^r* and *Tc^r* genes in *S. epidermidis* have not spread to *S. aureus* strains. Future studies in which these two species are isolated from the same host or environment may allow the detection of more strains which have similar resistance determinants.

The data presented here do not refute the theory that *Pc^r*, *Tc^r*, and other plasmid genes may be exchanged between staphylococci in vivo via transduction. Further studies by DNA/DNA hybridization or heteroduplex analysis may clarify the physical relationships of staphylococcal plasmids and could be used to further examine their epidemiology.

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