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' plasmid been identified in S. epidermidis (18). Before this time, the only evidence for S. epidermidis Pc' plasmids was cotransduction or loss of penicillin resistance with other traits (14, 19, 21, 22).

The Tc' 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' 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' and Pc' 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 (Trobinicin; 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 disk 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-
TABLE 1. Characteristics and source of strains of S. epidermidis used in transduction experimentsa

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotypic response*</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cd Asa Hg PMA EB Pc Tc Bla</td>
<td></td>
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<td></td>
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<tr>
<td>Recipient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JB216</td>
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<td>UGa</td>
</tr>
<tr>
<td>JB248</td>
<td>S S S S S S NT -</td>
<td>JTP</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>PS29</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>JB104</td>
<td>S R S S S S R NT +</td>
<td>UGa</td>
</tr>
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</table>

*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.

nitol and ribose was determined by the method of Schaeffer (22). Deoxyribonuclease test agar and Christianson ures 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 μg 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 μM 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 (p258), 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 μg of methicillin per ml and 0.5% glycine. The concentrations of lysostaphin used were 15 μg/ml for S. aureus and 30 μg/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 ethylenediaminetraacetic 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.

Aagarose 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 μg 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 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' genes were not present on these Pc 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' transductants produced penicillinase.

Cotransduction of Pc' with other traits reported to be associated with S. epidermidis Pc' 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' donors did not ferment manitol or produce nuclease, these traits were not linked with penicillin resistance in these strains.
Tc’ 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’ transductants from donor strains PS29, PS108, and 12228 showed additional plasmid bands; the other seven Pc’ 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’ 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 lysozyme digestion followed by CsCl-EB centrifugation. Analysis of the Tc’ transductants revealed plasmids (pAJ1004 and pAJ1005) which migrated similarly to the S. aureus Tc’ plasmid (PCW6).

Electron microscopy. Molecular masses of Pc’ 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’ 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’ 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, HaeIII 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’ plasmids of S. aureus after digestion with restriction enzymes were compared with digests of Pc’ 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 EcoRI. 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.

HaeIII digestion patterns of S. aureus and S. epidermidis Pc’ plasmids were also compared (Fig. 3). The cryptic plasmid present in the S. epidermidis strains was not digested by HaeIII; 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’ 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’ plasmids were quite different, many of the plasmids appeared to have bands in com-
Plasmid  | Resistance  | Molecular mass (Mdal) | Donor strain | Transductant | Source |
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<td></td>
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</tr>
<tr>
<td>pAJ1001</td>
<td>Pc', EB'</td>
<td>15.5 ± 0.4</td>
<td>PS29</td>
<td>JB217</td>
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<td>PS108</td>
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<td>PS29</td>
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</tr>
<tr>
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<td>2.9</td>
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<tr>
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<td>7 strains: JB117, JB20, JB41, JB44, JB36, JB49, JB104</td>
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S. aureus

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<th>pAJ1</th>
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</tr>
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<td>pAJ5</td>
<td>Pc', Cd', Asa', Hg'</td>
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<tr>
<td>p1258</td>
<td>Pc', Cd', Asa', Hg', Em'</td>
<td>18.6</td>
<td>Transformant of 8325-4</td>
</tr>
<tr>
<td>pCW6</td>
<td>Tc'</td>
<td>2.9</td>
<td>Transformant of 8325-4</td>
</tr>
</tbody>
</table>

* 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' plasmids are in slots 1 through 5: (1) pAJ3, (2) p1258, (3) pAJ1, (4) pAJ2, and (5) pAJ5. Digests of S. epidermidis Pc' 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' 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' 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' 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' plasmids could be
FIG. 3. HaeIII 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 HindIII, slot 2) are listed (in megadaltons) on the left. Plasmid DNA of S. epidermidis digested with HaeIII 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) p1258. (B) HaeIII 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) p1258, (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 HindIII (A) and HpaII (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 de-
tect plasmid DNA similar to the DNA of the Pc' plasmids of S. aureus or of Pc' plasmids identi-
fied in the other strains of S. epidermidis. Per-
haps the Pc' genes in these strains are on plas-
mids 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 pos-
sible that a large plasmid in the donor could be
fragmented when transduced and incorporated
into the recipient chromosome. Although chromo-
somal Pc' genes are rare in S. aureus (8),
their presence in these seven strains of S. epi-
dermidis 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 pro-
duction (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 con-
trol (2) or membrane attachment sites (6) for
these molecules.

The Pc' plasmids of S. epidermidis were simi-
lar to those found in S. aureus in several re-
spects. One S. epidermidis plasmid encoded for
EB resistance and another encoded for resist-
ance 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' plasmids and could possibly indicate a conserved region in these plasmids. Recently, Novick et al. also reported the physical heterogeneity of S. aureus Pc' 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' 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' plasmids of S. aureus are similar in several other strains tested (23, 32). The reason for the conservation of the sequences of the Tc' plasmids opposed to the heterogeneity of the Pc' 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' 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' 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' 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' and Tc' 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', Tc', 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.

ACKNOWLEDGMENTS

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LITERATURE CITED


