Plasmid Pattern Analysis of *Staphylococcus epidermidis* Isolates from Patients with Prosthetic Valve Endocarditis

GORDON L. ARCHER,†* NAHUM VISHNIAVSKY,† AND H. GRANT STIVER‡

Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298,† and Department of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada‡

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The electrophoretic pattern formed by individual bacterial plasmid DNA molecules of differing molecular size was evaluated as an epidemiological marker among isolates of *Staphylococcus epidermidis* from patients with prosthetic valve endocarditis (PVE). Purified covalently closed circular plasmid DNA was obtained from selected isolates, and 79% of the plasmids were found to be <10 megadaltons in size; only these small plasmids were sought in subsequent screening gels. Crude cell lysates obtained by a rapid lysis technique and screened by agarose gel electrophoresis revealed the presence of one or more small plasmids in 54 of 58 (93%) PVE isolates; 79% contained two or more. Among 45 plasmid-containing isolates from cases of sporadic PVE at three institutions there were no identical plasmid patterns, although several isolates differed by a single plasmid. In contrast, among nine isolates from a cluster of cases of PVE in Canada, two groups of three isolates each had identical plasmid patterns. Additional clinical data suggested that these isolates were epidemiologically related. Phage typing distinguished one of the groups with plasmid pattern identity, but not the other, from the three isolates with dissimilar patterns. Plasmid pattern analysis shows promise as an epidemiological marker for clinically important isolates of *S. epidermidis*.

*Staphylococcus epidermidis,* one of the most common bacteria which infect prosthetic heart valves (10, 13), may be introduced into the tissue around the valve prosthesis at the time of surgery (2). Identification of the source of hospital-associated *S. epidermidis* isolates responsible for contamination of cardiac tissue would, therefore, be important for preventing prosthetic valve endocarditis (PVE). To trace *S. epidermidis* isolates from the environment to the patient, it is necessary to find markers which establish a common origin for bacteria recovered from different sources. It is also desirable to be able to screen among many isolates for related strains by easy and rapid methods. Biotyping and phage-typing techniques have been devised for *S. epidermidis* (3, 16) but have proved inadequate for general use; biotyping is nonspecific (3), and isolates are frequently not typable by using available phage-typing sets (16). The determination of plasmid DNA profiles by agarose gel electrophoresis has been reported to be useful for comparing *S. epidermidis* isolates from clinical sources (12). In this study, we evaluated the utility of plasmid pattern analysis as an epidemiological tool by examining isolates both from a known outbreak of *S. epidermidis* PVE and from sporadic cases of *S. epidermidis* PVE at three institutions.

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MATERIALS AND METHODS

Bacteria. *S. epidermidis* isolates from 49 consecutive cases of PVE were obtained over a 5-year period (June 1975 through May 1980) from the Medical College of Virginia Hospitals (14 isolates), Massachusetts General Hospital (21 isolates obtained from A. W. Karchmer), and the University of Alabama Medical School (14 isolates obtained from W. E. Dismukes). Nine additional isolates were obtained from a cluster of cases of *S. epidermidis* PVE which occurred at St. Boniface Hospital in Winnipeg, Manitoba. The clinical aspects of these Canadian cases have been reported previously (6). *S. epidermidis* isolates fermented glucose, but neither coagulated rabbit plasma nor fermented mannitol. All organisms had been recovered from either blood cultures or perivalvular tissue of patients with documented PVE.

Preparation of cell lysates. The rapid cell lysis technique of Macrina et al. (9) was modified for *S. epidermidis*. The modifications were the use of brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) instead of Todd-Hewitt broth, the omission

† Present address: Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada.
of glycine pretreatment, and the substitution of lyso-
staphin (Sigma Chemical Co., St. Louis, Mo.) for
lysozyme. Lysostaphin was freshly prepared in TES
buffer (9), and 5 µl of a 1.5-mg/ml solution was added
to log-phase bacteria pelleted from an initial volume of
3.0 ml. The remaining lysis procedure was as de-
scribed. Sheared lysates were analyzed by electropho-
resis through 0.7% agarose on a vertical slab gel (0.3
by 10 by 10 cm) with a Tris-borate buffer system (11).
The gel contained 16 wells, and 25 µl of lystate was
deposited in each well. The gel was run at 20 V for 16 h at
room temperature. Low-voltage electrophoresis was
used for screening to allow small (<10 megadalton)
plasmids to be preferentially identified. Escherichia
coli strain V517 (7) was used as a standard in each gel.
Gels were stained with 100 µl of a 10-mg/ml solution of
tetidium bromide for 20 min and destained in running
tap water for 45 min.

Purified plasmid DNA was prepared by tetidium
bromide-cesium chloride (EB-CsCl) density gradient
centrifugation. The method used was that previously
described for the isolation of bulk cellular DNA from
oral streptococci (8) but with a few modifications.
These modifications were (i) the omission of glycine
and Sarkosyl and (ii) the substitution of lysozymin
(1.75 ml of a 1.5-mg/ml solution in TES buffer added to
150 ml of log-phase bacteria) for lysozyme. The plas-
mid DNA was dialyzed against TE buffer (9) and
subjected to electrophoresis at 100 V for 2 h on a 0.7%
agarose vertical slab gel.

In vitro studies. S. epidermidis isolates were bio-
typed according to the scheme of Baird-Parker (3).
Isolates were tested for antimicrobial susceptibility by
the microtiter broth dilution method as previously
described (1), using the following antibiotics: penicillin
G potassium (Eli Lilly and Co., Indianapolis, Ind.),
cephalothin sodium (Eli Lilly), gentamicin sulfate
(Schering Laboratories, Bloomfield, N.J.), clindamycin
hydrochloride (The Upjohn Co., Kalamazoo,
Mich.), rifampin (Dow Chemical Co., Indianapolis,
Ind.), vancomycin hydrochloride (Eli Lilly), erythromycin
base (Eli Lilly), sodium methicillin (Bristol
Laboratories, Syracuse, N.Y.), tetracycline hydro-
chloride (Pfizer Laboratories, New York, N.Y.), and
chloramphenicol (Parke, Davis and Co., Detroit,
Mich.). Phage typing was performed by Joseph Parisi,
using phages and methods previously described (14).

RESULTS

Analysis of isolates. The utility of the rapid
lysis technique for identifying plasmids in S. epidermidis
was evaluated by comparing plasmids seen in crude lysates from 10 isolates with
those seen in the same isolates after extraction using EB-CsCl density gradient centrifugation. There were 47 total plasmids (range, 1 to 9 per isolate) seen in the purified preparations, and 37 of these (79%) were less than 10 megadaltons in
molecular size; all 37 were easily seen when crude lysates were electrophoresed overnight at
low voltage. Thus, because low-voltage electrophoresis of crude lysates identified most of the
plasmids in S. epidermidis, it was used to screen the remaining isolates for plasmid pattern identi-
ty. Crude lysates from isolates with identical
patterns of small plasmids could be electrophoresed at higher voltage (100 V) for 3 h in order to
identify bigger (>15 megadaltons) plasmids. However, plasmids running with the chromo-
some (10 to 15 megadaltons) could not be identified in crude lysates.

Analysis of crude lysates from all 58 PVE
isolates demonstrated the presence of one or
more small plasmids or plasmid components in
54 of the 58 (93%). The isolates contained from 1
to 12 small plasmids, with 79% having 2 or more.

Of the 49 isolates from sporadic cases of PVE,
45 contained small plasmids and none had identi-
cal plasmid patterns. Figure 1 is a representative
gel of crude lysates of 14 isolates from cases of
S. epidermidis PVE at the Medical College of
Virginia showing an absence of plasmid pattern
identity. The isolates in lanes A and B and in
lanes M, N, and L appeared similar but differed
by a single plasmid.

In contrast to the isolates from sporadic cases
of PVE, two groups of three isolates each from
the cluster of nine PVE cases at St. Boniface
Hospital appeared to have identical plasmid
patterns when crude lysates were examined (Fig.
2, lanes D, E, and F and lanes H, I, and J). The
three isolates in lanes A, B, and C appeared
dissimilar from each other and from the other six
isolates. The two groups of three identical pat-
terns were confirmed as identical and the other
three isolates as nonidentical when plasmid
DNA was purified by EB-CsCl density gradient
centrifugation (Fig. 3). The two gels were some-
what dissimilar because of the presence of dif-
ferent forms of the same plasmid (covalently
closed circular and nicked open circular DNA)
on crude preparations. The characteristic of S.
epidermidis plasmids to appear in multiple forms

FIG. 1. Agarose gel electrophoresis of crude plasmid DNA of S. epidermidis isolates from 14 sporadic
cases of PVE at the Medical College of Virginia. Plasmid DNA was electrophoresed through a vertical
slab gel at 20 V for 18 h. Lane F contains size
reference molecules from E. coli strain V517. The
bright bands represent molecular sizes of 1.4, 1.8, 2.0,
2.6, 3.4, 3.7, and 4.8 megadaltons from bottom to top,
respectively.
surgery had one of two identical plasmid patterns, and the ninth patient's pattern matched that of two isolates from the cluster (Fig. 4). In contrast, no PVE patients clustered by time of surgery at the three other institutions had identical plasmid patterns, and no more than four patients with PVE underwent surgery during any one 8-month period over the 5 years that isolates were collected. Although some patients' isolates differed by only a single plasmid (Fig. 1, lanes M and N, L and M, and A and B), none of these patients could be linked by the date of surgery or common personnel.

The time from surgery to the first positive blood culture ranged from 10 days to 13 months (mean, 5.3 months) in the six patients with one of the two common plasmid patterns. In the three patients with dissimilar plasmid patterns, the time to first blood culture was 1 month (case 8), 18 months (case 6), and 16 months (case 4). The same chief surgeon performed all surgery at the St. Boniface Hospital, and no assistant surgeon was common to only cases with the same plasmid pattern. Random nasal swabs from five of the six surgeons performing surgery during the period of the cluster yielded *S. epidermidis* which had no plasmid patterns in common with any of the PVE isolates.

**Comparison of epidemiological markers.** Table 1 shows a comparison of plasmid typing with other methods of relating organisms of epidemiological significance. All nine St. Boniface iso-

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**FIG. 2.** Agarose gel electrophoresis of crude plasmid DNA of *S. epidermidis* isolates from the nine cases of PVE at St. Boniface Hospital. Plasmid DNA was electrophoresed through a vertical slab gel at 20 Volts for 18 h. The case numbers are identified in Fig. 4. Lanes A, B, and C correspond to cases 4, 8, and 5, respectively; lanes D, E, and F correspond to cases 1, 2, and 3, respectively; lanes H, I, and J correspond to cases 6, 7, and 9, respectively. Lane G contains *E. coli* strain V517 with reference molecules as described in Fig. 1. The top arrow (c) indicates the bright chromosome band. The other arrows indicate covalently closed circular DNA molecules in lanes I, J, and K which correspond to bands indicated by arrows in Fig. 3. The other bands are open circular forms.

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**FIG. 3.** Agarose gel electrophoresis of purified plasmid DNA extracted by using EB-CsCl density gradient centrifugation of *S. epidermidis* isolates from nine cases of PVE at St. Boniface Hospital. Plasmid DNA was electrophoresed through a vertical slab gel at 100 V for 3 h. Lanes are identical to those described in Fig. 2. The top band in the reference strain corresponds to a molecular size of 38.5 megadaltons. The arrows on the right are covalently closed circular DNA molecules in lanes I, J, and K which correspond to those in Fig. 2. The arrow on the left (c) indicates contaminating chromosome in the lysate in lane B.
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were
differentiated
three
differentiated three isolates with identical plasmid patterns from those with dissimilar patterns; none of the other six isolates were typable. 
Antibiograms were not helpful in grouping any of the nine isolates. Of the 49 isolates from sporadic cases of PVE, 91.3% were biotype 1, 83% had similar antibiograms, and only 42% were phage typable.

DISCUSSION
Plasmid pattern analysis has been used to establish the similarity of both gram-negative (15) and gram-positive (12) bacteria isolated from different sources. To be a useful screening tool, this technique must fulfill several criteria. First, it is desirable for the bacterial species in question to contain multiple plasmids. The possibility that organisms contain plasmids of the same molecular size by chance alone decreases as the number of plasmids increases. Single plasmids of the same molecular size have been used successfully for establishing the similarity of two organisms when the plasmids contain identical resistance determinants or identical restriction endonuclease digests (14). However, these are more complicated analyses requiring some expertise in molecular genetics and would not be classified as screening procedures. S. epidermidis is well suited to plasmid pattern analysis, since 73% of the clinical isolates examined in this study contained at least three small plasmids.

Second, the methods used must be rapid and must be adaptable to nonspecialized laboratories. The rapid technique for extraction of DNA used in this study employed easily obtainable reagents which were used in small quantity and needed only a standard electrophoresis apparatus. Single colonies were picked from agar, grown in broth, lysed, electrophoresed overnight, and examined in 36 h. As many as 32 isolates could be lysed and examined at one time.

Third, the technique must be validated by using isolates from adequately documented infections which appear to be epidemiologically related. Parisi and Hecht (12) have reported data similar to ours in that plasmid profiles of S. epidermidis were used to establish the similarity and difference of isolates from clinical sources. However, the clinical and epidemiological significance of their isolates with similar plasmid patterns was unclear. In contrast, S. epidermidis

FIG. 4. Schematic representation of the plasmid patterns of S. epidermidis isolates from a cluster of cases of PVE at St. Boniface Hospital arranged by date of surgery. The schematic plasmid bands correspond to the bright bands seen in Fig. 3.

<table>
<thead>
<tr>
<th>CASE NO</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLASMID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1. Epidemiological markers of S. epidermidis isolates from a cluster of PVE patients at St. Boniface Hospital

<table>
<thead>
<tr>
<th>Isolate (case) no.</th>
<th>Minimal inhibitory concn (µg/ml)</th>
<th>Phage type</th>
<th>Bio-Plasmid type</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>CYP A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me</td>
<td>Vc</td>
<td>Cl</td>
<td>Er</td>
</tr>
<tr>
<td>1</td>
<td>6.25</td>
<td>1.6</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>&gt;100</td>
<td>6.25</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>6.25</td>
<td>12.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4</td>
<td>12.5</td>
<td>3.12</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>3.12</td>
<td>0.8</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>3.12</td>
<td>3.12</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
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<td>&gt;100</td>
</tr>
<tr>
<td>8</td>
<td>3.12</td>
<td>6.25</td>
<td>25</td>
</tr>
</tbody>
</table>

* These numbers correspond to those depicted in Fig. 4.
* P, Penicillin G; CP, cephalothin; Me, methicillin; Vc, vancomycin; Cl, clindamycin; Er, erythromycin; Te, tetracycline; Rf, rifampin; Chl, chloramphenicol; G, gentamicin.
* Isolates with the same number have identical plasmid patterns.
* NT, Nontypable.
is the most common bacterium which caused PVE. The organisms examined in our study were proven to be etiological by their isolation from multiple cultures in patients with clinically compatible illnesses. The cluster of cases in Canada appeared to be an outbreak on the basis of the occurrence of eight cases of PVE in patients who underwent surgery during an 8-month period, the absence of cases before this time, and the discovery of contamination of the bypass equipment with S. epidermidis. Thus, the finding that there was plasmid pattern identity in S. epidermidis isolates associated with an outbreak of PVE but not in isolates from sporadic cases helps validate the use of plasmid pattern analysis for epidemiological purposes.

Three of the isolates with identical plasmid patterns from the outbreak also had an identical phage type which confirmed their identity. However, the other three isolates from the outbreak having an identical plasmid pattern were not distinguishable from the three unrelated isolates by conventional methods. None of the six was typable by phage typing, all biochemical characteristics examined were the same, and their antibiotic susceptibility characteristics formed no pattern. Although phage typing of S. epidermidis has been useful in relating organisms of epidemiological significance in other studies (5), 40 to 60% of isolates may not be typable (5, 16); biochemical characteristics are also frequently similar, even when many reactions are tested (4); and, since most S. epidermidis isolates from PVE and cardiac surgery patients are resistant to multiple antibiotics and often have the same patterns of resistance (1), antibiograms will be of little value. Thus, since plasmid pattern analysis will be applicable to at least the 80% of S. epidermidis isolates which have two or more small plasmids and the technique is easy and can be used to screen many isolates, it may be superior to methods currently available for use as an epidemiological tool.

Caution should be exercised, however, in attaching epidemiological significance to S. epidermidis isolates which have similar, but not identical, plasmid patterns. Parisi and Hecht (12) related two S. epidermidis isolates with only one plasmid difference by identifying the probable acquisition of a tetracycline resistance plasmid in one of the isolates. However, most of the plasmids we found in S. epidermidis PVE isolates had unidentified gene products, and 4 of the 14 isolates from cases of PVE at the Medical College of Virginia shown in Fig. 2 differed by a single plasmid, yet had no epidemiological link. Thus, only complete plasmid pattern identity correlated with epidemiological data in this study. The epidemiological significance of the presence of single plasmids of the same molecular size or of similar but not identical plasmid patterns in different isolates should be assessed by prospective studies.

We have shown that plasmid pattern analysis of S. epidermidis isolates from patients with PVE can be performed rapidly and easily and can identify infections which may have arisen from a common source. The technique of plasmid pattern analysis shows promise of answering questions about the hospital epidemiology of S. epidermidis and its role as a pathogen.

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LITERATURE CITED
