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Molecular Relatedness of *Staphylococcus epidermidis* Isolates Obtained during a Platelet Transfusion-Associated Episode of Sepsis

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***Staphylococcus epidermidis* was isolated from the blood of a 25-year-old pregnant woman following the administration of eight units of platelets. She had developed chills and a fever of 41.4°C soon after the transfusions were completed. *S. epidermidis* was also obtained from all eight platelet units, as well as from the packed-erythrocyte unit associated with the first unit of platelets. The isolation of the same organism from these epidemiologically related sources provided us with the opportunity to phenotypically and genetically characterize the isolates. Several typing methods, including four molecular techniques, were used to increase our chances of finding any differences between the isolates under investigation. Phenotypic analyses demonstrated that *S. epidermidis* isolates from the patient, platelet units, and erythrocyte unit reacted in exactly the same manner in 15 biochemical tests, exhibited slime production, and had the same antibiotic susceptibility pattern. Genetic analyses, which included plasmid profiles, plasmid cross-hybridization, field inversion gel electrophoresis, and ribotyping, substantiated the relationships between the *S. epidermidis* isolates from the patient, platelet units, and erythrocyte unit. Eight *S. epidermidis* control strains unrelated to the case were found to differ significantly from the platelet-related strain.**

Historically, *Staphylococcus epidermidis* and other coagulase-negative staphylococci were considered to be nonpathogenic members of the normal skin flora and not clinically significant pathogens. In recent years, *S. epidermidis* has emerged as an important nosocomial pathogen (7, 13, 15, 17, 24) and is now the most common coagulase-negative staphylococcus isolated from human infections (15). Bacteremias of various severities caused by *S. epidermidis* have been reported (10, 22). In addition, transfusion of contaminated platelets containing large numbers of *S. epidermidis* have caused severe symptoms in recipients (2, 8, 20).

Platelets are unique among commonly used blood products in that they are usually stored at 20 to 24°C to maintain function (21). This lack of refrigeration results in a bacterial contamination incidence of 0 to 7% in platelet concentrates (9, 12, 28). Contamination most likely occurs at the donor venipuncture site at the time of collection (2, 20), a hypothesis strengthened by culture results demonstrating that coagulase-negative staphylococci compose up to 83% of the organisms found in contaminated platelets (9, 12, 28). In most cases of transfusion reaction caused by bacterially contaminated platelets, the platelet units had been stored at room temperature for more than 5 days, allowing time for the growth of bacteria. Contamination of the platelet units at the time of transfusion is considered unlikely, since transfused blood products containing only a few organisms (<20 per ml) were retrospectively found to produce no transfusion reactions in 29 recipients (4).

Because of the ubiquitous nature of *S. epidermidis* on skin and mucosal surfaces and because of its pathogenic potential, the strain delineation of this organism has become increasingly important. Epidemiologic typing methods for *S. epidermidis* include biotyping, antibiogram analysis, bacteriophage typing, serological typing, slime production, polypeptide analysis, and molecular typing, including plasmid analysis, DNA hybridization, and restriction enzyme analysis of chromosomal DNA (15, 24). Because no single technique has been found to be optimal for epidemiologic studies of *S. epidermidis*, a combination of methods may be most useful. In our laboratory, when typing isolates implicated in disease outbreaks, we routinely begin with biochemical and antimicrobial susceptibility assays. We then use serologic and bacteriophage typing tests and/or related virulence assays for selected organisms. Finally, we progress to the use of genetic analyses if the isolates in question still cannot be differentiated from each other or from control strains.

In the present report, we describe the methods used to type *S. epidermidis* isolates obtained from the following sources: (i) blood cultures drawn from a young pregnant woman who became seriously ill after receiving multiple units of platelets, (ii) the platelet units cultured after the transfusions, and (iii) the erythrocyte unit associated with the first unit of transfused platelets. Biotyping, antibiotic susceptibility pattern analysis, slime production, plasmid profiles, and plasmid cross-hybridization, as well as field inversion gel electrophoresis (FIGE) and ribotyping of chromosomal DNA digests, were used to study the relatedness of *S. epidermidis* isolates from the patient, the platelet units, the associated erythrocyte unit, and several unrelated strains.

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CASE REPORT

A 25-year-old pregnant woman was admitted to her local hospital with a diagnosis of preeclampsia. Because of a low platelet count, she received a transfusion of 8 units of random-donor platelet concentrates. The platelets were administered sequentially, without pooling, over a period of 45 min, with the same infusion set being used for the entire transfusion. Fifteen minutes after completion of infusion of the last platelet unit, the patient developed violent shakes and chills, and after 2 h her temperature had increased to 41.4°C. A transfusion reaction workup which included culture and Gram stain of each platelet unit and blood cultures from the patient was initiated. The patient was treated with multiple antibiotics, underwent induction of labor, and received additional platelets and erythrocytes. She ultimately required a 5-day course of hemodialysis prior to recovery.

MATERIALS AND METHODS

Bacterial isolation and identification. The remaining fluid from each platelet unit was cultured and Gram stained within 4 h of the time of transfusion. An aliquot of each sample was immediately plated on blood agar plates and incubated, while another aliquot was enriched by incubation in thioglycolate broth prior to plating. Blood cultures were obtained from the patient both before and after the transfusion. Preliminary identifications of all isolates from the platelet units and the patient were made in the hospital laboratories. All isolates, including four isolates subsequently found to be duplicates, were then submitted to the Bacteriology Laboratories of the Wadsworth Center for confirmation. In addition, the packed-erythrocyte unit associated with the first unit of platelets was received by this reference laboratory and was cultured by standard blood culture techniques.

Biotyping and antibiotic susceptibility testing. All *S. epidermidis* isolates were examined for colony size; hemolysis; fermentation of glucose, maltose, lactose, mannitol, trehalose, sucrose, and xylose; production of urease, catalase, and coagulase; decarboxylation of arginine; reduction of nitrate; reactivity in the Voges-Proskauer test; and anaerobic growth in thioglycolate. Standard disk diffusion methodology (5) was used to determine susceptibility to amikacin, ampicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, methicillin, oxacillin, penicillin, polymyxin B, streptomycin, tetracycline, and vancomycin.

Adherence to smooth surfaces. Isolates were examined for slime production, a virulence-associated phenotype, as previously described (11). Bacteria were grown overnight at 37°C in Trypticase soy broth in glass tubes. The cultures were decanted, and the growth adhering to the inner surface of the tube (slime production) was visualized by staining the tube with 5% safranin.

Plasmid isolation procedure. Plasmid DNA from isolates of *S. epidermidis* (Table 1) was obtained as previously described (16, 23) by using lysostaphin, Brij 58, and deoxycholate to lyse the staphylococci. DNA samples were subjected to horizontal electrophoresis in 0.8% agarose gels. DNA bands were visualized by UV transillumination following staining with ethidium bromide.

Cross-hybridization of plasmid DNA. A well-separated 7.6-kb plasmid DNA band from a patient isolate was recovered from an agarose gel by the glass milk procedure (GeneClean; Bio 101, Inc., La Jolla, Calif.). This 7.6-kb band was labeled with ^{32}P by nick translation for use as a probe. Plasmid DNA from all *S. epidermidis* isolates was transferred from agarose gels to nylon membranes (Hybond; Amersham, Arlington Heights, Ill.) by the method of Southern as previously described (26). Hybridization with the ^{32}P-labeled probe was performed overnight at 60°C (26). Filters were washed at 60°C with 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) for 30 min and then underwent three 30-min washes in 0.1× SSC–0.5% SDS. Bound probe was then visualized by autoradiography.

TABLE 1. Outbreak-related isolates of *S. epidermidis*

Isolate(s)[a]	Source
B479, B436,[b] B437, B480, B438,[b] B466	Patient's posttransfusion blood cultures
B454, B434,[b] B456	First platelet unit transfused
B457	Second platelet unit transfused
B458	Third platelet unit transfused
B459, B460	Fourth platelet unit transfused
B461	Fifth platelet unit transfused
B462, B463	Sixth platelet unit transfused
B455, B464	Seventh platelet unit transfused
B465, B435[b]	Eighth platelet unit transfused
B451-A, B451-C[c]	Packed erythrocyte unit associated with first platelet unit transfused

[a] Control strains were B2132-79, B530-80, B2892-80, B2324-82, B1210-81, B218-90, B252-90, and B59265-59. All eight were clinical isolates obtained from the lyophilized culture collection of the Bacteriology Laboratories of the Wadsworth Center for Laboratories and Research.

[b] These isolates were subsequently found to have been duplicates submitted by different hospitals.

[c] In addition, B451-B, also isolated from the erythrocyte unit, was identified as *S. haemolyticus*.

Chromosomal DNA analysis by FIGE. Genomic DNA was prepared in agarose blocks (ca. 10 by 5 by 1 mm) essentially as described by Goering and Duensing (14). For restriction endonuclease digestion, a single agarose block was placed in a microcentrifuge tube containing 20 U of *Sma*I in 250 μl of restriction buffer and incubated at 25°C for 2 h.

Chromosomal restriction fragment patterns were analyzed by loading small slices of agarose blocks into the wells of horizontal 0.8% agarose (SeaKem HGT Agarose; FMC BioProducts, Rockland, Maine) minigels (15 by 10 cm) prepared with 0.5× Tris-borate-EDTA buffer (18). Lambda oligomers were used as DNA size standards. FIGE at 16 V/cm and 18°C was regulated with a FIJI HV600 pulse controller (IBI, New Haven, Conn.). Restriction fragments >50 kb in size were separated by initial 1.2- and 0.4-s forward and reverse electrical pulses, respectively, which were linearly increased over 3.5 h to 12 and 4 s, respectively. For restriction fragments <50 kb in size, constant 0.4-s forward and 0.2-s reverse electrical pulses (2:1 ratio) were used over a 3.25-h period. The gels were then stained and viewed as described above.

Ribotyping. Total cellular DNA, obtained as previously described for plasmid DNA isolation (16, 23), was treated with proteinase K (final concentration of 20 μg/ml), extracted with phenol-chloroform, and ethanol precipitated. Three micrograms of total cellular DNA was then digested with 10 U of *Eco*RI, *Bgl*II, *Sau*3A, or *Msp*I at 37°C for 5 h. Digested DNA was electrophoresed through either 0.7 or 0.5% agarose gels for 16 h at 35 V, with *Bgl*II-digested DNA being electrophoresed for an additional 4 h at 120 V. DNA fragments were transferred to nylon membranes with a Vacublot apparatus (LKB, Piscataway, N.J.) by following the manufacturer's directions.

Escherichia coli (16S and 23S) rRNA (Boehringer Mann-

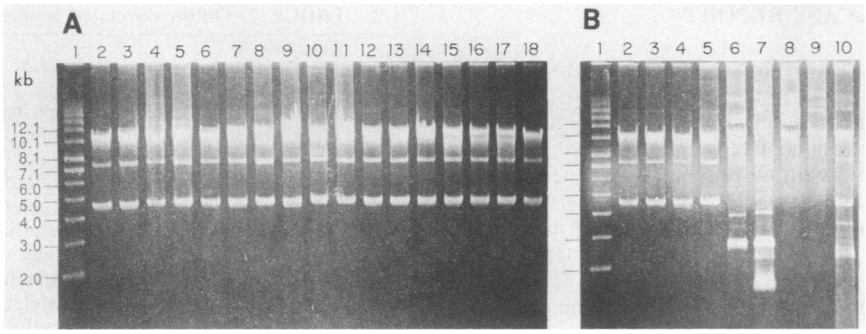

FIG. 1. Plasmid profiles of *S. epidermidis* isolates from the eight platelet units, the patient, and the erythrocyte unit. (A) Lane 1, supercoiled ladder standard (BRL); lanes 2 and 3, platelet unit isolates B434 and B435; lanes 4 to 6, patient isolates B436 to B438; lanes 7 to 18, platelet unit isolates B454 to B465. (B) Lane 1, supercoiled ladder standard; lanes 2 to 4, patient isolates B466, B479, and B480; lanes 5 to 7, erythrocyte unit isolates B451-A, B451-B, and B451-C; lanes 8 to 10, unrelated isolates B218-90, B252-90, and B2132-79.

heim, Indianapolis, Ind.) was labeled with ^{32}P according to the method of Stull et al. (27). Labeled rRNA was separated from unincorporated nucleotides with a Sephadex G-50 Quick Spin column (Boehringer Mannheim). Blots were hybridized overnight with ^{32}P-labeled rRNA at 50°C and then washed at 50°C with 2× SSC–0.1% SDS. Bound probe was visualized by autoradiography.

RESULTS

Culture results. Gram stains of samples taken from both the proximal and distal segments of the unit of platelets first transfused showed many positive cocci, while a sample from the proximal segment of the second unit showed only a few positive cocci. None of the samples from the rest of the units revealed organisms by Gram stain. *S. epidermidis* was grown in pure culture from all eight platelet units. With some units it was necessary to use enrichment techniques to isolate the small number of CFU present. A blood culture obtained from the patient prior to the transfusions was negative; posttransfusion blood cultures grew pure cultures of *S. epidermidis*. In addition, the unit of packed erythrocytes associated with the first unit of platelets was cultured, and following enrichment techniques, three different colony types were isolated and subsequently identified as *Staphylococcus haemolyticus* (B451-B) and two strains of *S. epidermidis* (B451-A and B451-C). The accession numbers of the isolates and their sources are listed in Table 1.

Biotyping and antibiograms of the *S. epidermidis* isolates. In order to determine whether all of the organisms obtained from the patient, platelet units, and packed-erythrocyte unit had the same phenotype, biochemical and antibiotic susceptibility analyses were performed. Of the 23 isolates, 22 (14 from the platelet units, 6 from the patient's blood cultures, and 2 from the erythrocyte unit [B451-A and B451-C]) were identified as *S. epidermidis* by their small, nonhemolytic colonies; positive reactions in the glucose, maltose, lactose, sucrose, urease, arginine, nitrate, Voges-Proskauer, and catalase tests; and negative reactions in the coagulase, mannitol, xylose, and trehalose tests. The third isolate from the erythrocyte unit (B451-B) was identified as *S. haemolyticus* on the basis of the following reactions which differed from those of *S. epidermidis*: positive reactions in the mannitol and trehalose tests; negative reactions in the lactose, urease, and nitrate tests; and delayed beta-hemolysis.

By antibiotic disc diffusion methodology, 21 of the 22 *S. epidermidis* isolates had the same antibiotic susceptibility pattern (resistance to ampicillin, penicillin, polymyxin B, and tetracycline and susceptibility to amikacin, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, methicillin, oxacillin, streptomycin, and vancomycin). The one *S. epidermidis* isolate that differed was B451-C isolated from the erythrocyte unit. This strain was resistant to ampicillin and penicillin and susceptible to the other 13 antibiotics. The *S. haemolyticus* isolate was susceptible to all 15 antibiotics tested.

Slime production. Because many isolates of *S. epidermidis* associated with clinical infections have previously been shown to produce extracellular polysaccharide, or slime (11), the 22 *S. epidermidis* isolates associated with this case were tested for this ability. The 6 patient isolates, the 14 isolates from the platelet units, and B451-A from the erythrocyte unit all produced slime, while B451-C from the erythrocyte unit and 3 nonrelated *S. epidermidis* isolates did not.

Plasmid DNA analyses. Plasmid DNA analysis of the 22 *S. epidermidis* isolates (Fig. 1) showed that 21 of these isolates, the 6 patient isolates, the 14 isolates from the platelet units, and B451-A from the erythrocyte unit, contained the same plasmid pattern. This pattern consisted of two distinct bands of approximately 4.5 and 7.6 kb. The second *S. epidermidis* isolate from the erythrocyte unit (B451-C), which differed in its antibiotic susceptibility pattern, also had a different plasmid pattern, as did three nonrelated *S. epidermidis* control isolates and the *S. haemolyticus* isolate obtained from the erythrocyte unit (B451-B).

When the radiolabeled 7.6-kb band from a patient isolate was used as a probe, it was found to hybridize to multiple plasmid bands (possibly different conformations of the 4.5-kb plasmid) in the 6 patient isolates, the 14 isolates from the platelet units, and B451-A from the erythrocyte unit. No hybridization was seen with plasmids from any other isolates (Fig. 2).

FIGE of chromosomal DNA. *Sma*I digestion of chromosomal DNA of *S. epidermidis*, analyzed by FIGE, showed an identical banding pattern for representative patient and platelet unit isolates and for B451-A from the erythrocyte unit, but a different pattern was seen for B451-C from the erythrocyte unit and two control isolates unrelated to the case (Fig. 3).

Chromosomal digests of *S. epidermidis* hybridized with *E. coli* rRNA. When total cellular DNA samples were digested with *Bgl*II, run on agarose gels, blotted, and probed with radiolabeled *E. coli* rRNA, identical banding patterns were

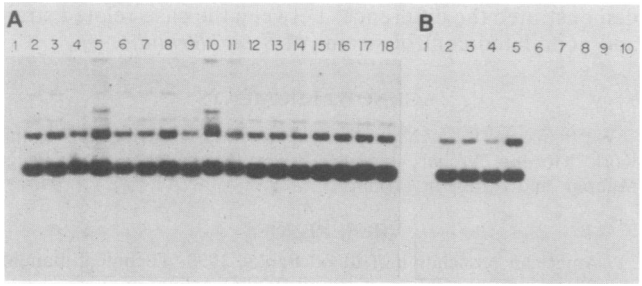

FIG. 2. Cross-hybridization of the 7.6-kb band from a patient isolate (B437) with genetically related plasmids. Panels and lanes are as described in the legend to Fig. 1.

seen with representative patient and platelet unit isolates and with B451-A from the erythrocyte unit (Fig. 4). The same relationship was seen after blotting and probing when total DNA samples were digested with *Eco*RI, *Sau*3A, and *Msp*I (data not shown). B451-C from the erythrocyte unit and 10 unrelated control strains of *S. epidermidis* revealed banding patterns different from that of the patient-related strain with at least one enzyme each. Additional control strains were used with this procedure to ensure that a variety of ribotypes were demonstrated.

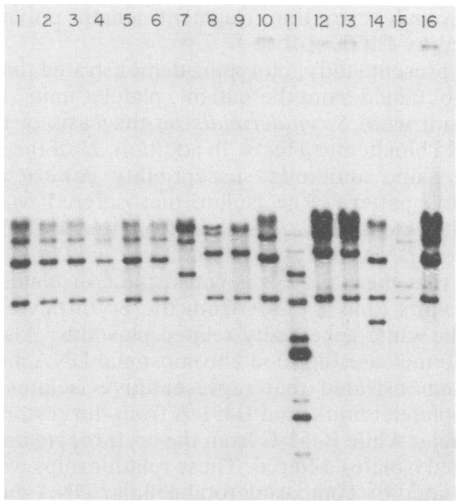

FIG. 4. *Bgl*II-digested chromosomal DNA of *S. epidermidis* hybridized with *E. coli* rRNA. Lanes 1 to 3, platelet unit isolates B454, B456, and B465; lanes 4 and 5, patient isolates B466 and B479; lanes 6 to 8, erythrocyte unit isolates B451-A, B451-B, and B451-C; lanes 9 to 16, unrelated isolates B218-90, B252-90, B2132-79, B59265, B530-80, B2324, B1210-81, and B2892-80.

DISCUSSION

Platelets must be stored at 20 to 24°C to maintain function (21), but following storage at this temperature, up to 7% of platelet units have been found to be contaminated (9, 12, 28). This risk of bacterial growth has resulted in a required 5-day shelf life for platelet units (1, 8). In the present case, the patient was transfused with eight units of platelets which were in their fifth and final day of storage. Since the patient experienced extreme fever and symptoms of shock immediately after the platelet transfusions, the infusion of a large quantity of bacteria with the platelets was suspected. Thus, when *S. epidermidis* was subsequently isolated from the patient's blood, the segments of the platelet units, and the erythrocyte unit associated with the first-transfused unit of platelets, we sought to phenotypically and genetically characterize these epidemiologically associated isolates.

Biotyping, phage typing, and antimicrobial susceptibility testing have often been used in investigations dealing with nosocomial *S. epidermidis* infection (15, 24). However, these methods do not always adequately discriminate between different strains of *S. epidermidis* (3, 6, 19, 23, 25). In several studies, plasmid profiles have been able to differentiate isolates with the same antibiogram, biotype, or phage type (3, 19, 23). However, because plasmids may not be present in all strains of *S. epidermidis* or may be difficult to demonstrate, other genetic assays have been utilized. Restriction endonuclease digestion of chromosomal DNA with restriction fragment length polymorphisms analyzed by constant-voltage gel electrophoresis has been found to be useful in some studies (6, 25). Also, a more recent investigation has demonstrated that interrelationships of *S. epidermidis* strains could be established on the basis of *Sma*I-generated

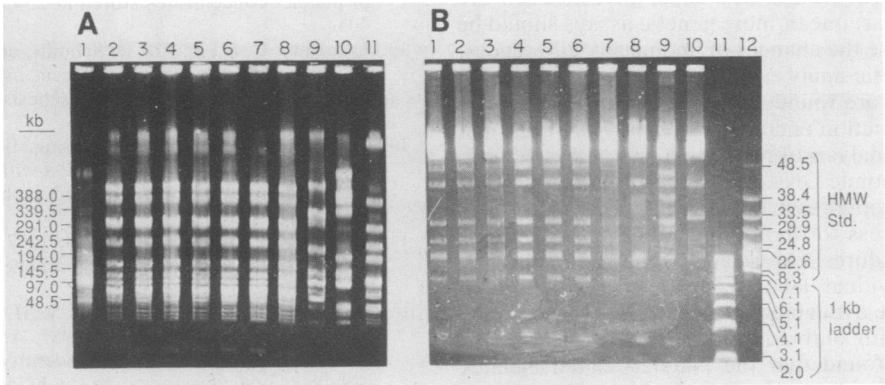

FIG. 3. FIGE analysis of *Sma*I-digested chromosomal DNA from *S. epidermidis* isolates. (A) Gel for DNA fragments greater than 50 kb in size. Lane 1, lambda (48.5-kb) oligomers (BRL); lane 2, erythrocyte unit isolate B451-A; lanes 3 to 5, platelet unit isolates B454, B456, and B465; lanes 6 to 8, patient isolates B466, B479, and B480; lane 9, erythrocyte unit isolate B451-C; lanes 10 and 11, unrelated isolates B218-90 and B252-90. (B) Gel for DNA fragments less than 50 kb in size. Lanes 1 to 10, samples as in lanes 2 to 11 in panel A; lanes 11 and 12, 1-kb ladder and high-molecular-weight standards (HMW Std.) (BRL), respectively.

chromosomal restriction fragment length polymorphisms separated by FIGE (14).

In the present study, biotyping demonstrated that 22 of the isolates obtained from the patient, platelet units, and erythrocyte unit were *S. epidermidis* on the basis of their reactions in 15 biochemical tests. In addition, 21 of the 22 isolates had the same antibiotic susceptibility pattern and slime production pattern. The isolate that differed was B451-C, one of the *S. epidermidis* isolates from the erythrocyte unit. Moreover, DNA analyses of the 22 *S. epidermidis* isolates showed that the 6 patient isolates, the 14 isolates from the platelet units, and B451-A from the erythrocyte unit contained the same genetically related plasmids. Also, restriction endonuclease-digested chromosomal DNA analyzed by FIGE demonstrated that representative isolates from the patient, platelet units, and B451-A from the erythrocyte unit were similar while B451-C from the erythrocyte unit and two nonrelated isolates differed. These relationships were further substantiated by comparing total cellular DNA digests from representative isolates hybridized with an *E. coli* rRNA probe. Thus, even though many methods of typing were used, no differences were seen in the organisms obtained from the original erythrocyte unit, the eight platelet units, and the patient. However, the nonrelated controls used in each typing method were distinctly different from each other and from the isolates obtained from the erythrocyte unit, the platelet units, and the patient.

The patient's septicemia was apparently related to infusion of the eight platelet units, the first of which was most likely the source of the contamination. The other units were probably contaminated at the time of transfusion, since the same infusion set was used for all eight units infused. Because we isolated three different strains of normal skin flora from the erythrocyte unit which was associated with the first unit of platelets, we feel that the contamination most likely occurred at the donor venipuncture site at the time of collection of this unit. The skin of the donor has also been the suspected source of contamination of platelets in other studies (2, 8). However, in this case, the donor was not available for further testing.

This case allowed us to use a number of molecular epidemiologic techniques for the study of *S. epidermidis*, all of which, ultimately, supported the same conclusion. In general, when the results of phenotypic assays demonstrate differences between bacterial isolates obtained during an outbreak investigation, genetic fingerprinting is not necessary. However, when the isolates under investigation are phenotypically similar, one or more genetic assays should be performed to increase the chances of finding any differences. The number of genetic analyses performed is dependent on whether differences are found. In our laboratory, we begin with sizing and restriction endonuclease analysis of plasmid DNA, followed by analysis of restriction endonuclease fragment patterns of genomic DNA separated either by FIGE or pulse field gel electrophoresis. Finally, if further analysis is necessary, we progress to ribotyping and/or plasmid cross-hybridization, procedures which require the use of radiolabeled probes. Individual laboratories need to determine which techniques are available and appropriate for their use when confronted with outbreak situations. In the present study, because we found that the platelet-related isolates were phenotypically identical, we tried to differentiate among them using four different genetic analyses. We subsequently found no evidence of genetic variability. Therefore, the results of the genetic techniques not only confirmed the relatedness of the platelet-associated isolates but also demonstrated the differences between the case-related strain and nonrelated control strains of *S. epidermidis*.

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