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Phenotypic Variation of *Staphylococcus epidermidis* in Infection of Transvenous Endocardial Pacemaker Electrodes

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Coagulase-negative staphylococci isolated from a patient with a pacemaker electrode infection were extensively evaluated by phenotypic and genotypic characterization. Findings from this evaluation were striking because different colony morphologic subtypes were recovered from blood and resected pacemaker electrodes. Staphylococci from each colony subtype (LBL, LBV, LBP, LBS) were identified as slime-producing strains of *Staphylococcus epidermidis* sensu stricto. Direct plating of isolates from a resected electrode revealed a mixture of colony phenotypes when examined on a high-salt, low-glucose medium, Memphis agar. Bacteriophage typing employing 17 different phages and plasmid profile analysis were largely unsuccessful in further characterizing bacterial cells of each of the four colony morphotypes. On the other hand, restriction endonuclease analysis by *Eco*RI digestion of the chromosomal DNA demonstrated the probable common clonal origin of the four colony phenotypes.

Colony morphologic variation within strains of staphylococci isolated from clinical specimens and animal infection models has been described by several groups of investigators recently (1–4, 7, 10, 11, 13, 17, 18, 21, 24, 25). Travis and MacLowry (25), for example, surveyed isolates recovered from patient blood cultures and reported that at least two or more colony morphologic subtypes were seen in over 6% of the isolates. Moreover, coagulase-negative staphylococci had the highest percentage (9.54%, 115 of 1,205) of isolates with at least two colony morphotypes. Four different colony morphologic subtypes were recovered from blood and resected pacemaker electrodes from a patient described herein with a coagulase-negative staphylococcal infection of two transvenous endocardial pacemaker electrodes. These subtypes seemed to undergo phenotypic variation, and an analysis of chromosomal DNA digests of the different colony subtypes provided evidence that they were probably of the same clonal origin.

CASE REPORT

A 73-year-old man was admitted to the Harry S. Truman Memorial hospital with a 3-month history of fever and chills which increased in severity over the week prior to admission. These symptoms began approximately 10 days after the replacement of a cardiac pacemaker which had been inserted over 2 years previously. At the time of the replacement, the initial transvenous endocardial pacemaker electrode was so firmly imbedded in the endocardial wall that transvenous removal was not successful. Therefore, this electrode was left imbedded and a second electrode with a new generator was surgically implanted.

Physical examination at admission revealed a soft, grade 1/6 systolic murmur at the apex and a subungual hemorrhage of the right thumb. There was no evidence of infection of the skin and soft tissue overlying the newly implanted generator.

Multiple sets of blood cultures were obtained at admission, and the patient was given vancomycin hydrochloride intravenously. Echocardiographic examination failed to demonstrate vegetations or evidence of valvular insufficiency. Oral rifampin was added to his therapy after the isolation of *Staphylococcus epidermidis* from all three sets of blood cultures.

Although the patient showed a clinical response to therapy, he underwent removal of both pacemaker electrodes and generator 10 days after admission. Median sternotomy was required for removal of the initial pacemaker electrode because of entrapment of the electrode tip in the endocardial wall at the juncture of the right atrium and superior vena cava. No vegetations were evident on inspection of this site. Following surgery, the patient received 2 additional weeks of antimicrobial therapy and made a gradual recovery.

MATERIALS AND METHODS

Culture methods. Blood cultures were processed with an automated broth culture system (BACTEC 460; Becton Dickinson and Co., Towson, Md.). Aliquots of the broth were subcultured on Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.), and the recovered colonies were characterized after overnight growth at 37°C. The intracardiac tips of the resected electrodes were placed in sterile phosphate-buffered saline (0.005 M phosphate, 0.15 M NaCl, pH 6.8) and vortexed for 1 min, and then 0.1-ml aliquots of the suspension were plated on sheep blood agar and a high-salt, low-glucose medium (Memphis agar) (4). After 24 h of incubation at 37°C, plates were examined by surface illumination and transillumination with a binocular dissecting microscope.

Species identification. An automated system (Vitek AMS; Vitek Systems, St. Louis, Mo.) with a gram-positive organism identification card was used to identify the clinical isolates. In addition, the API Staph-Ident and Staph-Trac systems (Analytab Products, Plainview, N.Y.) were performed according to the instructions of the manufacturer for species identification of each of the four colony morphotypes.

Antimicrobial susceptibility testing. Susceptibility testing was performed by the same automated system (Vitek AMS).

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TABLE 1. Clinical and microbiologic characteristics of the four phenotypic isolates examined in this survey

Isolate	Source of isolate	Identification system/code	Species designation	Slime production (±SE)	Antimicrobial phenotype[a]	Phage type	Plasmid profile[b]
LBP	Blood	Vitek/57506040010	Unidentified	7.29 ± 0.08	Pc^r Am^r Ox^r	188	Negative
		Staph-Ident/7040	*S. epidermidis*				
		Staph-Trac/6706013	*S. epidermidis*				
LBV	Blood	Vitek/53402040010	Unidentified	7.22 ± 0.13	Pc^r Am^r Ox^r	NT[c]	Negative
		Staph-Ident/7040	*S. epidermidis*				
		Staph-Trac/6706013	*S. epidermidis*				
LBL	Electrode	Vitek/77546040010	*S. epidermidis*	7.43 ± 0.0004	Pc^r Am^r Ox^r	NT	Negative
		Staph-Ident/7040	*S. epidermidis*				
		Staph-Trac/6706013	*S. epidermidis*				
LBS	Electrode	Vitek/73546040010	*S. epidermidis*	7.34 ± 0.08	Pc^r Am^r Ox^r	NT	Negative
		Staph-Ident/7040	*S. epidermidis*				
		Staph-Trac/6706013	*S. epidermidis*				

[a] By disk diffusion susceptibility testing, resistance to penicillin G (Pc), ampicillin (Am), and oxacillin (Ox).
[b] Negative, No plasmid bands were demonstrated on agarose gel electrophoresis.
[c] NT, Nontypable with 17 phages available.

In addition, disk diffusion susceptibility testing was done by the method of Bauer and Kirby (6).

Adherence measurements. Slime production was quantitatively examined by a modification (G. D. Christensen, L. M. Baddour, B. N. Madison, S. N. Abraham, J. T. Parisi, D. L. Hasty, J. H. Lowrance, J. A. Josephs, and W. A. Simpson, J. Infect. Dis., in press) of a spectrophotometric technique as previously described (5) which employed a Micro-Elisa AutoReader (model MR580; Dynatech Laboratories, Inc., Alexandria, Va.). In the present investigation, the optical density of stained bacterial films adherent to tissue culture plates (Cell Wells, no. 25860; Corning Glass Works, Corning, N.Y.) was measured with a model 2550 EIA Reader (Bio-Rad Laboratories, Richmond, Calif.) with a 600-nm filter and a data analysis system. The density scale of the EIA Reader has a maximum value of 8.000, which increased the quantitative distinction between slime-producing and slime-nonproducing strains. Therefore, the densities of the adherent bacterial films were reported herein only when the bacteria were grown in Trypticase soy broth (BBL). Each measurement was performed in quadruplicate, repeated three times, and then averaged.

Colony phenotypic characterization. One colony of each phenotype obtained from blood culture isolates that had been streaked onto blood agar plates was selected for subculture on Memphis agar. The use of Memphis agar has previously allowed us to distinguish among various colony forms of *S. epidermidis* (2, 4).

Bacteriophage typing. Phage typing was performed with 17 phages as described previously (20).

Plasmid profile determinations. The lysis of cells and preparation of cleared lysates for plasmid profile analysis were performed as previously reported (15, 20).

Restriction endonuclease analysis. Chromosomal DNA digest patterns of the four colony subtypes obtained from the blood and pacemaker electrodes of the patient and three control strains (H1, ATCC 15305, BENS no. 2) were performed by a modification of the methods of Berger-Bächi (8) with *Eco*RI restriction endonuclease (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Our modification included using chloroform for lysate extractions rather than ether. The control strain H1 refers to a slime-producing colony phenotype of the clinical isolate RP62A (ATCC 35984); strain ATCC 15305 is the type strain for *Staphylococcus saprophyticus* from the American Type Culture Collection (Rockville, Md.); strain BENS no. 2 represents a nitrosoguanidine-induced mutant derived from strain ATCC 15305. The application of restriction endonuclease analysis as an epidemiologic tool to examine strains of coagulase-negative staphylococci was reported initially by Burnie and Lee (9) and Renaud and co-workers (23).

RESULTS

Colony subtypes. Two colony morphotypes, designated LBP and LBV, were seen when blood culture isolates were grown on Trypticase soy agar with 5% sheep blood. After overnight incubation at 37°C, the predominant colony phenotype (LBP) was greater than 1 mm in diameter with a pearly white color and smooth borders when examined with surface light. The variant colonies (LBV) were much fewer in number and were similar in diameter and shape to the predominant colonies, but they were grey when examined with surface light. Two different colony subtypes, designated LBL and LBS, were also identified from cultures of both resected electrodes. One colony subtype (LBL) was similar in appearance to the predominant colony phenotype recovered from the blood cultures of the patient. In contrast, the other subtype (LBS) grew as a small colony variant form with colony diameters of less than 0.2 mm. Borders of the small colony forms were highly irregular when viewed with transillumination and a dissecting microscope. All four colony subtypes were nonhemolytic on sheep blood agar.

The automated Vitek system failed to identify to the species level both colony phenotypes from blood cultures; however, the two colony subtypes isolated from electrode cultures were identified as *S. epidermidis* sensu stricto (Table 1). All four isolates were identified as *S. epidermidis* sensu stricto with identical code numbers with the API Staph-Ident and Staph-Trac strips.

Similar susceptibility patterns were seen for the four colony subtypes (Table 1) with the automated system, which demonstrated that the isolates were resistant to penicillin G and ampicillin and susceptible to eight other antimicrobial agents tested (Table 1). Disk diffusion susceptibility testing disclosed that the four subtypes were also resistant to oxacillin.

Blood and electrode isolates demonstrated high-level adherence (mean, 7.22 to 7.43). The adherence values for each of the isolates did not differ significantly ($P > 0.05$ by Student's t test). This high level of adherence has been characteristic of many coagulase-negative staphylococcal strains causing various types of foreign body-related infections (5, 12). One of the three control strains (RP62A, ATCC

TABLE 2. Colony phenotypes on Memphis agar among pacemaker electrode isolates

Source	Morphology[a] (no. [%] of total colonies counted)
Electrode 1	LBE (381 [41.7])
	GRE (45 [4.93])
	Intmd (114 [12.5])
	SCV (373 [40.9])
Electrode 2	LBE (121 [8.26])
	GRE (33 [2.25])
	Intmd (822 [56.1])
	SCV (489 [33.4])

[a] LBE, Light blue epsilon; GRE, green epsilon; Intmd, intermediate; SCV, small colony variant.

35984) had adherence values similar to those of the isolates in the present survey.

Subcultures of LBP and LBV grew as predominately (>99%) light blue epsilon phenotypes (4) on Memphis agar (data not shown). Organisms colonizing the surface of both electrodes were directly plated onto the Memphis agar for colony phenotypic characterization. As detailed in Table 2, several colony phenotypes were obtained and sectoring among the epsilon colony phenotypes was prevalent. In addition, the saline suspensions contained organisms that grew predominantly as either small colony variant forms or intermediate forms on the Memphis agar. Single colonies of light blue epsilon, intermediate, and small colony variants from electrode 1 cultures were chosen for subculture. After 24 h of incubation at 37°C, the frequencies of recovering aberrant types of colonies were high and ranged from 6.8×10^{-2} to 8.6×10^{-1} (data not shown).

Phage typing and plasmid pattern analysis were of limited value in this survey of isolates. Cells from one colony morphotype, LBP, were weakly lysed by phage 188 (Table 1). Otherwise, the isolates proved nontypable with the phage types used in this investigation. Similarly, no plasmids were identified among the four colony phenotypes.

Restriction enzyme analysis demonstrated the same pattern of restriction enzyme fragments of chromosomal DNA among the four colony types (Fig. 1). In contrast, the patterns seen for control strains BENS no. 2, ATCC 15305, and H1 were markedly different.

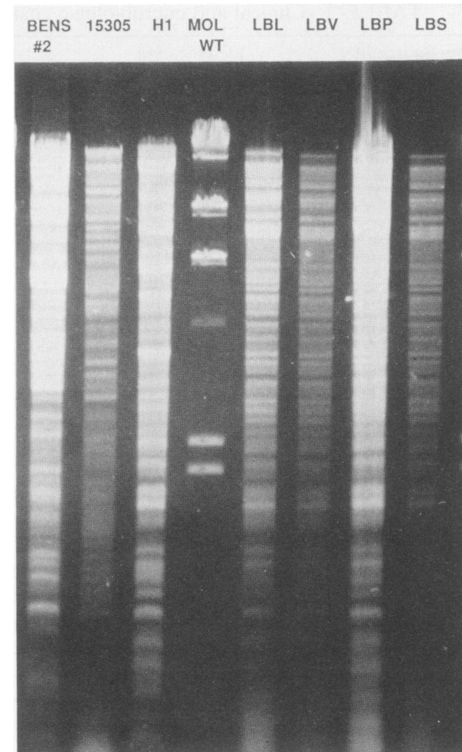

FIG. 1. Chromosomal DNA restriction endonuclease analysis of colony phenotypes (LBL, LBV, LBP, LBS) isolated from the present patient and control strains (BENS no. 2, ATCC 15305, H1) with molecular weight size markers (phage lambda DNA digested with *Hin*dIII).

DISCUSSION

The isolation of multiple colony phenotypes from a clinical specimen is of keen interest because it suggests that the culture contains a mixture of strains, some of which may be contaminants. Alternatively, these different colonies could reflect different phenotypes of the same bacterium which might be important in the pathogenesis of intravascular infections caused by coagulase-negative staphylococci. This is particularly true for coagulase-negative staphylococci, which are extremely common contaminants isolated from blood cultures (27), and uniformity of phenotype is usually an initial assumption in claiming that these bacteria are pathogens.

Multiple epidemiologic tools, including phage typing and plasmid analysis, proved useful in determining strain relatedness of coagulase-negative staphylococci (19). Unfortunately, some strains, as evidenced in the present work, are nontypeable and do not possess characteristic plasmid profiles. In this case, restriction endonuclease analysis has been extremely helpful in determining strain relatedness among *S. epidermidis* isolates by providing good typeability, reproducibility, and discrimination. Moreover, restriction endonuclease analysis (9, 23) has demonstrated patterns that remained stable (in vivo reproducibility) (23) among strains sequentially isolated over several days from individual patients, which was characteristic of the four colony subtypes examined in this study.

Surprisingly little is known about the pathogenesis of staphylococcal pacemaker-related infections considering that (i) an estimated 1 in 500 Americans have a permanent pacemaker and more than 120,000 new implants are inserted annually (14); (ii) infection may complicate up to 15% of implanted permanent pacemakers (26); and (iii) staphylococcal species account for the large majority of these foreign body-related infections (26). One proposed staphylococcal virulence factor, slime production, was characteristic of all four colony morphotypes isolated in the present case report. Indeed, recent case reports (16, 22) and experimentally induced infections (22) of pacemaker electrodes provided electron microscopic evidence that staphylococci were attached to the electrodes and produced a slimy amorphous matrix in which the bacterial cells were embedded. Some (16, 22) have speculated that slime is responsible for maintenance of foreign body-associated infections by interfering with both natural host defense mechanisms and antimicrobial agent efficacy.

After the recognition (1) of a patient with prosthetic valve endocarditis caused by small colony variant forms of *S. epidermidis*, we used an animal model of experimental endocarditis to further investigate the role of the small

colony variants in foreign body-related intravascular infections (4). By analyzing colony populations from inocula and respective infected vegetations, we obtained data providing strong evidence that there was a selection for small colony forms in endocardial lesions which was not merely an in vitro phenomenon (4). Since pretreatment blood was not available in the present case for direct culture on Memphis agar, no analysis could be made comparing the colony populations obtained from pretreatment blood cultures and resected electrodes. At the same time, it is interesting that small colonies were prominent among electrode isolates.

The potential role of colony phenotypic variation in the pathogenesis of foreign body-associated infections caused by coagulase-negative staphylococci is a focus of study for future investigations. At the same time, data presently available would support the use of restriction endonuclease analysis as an important epidemiologic tool in determining the strain relatedness of different colony phenotypes of coagulase-negative staphylococci, particularly when phage typing and plasmid analysis prove unrewarding.

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