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Coagulase-Negative Staphylococci in Multiple Blood Cultures: Strain Relatedness and Determinants of Same-Strain Bacteremia

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The frequency of strain relatedness was determined among randomly selected patients with coagulase-negative staphylococcal infections as determined in multiple blood cultures by plasmid typing, determination of species, and antibiotyping. Strain relatedness was demonstrated in 21 of 47 episodes of bacteremia (44.7%) among 34 patients, with a similar percentage among patients with two or one positive blood culture in 24 h (14 of 30 [46.7%] versus 7 of 17 [41.2%], respectively). Same-strain bacteremia was more frequent in cases of infection among patients with a corresponding fever (15 of 21 [71.4%]), among patients infected with organisms from an identifiable source (7 of 9 [77.8%]) and with non-*Staphylococcus epidermidis* species (9 of 11 [81.8%]), and among patients with nosocomially acquired infections (18 of 36 [50%]). Comparing episodes with or without strain relatedness, no difference was noted in the time to growth (2.1 ± 1.4 versus 1.9 ± 0.9 days, respectively), in bacterial growth in two culture bottles (5 of 14 [35.7%] versus 8 of 24 [33.3%], respectively), and in the presence of additional negative blood cultures (9 of 21 [42.9%] versus 11 of 26 [42.3%], respectively). The antibiotypes of all related strains and 7 of 44 (15.9%) unrelated pairs were identical. These findings demonstrate that coagulase-negative staphylococci from multiple blood cultures are frequently unrelated, suggesting a high prevalence of contamination. In the absence of precise measures for demonstrating strain relatedness, the combination of a clinical assessment with antibiotype determination appears to be a suitable alternative.

Coagulase-negative staphylococci are the most frequently isolated organisms from blood cultures (BCs). They are usually considered contaminants (1, 16), although they have been recognized as true pathogens (4, 6, 13) and at times they may be associated with severe morbidity and identifiable virulence factors (9, 14, 18). The rate of contamination of BCs varies, but it is estimated to be about 2 to 3% in most laboratories (5). For this reason, several clinical and microbiologic guidelines were established to differentiate true bacteremia from contamination (1, 3, 8, 11, 12, 15). For instance, growth in both aerobic and anaerobic bottles and growth within 48 h are considered suggestive of true bacteremia (1, 3), whereas isolation of other skin contaminants (11, 12), growth of organisms in blood drawn for culture during effective antibiotic therapy (12), and negative additional BCs (11, 12) are viewed as more characteristic of contamination. Most of these guidelines were based on clinical determination of the likelihood of true bacteremia. Whether they apply to patients with multiple positive BCs is uncertain. On the basis of probabilities, if the contamination rate of one BC is estimated to be 2 to 3%, the likelihood that two coagulase-negative staphylococcal isolates will be unrelated should fall between 2 and 3% (if one positive BC represents a true bacteremia) and 0.04 and 0.09% (if both isolates are contaminants). Yet, using clinical assessment, Kirchhoff and Sheagren (11) estimated that 52.9% of patients with coagulase-negative staphylococci in multiple cultures of their blood do not have corresponding clinical signs that suggest true bacteremia. Since the accuracy of clinical assessment has not been validated and since transient bacteremia may not always be recognizable, their conclusion could not be substantiated without the use of more precise measures that determine whether these isolates are related, which would suggest that true bacteremia is possible, or whether they represent multiple unrelated strains, which would imply that all or some of these isolates are contaminants. Unfortunately, most routine methods such as determination of species, phenotypic characteristics, and antibiotic resistance pattern (antibiotype) fall short of this goal. For instance, determination of the species of the isolates is not likely to be helpful except for patients infected with diverse species because it does not determine strain relatedness within the same species. Likewise, phenotypic characteristics are unreliable because variation was documented among several genetically related isolates (2, 12, 17). With respect to antibiotype, its specificity in detecting genetically related coagulase-negative staphylococcal strains has not been verified. To address all of these issues, we elected to study the frequency of strain relatedness among patients infected with coagulase-negative staphylococci, as shown in multiple cultures of their blood, to assess whether commonly used guidelines for distinguishing true bacteremia from contamination are applicable to patients with multiple positive BCs and to define a strategy for the clinical determination of same-strain bacteremia.

MATERIALS AND METHODS

Patient selection. Five patients with multiple positive BCs seen by the Infectious Diseases Service in October 1991 were selected for an initial pilot study. The remaining patients were randomly selected through prospective review of BC results in the microbiology laboratory between 1 June and 1 December 1993 without any knowledge of the patients' clinical conditions. Selection criteria included two or more BCs positive for coagulase-negative staphylococci within a 7-day period. Exclusion criteria were concomitant isolation of other organisms from the same BC and the fact that all blood for culture had to be drawn from an accessible intravascular device. All selected isolates were frozen at −70°C until further testing. Patients' medical records were evaluated without any knowledge of plasmid typing results. The following information was abstracted: the patient's age, sex, and underlying condition, the presence of fever, the possible

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source of bacteremia, whether there were other causes of fever, whether cultures were drawn while the patient was receiving antibiotics, the interval between positive cultures, the number and type of bottles with bacterial growth, the time to bacterial growth, antibiotype results, and the total number of blood samples for culture drawn within 48 h of the occurrence of the implicated bacteremia.

BC. Venipuncture sites were cleansed with alcohol and then 2% tincture of iodine and were allowed to dry for 30 to 60 s. Then, by using a sterile needle and syringe or butterfly with a VACUTAINER adapter, 10 to 20 ml of blood was drawn from adults and older children and was dispensed into the anaerobic bottle and then into the aerobic bottle. Among infants and young children, 0.5 to 3 ml of blood was drawn, with an optimum being 1.5 ml, and the blood was dispensed into one aerobic bottle. Each individual BC was performed with blood obtained at a separate venipuncture. Cultures were incubated at 35°C and were monitored by Bactec NR 660 or 9240 blood culture instruments (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) for 5 to 7 days according to the manufacturer's instructions. Both systems relied on CO_2 production for the detection of positive cultures.

Organism identification and susceptibility testing. Positive BC bottles were Gram stained and were examined for growth showing the typical morphology of gram-positive cocci. Organisms were grown on Trypticase soy agar (TSA) with 5% sheep blood at 37°C and 5% CO_2. They were then characterized as catalase positive and coagulase negative by Staphaurex latex agglutination (Murex Diagnostics Limited, Dartford, England). Species identification was accomplished with Vitek Gram-Positive Identification Cards (bioMerieux Vitek, Inc., Hazelwood, Mo.).

Antimicrobial susceptibility testing was carried out with the Vitek automated system with Gram-Positive Susceptibility Cards. Antibiotypes were considered identical if the MICs of all antibiotics tested were identical or within 1 dilution variation. These antibiotics were penicillin, oxacillin, cephalothin, vancomycin, ampicillin-sulbactam, tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin, erythromycin, and clindamycin.

Plasmid isolation. Coagulase-negative staphylococcal isolates were frozen in skim milk at −70°C until plasmid isolation. They were then grown on TSA with 5% sheep blood overnight at 37°C in a 5% CO_2 atmosphere. The Magic Miniprep DNA Purification System (Promega, Madison, Wis.) was used to isolate plasmid DNA.

DNA digestion and electrophoresis. Five microliters of the plasmid preparation was combined with 1 μl of restriction enzyme *Eco*RI or *Hin*dIII, 1 μl of appropriate 10× restriction buffer, 1 μl of RNase A (0.1 mg/ml), and 2 μl of sterile distilled water. The mixture was then incubated for 2 h at 37°C. Then, 3 μl of loading dye was added to each individual *Eco*RI and *Hin*dIII digest, samples were pipetted onto wells of 0.7% SeaKem GTG agarose gel (FMG BioProducts, Rockland, Maine), and the gels were run on the Horizon 11-14 Gel Electrophoresis Apparatus (Gibco BRL, Life Technologies, Inc., Grand Island, N.Y.) for 4 h at 55 to 60 V in Tris-borate electrophoresis buffer. *Hin*dIII digests of bacteriophage lambda (Gibco BRL) were loaded onto each gel for use as molecular size standards, and a *Staphylococcus aureus* control organism (University of Iowa, Iowa City) was included as a known plasmid control. Gels were stained with ethidium bromide, destained in water, and then viewed and photographed under transillumination.

Definitions. An episode of bacteremia signifies two or more positive BCs of blood drawn within a 24-h period (defined as a continuous bacteremia) or spaced out by a 1- to 7-day interval with at least one negative BC in between (defined as an intermittent bacteremia). Any additional positive BC that was separated by 2 or more days from a continuous episode of bacteremia or by 8 or more days from an intermittent episode of bacteremia was considered identification of a separate episode of bacteremia.

Strains were considered related if plasmid bands were identical or, if the isolates lacked plasmids, they were of the same species and had identical antibiotypes. Same-strain bacteremia indicates that the coagulase-negative staphylococcal isolates were related. Distinct-strain bacteremia signifies that the isolates were unrelated.

One of the following was considered a probable source of bacteremia: an intravascular device with ≥15 CFU of coagulase-negative staphylococci, an intravascular device for which there was no microbiologic data if the persistent bacteremia cleared after the device was removed, or any sterile body site at which coagulase-negative staphylococci were detected.

A community-acquired episode indicates bacteremia that was present on admission to the hospital or that developed within 2 days after admission to the hospital. A nosocomial episode signifies bacteremia that occurred 3 or more days after admission to the hospital or that was present on admission to the hospital in patients with prior hospitalization within 30 days of the implicated bacteremia.

Fever is defined as any oral temperature of ≥38.5°C or a daily temperature greater than 38°C over 2 consecutive days. Corresponding fever implies any fever that started within 2 days of coagulase-negative staphylococcal bacteremia preceded by at least 3 days without fever or, in patients with prior fever, an increase in temperature above the baseline temperature of 2°C at about the time of bacteremia.

Another cause of fever indicates coexisting clinically or microbiologically documented infections or other conditions that are known to cause fever such as disseminated malignancies, collagen vascular disorders, hypersensitivity reactions, or thromboembolic events.

TABLE 1. Growth characteristics of coagulase-negative staphylococci in BCs

Characteristic	Value
Total no. (%) of BCs studied	87
No. (%) of blood samples inoculated into two bottles[a]	67 (77.0)
No. (%) of BCs with growth in aerobic bottles only	40 (59.7)
No. (%) of BCs with growth in anaerobic bottles only	14 (20.9)
No. (%) of BCs with growth in both bottles	13 (19.4)
No. (%) of BCs with the following time to growth:	
1 day	30 (34.9)
2 days	41 (47.7)
3 or more days	15 (17.4)[b]

[a] For 20 pediatric patients, aerobic bottles only were inoculated with blood.
[b] The time of growth was not recorded for one culture.

After all tests were completed, the results were averaged in patients with infections caused by strains with and without strain relatedness, and the findings were then compared. Any statistically significant difference was considered a distinguishing feature. The sensitivity and specificity plus the positive and negative predictive values of each presumed determinant of same-strain bacteremia were calculated by standard methods.

Statistical methods. The data were evaluated by 2-by-2 contingency tables by using Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) were calculated according to the Mantel-Haenszel inference. A P value of 0.05 or less was considered statistically significant.

RESULTS

A total of 896 positive BCs were detected among 11,092 BCs performed during the study period (8.1%); coagulase-negative staphylococci were the organisms detected in 369 of them (41.2% of positive cultures). These organisms were recovered from a single BC in 228 instances and from two or more BCs in the remaining instances (a total of 141 BCs among 57 patients). A total of 91 strains were isolated from the 87 BCs selected for the study. The majority of the coagulase-negative staphylococcal isolates (59.7%) grew in aerobic bottles only (Table 1). They were encountered in 47 individual episodes of bacteremia among 34 patients. Bacteremia was continuous in 30 episodes (the average interval between positive cultures was 88 min, with a range of 0 min to 24 h and a median of 15 min) and intermittent in 17 episodes. *Staphylococcus epidermidis* accounted for 25 episodes, single non-*S. epidermidis* species accounted for 11 episodes, and mixed species accounted for 11 episodes. The patients' ages ranged from newborn to 85 years (mean, 50.5 ± 25.4 years), and the ratio of males to females was 1.1. The duration of hospitalization prior to the implicated bacteremia varied between 0 and 111 days (mean, 14 ± 24.3 days); 11 episodes of bacteremia were community acquired. Plasmids were detected in 84 isolates (92.3%).

Strain relatedness was demonstrated in 21 episodes of bacteremia (44.7%). It was established by demonstrating the presence of identical plasmid bands in 18 of the episodes and by demonstrating the same species and antibiotypes among the plasmid-deficient isolates causing the remaining 3 episodes. The percentage of episodes caused by related strains was comparable in patients with continuous and intermittent bacteremias (14 of 30 [46.7%] versus 7 of 17 [42.2%], respectively; $P = 0.8$). No differences were noted between episodes caused by strains that were or were not related with respect to time to growth, growth in aerobic or anaerobic bottles, the percentage of patients whose blood grew organisms in both culture bottles, the interval between positive cultures, the presence of two or more additional negative BCs within 48 h of the implicated bacteremia, and the proportion of episodes occurring during antibiotic therapy (Table 2).

Strain relatedness was more common in nosocomial epi-

TABLE 2. Growth characteristics of coagulase-negative staphylococci in same-strain and distinct-strain bacteremias

Characteristic	No. (%) for the following type of bacteremia:	
	Same strain (21[a])	Distinct strain (26[a])
Episodes characterized by:		
Two or more BCs in 24 h (30)	14 (66.7)	16 (61.5)
One BC in 24 h (17)	7 (33.3)	10 (38.7)
BC during antibiotic therapy (29)	14 (66.7)	15 (57.7)
Negative additional BC[b] (20)	9 (42.9)	11 (42.3)
BCs with the following time to growth:	40	46[c]
1 day (30)	15 (37.5)	15 (32.6)
2 days (41)	17 (42.5)	24 (52.2)
3 or more days (15)	8 (20.0)	7 (15.2)
Growth in both bottles (13)	5 (12.5)	8 (17.4)

[a] Values are numbers of episodes.
[b] Two or more BCs within 48 h of the implicated bacteremia.
[c] The time of growth was not recorded for one BC.

sodes than in community-acquired episodes (18 of 36 [50%] versus 3 of 11 [14.3%], respectively; $P = 0.3$), in episodes with corresponding fever than in episodes without corresponding fever (15 of 21 [71.4%] versus 6 of 26 [23.1%], respectively; OR = 8.3; CI = 12.2 to 31; $P = 0.001$), in episodes with an identifiable source than in episodes without a documented source (7 of 9 [77.8%] versus 14 of 38 [36.8%], respectively; OR = 6; CI = 1.1 to 33; $P = 0.06$), and in episodes caused by non-*S. epidermidis* species than in episodes caused by *S. epidermidis* (9 of 11 [81.8%] versus 12 of 25 [48%], respectively; OR = 4.8; CI = 1.8 to 27.3; $P = 0.08$) (Tables 3 and 4).

Additional positive BCs were encountered in 12 episodes of same-strain bacteremia. The new isolates were related to the original strain in four instances (33.3%), and two of them were from a known intravascular source that had not been removed by the time that the BC was repeated.

Illustrative examples. (i) Patient 1. An 85-year-old female was admitted to the hospital because of a cerebrovascular accident. A single BC, performed with blood obtained on admission because of a low-grade fever, was positive for *S. epidermidis*. The patient's temperature increased further within 48 h of admission, and two additional BCs grew *S. epidermidis*. The antibiotypes of all isolates were similar. The patient was thought to have infective endocarditis on the basis of presumed continuous bacteremia with an apparent cerebral embolus. However, plasmid typing demonstrated that the two recent strains were related, whereas the earlier isolate was unrelated to the two subsequent isolates (Fig. 1, lanes c to e and j to l).

(ii) Patient 2. Patient 2 was a 57-year-old diabetic male in the intensive care unit with multiple organ failure and dialysis-related peritonitis. Peritoneal fluid grew numerous *Candida albicans* isolates and rare coagulase-negative staphylococci that were considered contaminants. *S. epidermidis* was isolated from two BCs obtained 4 days apart, with multiple negative BCs obtained between the two positive BCs. These blood isolates were also considered contaminants on the basis of multiple negative BCs, the patient's stable hemodynamic condition, and the lack of corresponding signs suggesting true bacteremia. However, plasmid typing demonstrated that these organisms were related (Fig. 1, lanes f, g, m, and n). Unfortunately, the isolate from peritoneal fluid was not typed. Although these isolates may represent repetitive contaminants from the patient's cutaneous flora, the possibility of true intermittent bacteremia could not be excluded.

The antibiotypes of all related strains and 7 of 44 unrelated pairs of strains were identical (OR = 335; CI = 18 to 6,095; $P = 0.0001$). Among pairs of strains with discordant antibiotypes,

TABLE 3. Strain relatedness of coagulase-negative staphylococcal causing bacteremia in various clinical settings

Clinical setting (no. of episodes)	No. (%) of episodes for the following type of bacteremia:	
	Same strain	Distinct strains
Community acquired (11)	3 (27.3)	8 (72.7)
Nosocomial (36)	18 (50.0)	18 (50.0)
Corresponding fever (21)	15 (71.4)	6 (28.6)
Identifiable source (9)	7 (77.8)	2 (22.2)
No other cause of fever (5)	4 (80.0)	1 (20.0)
All episodes (47)	21 (44.7)	26 (55.3)

TABLE 4. Species-related strain relatedness in coagulase-negative staphylococcal bacteremia

Staphylococcal species (no. of episodes)	No. of episodes for the following type of bacteremia:	
	Same strain	Distinct strains
S. epidermidis (25)	12	13
S. hominis (6)	6	0
S. haemolyticus (1)	1	0
S. saprophyticus (1)	0	1
S. capitis (1)	0	1
S. xylosus (2)	2	0
Multiple species (11)	0	11

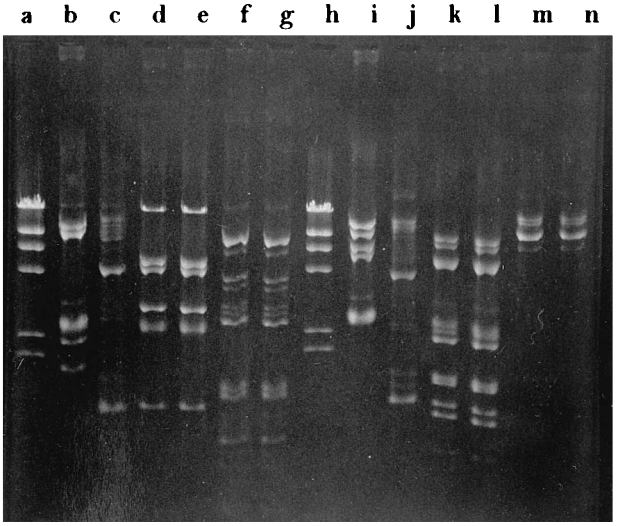

FIG. 1. Restriction endonuclease analysis of plasmid DNA from coagulase-negative staphylococci. Lanes a to g and h to n, *Hin*dIII and *Eco*RI digests, respectively; lanes a and h, molecular size standard (bacteriophage lambda); lanes b and i, *S. aureus* plasmid control; lanes c to e and j to l, three isolates from the same patient (patient 1) showing a distinct band pattern for an isolate obtained from the patient on admission (lanes c and j) and identical patterns for two isolates obtained 48 h later (lanes d, e, k, and l); lanes f, g, m, and n, two organisms with identical band patterns isolated from a single BC of blood drawn on each day 4 days apart from a patient without an obvious source of bacteremia.

TABLE 5. Value of antibiotype in predicting strain relatedness of coagulase-negative staphylococci

Characteristic	No. (%) for the following type of bacteremia:	
	Related strains	Distinct strains
No. of pairs compared	33	44
No. of strains for which MICs were identical for:		
10 drugs	33 (100)	7 (15.9)
4 drugs	33 (100)	14 (31.8)
No. of strains for which MICs are discordant	0	37
No. of strains for which discrepancy in MIC was limited to:		
One drug	0	8 (21.6)
Two drugs	0	8 (21.6)
Three or more drugs	0	21 (56.8)

discrepancies in MICs for the strains were limited to one drug in 8 of 37 episodes (21.6%), two drugs in 8 of 37 episodes (21.6%), and three or more drugs in 21 episodes (56.8%). An assessment of the value of the susceptibility to each antibiotic in distinguishing unrelated strains showed that ampicillin-sulbactam and cephalothin MICs were either ≤ 2 or ≥ 32 μg, without any intermediate values; these MICs corresponded to susceptibility to oxacillin and did not aid in detecting strain differences. In contrast, an isolated difference (fourfold or greater) in the MIC of tetracycline, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, clindamycin, or vancomycin was always predictive of strain variation. These differences would have changed the susceptibility category (from susceptible to resistant) for erythromycin, ciprofloxacin, ampicillin-sulbactam, cephalothin, and clindamycin for all strains, oxacillin for 90% of the strains, tetracycline for 80% of the strains, and trimethoprim-sulfamethoxazole for 68.8% of the strains but not that for vancomycin (range of vancomycin MICs, 0.5 to 4 μg). Determining the effect of selective reporting of susceptibility results for a few antibiotics (ciprofloxacin, oxacillin, vancomycin, and trimethoprim-sulfamethoxazole), a method that is used in our hospital to deter the use of potentially ineffective antibiotics, showed that this practice could have falsely labeled the antibiotypes of oxacillin-resistant isolates as identical for 14 of 31 unrelated oxacillin-resistant isolates (Table 5).

Same-strain bacteremia was noted in seven children and 14 adults. It was detected during assessment of corresponding fever in 15 patients, persistent fever in 5 patients, or corresponding leukocytosis without fever in 1 patient. Transient hypotension was noted in one patient. In the majority of these patients, bacteremia caused by coagulase-negative staphylococci did not affect the course of illness or the outcome for the patient.

Assessment of positive and negative predictive values of various clinical and microbiologic determinants showed that continuous bacteremia, the presence of a source of bacteremia, and growth within 48 h were not predictive of same-strain bacteremia, whereas new fever and identical antibiotypes of the causative strains were highly predictive of same-strain bacteremia (Table 6).

TABLE 6. Predictive values of clinical and microbiologic determinants of same-strain coagulase-negative staphylococcal bacteremia

Determinant	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV (%)[b]
Two or more positive BCs in 24 h	66.7	38.5	46.7	58.8
Identifiable source	33.3	92.3	77.8	63.2
Growth within 48 h	85.7	11.5	43.9	50.0
Same antibiotype	100.0	84.0	82.5	100.0
Corresponding fever	71.4	76.9	71.4	80.8
Same antibiotype and new fever	71.4	92.3	88.2	100.0

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

DISCUSSION

The differentiation of contamination of BCs from true bacteremia presents a common dilemma to clinicians and microbiologists. To minimize this problem, several guidelines for assessing the significance of positive BCs have been advocated on the basis of clinical and microbiologic criteria (3, 8, 12). These guidelines include the type of organism, the likelihood of a causal association of clinical findings with the incriminated pathogen, and various growth characteristics such as time to growth, number of bottles that are positive, the presence of multiple skin organisms, whether blood for culture was obtained during effective antibiotic therapy, and the number of additional BCs without growth (12). As to coagulase-negative staphylococci, the vast majority of single positive cultures are considered to be positive because of the presence of contaminants (1, 16). However, when these organisms are isolated from multiple BCs, they present a diagnostic challenge. Whether all or some of these cultures represent multiple contaminations with unrelated strains or whether they signify repetitive isolation of the same organism because of either true bacteremia or recontamination from the cutaneous flora of the patient could not be determined without precise typing methods (7, 10). Likewise, without such typing methods it would be difficult to clarify whether additional positive BCs among patients with true bacteremia signify persistent bacteremia with the same strain or a new, unrelated isolate. Unfortunately, most laboratories do not use these methods routinely. An example of this challenging dilemma is illustrated in our patient with a cerebrovascular accident and multiple positive BCs for *S. epidermidis* with identical antibiotypes.

Our findings demonstrate that coagulase-negative staphylococcal isolates from multiple BCs are often unrelated, especially in patients with community-acquired infections. They also show that additional positive BCs among patients with presumed true bacteremia are frequently caused by different strains. This conclusion is based on the results of plasmid typing for 92.3% of the organisms (84 isolates) and on species plus antibiotype determinations for the seven plasmid-deficient isolates. Although species determination and antibiotype correlation are not very precise, we presumed that the likelihood of strain relatedness among organisms within the same species and with identical antibiotypes and that also lack a plasmid should be very high.

Our study also shows that many of the guidelines that are advocated for use in differentiating contamination from true bacteremia are probably unreliable and do not appear to be applicable to patients with multiple positive BCs. For instance, growth within 24 and 48 h and growth in both aerobic and anaerobic bottles, which was encountered in only a few BCs, were just as common among patients with same-strain bacteremia and patients whose blood contained distinct isolates in each of multiple BCs. These findings differ from those of many previous studies (8, 11, 12). The reason for this discrepancy is

unclear. It is possible that as a result of automation of microbiologic procedures contamination takes place almost exclusively during the drawing of blood and inoculation of the blood into the bottle and not during processing within the laboratory. Then, contamination might affect both bottles, and growth can be detected within 24 to 48 h by an advanced laboratory technology that facilitates the early detection of bacteria, despite a low inoculum.

Our findings also show that identical antibiotypes, determined by using the individual MICs of 10 antibiotics, are highly predictive of strain relatedness, with a sensitivity of 100% and a specificity of 83.7%, and that a fourfold difference in a single MIC is always predictive of strain variation even if the difference does not change the susceptibility category for the strain. Moreover, our results illustrate that the specificity of the antibiotype is lower if nonquantitative methods are used and that the level of specificity correlates with the number of antibiotics being compared.

On the basis of these findings, we believe that true but probably insignificant cases of bacteremia caused by coagulase-negative staphylococci without an identifiable source may occur in a significant proportion of hospitalized patients. Additionally, our findings verify that routine determination of the species of coagulase-negative staphylococcal isolates found in blood is rarely useful in predicting strain relatedness. Hence, we recommend that if coagulase-negative staphylococci are isolated from two or more BCs, antibiotic susceptibility testing should be performed on all isolates and antibiotype similarities should be noted. Since the release of all antibiotic susceptibility data may be meaningless and may lead to the inappropriate use of antibiotics, a statement indicating antibiotype similarity or lack thereof may be sufficient. Then, if reliable typing methods are not available, a clinical assessment to look for corresponding fever unexplained by other causes and for the presence of a source of the bacteremia should also be used to determine the possibility of true same-strain bacteremia, because not all strains with identical antibiotypes are related and because the isolation of related strains from multiple BCs may still be the result of repetitive contaminations with the patient's cutaneous flora.

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