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Species Identification of Coagulase-Negative Staphylococcal Isolates from Blood Cultures

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Coagulase-negative staphylococci generally are not fully identified, are called *Staphylococcus epidermidis*, and are considered contaminants when isolated from blood cultures. In a cancer hospital during 6 months, 46 patients had multiple blood cultures (mean, 3.1) which yielded coagulase-negative staphylococci. Species identification of these showed that 10 of the 46 (22%) were not *S. epidermidis*. Similarly, 96 coagulase-negative staphylococci isolated from only one of multiple blood cultures from patients and thought to be skin contaminants were identified. Of 96 of the staphylococci, 14 (16%) of the latter group were not *S. epidermidis*. Species found included *S. haemolyticus*, *S. hominis*, *S. warneri*, *S. simulans*, and *S. xylosus*. Eight isolates of these species were methicillin resistant, and all eight were mannitol fermenters. The results suggest that these species invasively infect cancer patients with the same frequency at which the species colonize. No one species was identified as being more pathogenic than the others. Routine species identification of coagulase-negative staphylococci from blood cultures of cancer patients contributed little to management except to occasionally distinguish multiple-episode culture contamination by different species from sustained bacteremia with the same species.

Coagulase-negative staphylococci have been implicated as pathogens causing urinary tract infections (11) and infections of foreign bodies (9) such as indwelling intravenous catheters (1), cerebrospinal fluid shunts (5), prosthetic heart valves (A. W. Karchmer, W. E. Dismukes, W. D. Johnson, Jr., W. R. Wilson, G. L. Archer, and M. A. Sande, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Atlantic City, N.J., abstr. no. 365, 1979.), hip prostheses (13). Most laboratories call these organisms *Staphylococcus epidermidis* without further identification. Investigators have tried to subdivide these organisms to determine whether any identifiable subpopulation is more pathogenic (10). It has been determined that *S. saprophyticus* frequently causes urinary tract infections, but attempts to identify coagulase-negative staphylococcal blood culture isolates have not been reported. The diversity of staphylococcal species which infect humans has not been well established although the species which colonize normal human skin are known (6).

Since coagulase-negative staphylococci are the most common blood culture isolates in the Memorial Sloan-Kettering Cancer Center, we did a retrospective study of coagulase-negative staphylococcal isolates from blood cultures to establish which species caused bacteremia in cancer patients with indwelling intravenous catheters, which species were thought to be skin contaminants, and whether there was any correlation between the identified species and pathogenicity (as suggested by multiple positive cultures) or between the identified species and antibiotic resistance patterns.

MATERIALS AND METHODS

Staphylococcal isolates. Blood culture isolates were identified as staphylococci by Gram stain morphology, aerobic growth, and anaerobic acid production from glucose (12). A slide coagulase test was performed and later confirmed by a coagulase tube test (12). All coagulase-negative staphylococcal isolates were grown and saved on Trypticase soy agar slants (BBL Microbiology Systems, Cockeysville, Md.) until identified to the species level. Isolates collected over a 6-month period were classified according to whether they were repeatedly isolated from a patient over a 2-week period and had the same antibiogram (group 1) or whether there were single isolates from one blood culture bottle (group 2) and there were no subsequent blood cultures positive for staphylococci from those patients.

Two isolates each of 14 species of *Staphylococcus* (*S. aureus*, *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. hyicus*, *S. intermedius*, *S. lentus*, *S. saprophyticus*, *S. sciuri*, *S. simulans*, *S. warneri*, and *S. xylosus*) were obtained (courtesy of Wesley Kloos, North Carolina State University, Ra-

TABLE 1. Coagulase-negative staphylococci causing multiple episodes of bacteremia

Organism	No. isolated	Mannitol fermented (no.)	Methicillin resistant by DD[a] (no.)	Nafcillin resistant by BD[b] (no.)	Cephalothin resistant by DD[a] (no.)	Cephalothin resistant by BD[b] (no.)	Erythromycin resistant by DD[a] (no.)
S. epidermidis	36	0	30	28	3	5	31
S. haemolyticus	6	5	5	5	4	5	5
S. hominis	3	0	0	0	0	0	1
S. warneri	1	0	0	0	0	0	0

[a] DD, Disk diffusion test.
[b] BD, Broth dilution test; resistance is defined as MIC > 2 μg/ml.

leigh, N.C.) as controls for biochemical reactions and acid production from sugars.

Patient clinical information. Records of patients with multiple episodes of coagulase-negative staphylococci isolated from blood cultures were reviewed and their ages, underlying diseases (neoplasm), types of intravascular devices, peripheral leukocyte counts, and body temperatures at the time the blood cultures were compiled.

Identification of staphylococci. The identification scheme of Kloos and Schleifer (8) was followed. Acid production was tested aerobically in carbohydrate-phenol red medium (BBL), and the color was read after 72 h of incubation at 37°C. Resistance to novobiocin was determined by growth of the organism on P agar (8) containing 1.6 μg of novobiocin (Sigma Chemical Co., St. Louis, Mo.) per ml. Hemolysis was determined by surface streaking a loopful of organisms on Trypticase soy agar containing 5% human blood (single donor); a hemolytic zone around the streak ≥1.5 mm wide after 72 h of incubation was interpreted as positive. Nitrate reduction and phosphatase activity were determined by the methods of Kloos and Schleifer (8).

Susceptibility testing. Kirby-Bauer disk tests (3) for susceptibility to methicillin, cephalothin, vancomycin, erythromycin, clindamycin, penicillin, and gentamicin were performed on all isolates. Broth dilution susceptibility tests were also performed with microtiter plates (Linbro Titertek; Linbro Scientific Inc., Hamden, Conn.) by the method of Gavan and Barry (4), but with an inoculum of 10^6 organisms per ml. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs; 99.9% killed after 24 h) of the drugs nafcillin, cephalothin, rifampin, vancomycin, and gentamicin were determined.

RESULTS

During a 6-month period, 46 patients with neoplasms were found with multiple episodes (mean, 3.1 episodes) of recovery of coagulase-negative staphylococci with the same antibiogram in blood cultures (Table 1, group 1 organisms). Of these patients, 18 were 16 years old or younger, and 28 were older than 16. The patients had the follow underlying diseases: leukemia (12 cases), lymphoma (11 cases), and solid tumors (19 cases). The remaining five patients had diseases such as severe combined immunodeficiency, aplastic anemia, and mycosis fungoides. All of the patients were febrile when the blood cultures were obtained; 17 were leukopenic with less than 1,000 polymorphonuclear leukocytes per mm^3, and 29 had peripheral leukocyte counts of > 1,000/mm^3. All but four had intravenous catheters. Most had total parenteral nutrition catheters, subclavian intravenous catheters, or peripheral intravenous catheters (18 had catheters for total parenterial nutrition, and 24 used catheters for fluid and drugs). In the same period, there were 96 strains of coagulase-negative staphylococci isolated from only single blood culture bottles (Table 2, group 2 organisms). Blood cultures were obtained from this last group of patients because of fever, but no subsequent blood cultures yielded the same organism.

Of 46 isolates of group 1, 10 (22%) were not *S. epidermidis* and were identified as *S. haemolyticus*, *S. hominis*, and *S. warneri*. Among the

TABLE 2. Coagulase-negative staphylococci from blood cultures considered skin contaminants

Organism	No. isolated	Mannitol fermented (no.)	Methicillin resistant by DD[a] (no.)	Nafcillin resistant by BD[b] (no.)	Cephalothin resistant by DD[a] (no.)	Cephalothin resistant by BD[b] (no.)	Erythromycin resistant by DD[a] (no.)
S. epidermidis	82	ND[c]	30 of 50	ND	ND	ND	ND
S. haemolyticus	7	4	2	2	2	2	2
S. hominis	3	0	0	0	0	0	0
S. simulans	1	0	0	0	0	0	0
S. warneri	2	1	0	0	0	0	0
S. xylosus	1	1	1	1	1	1	1

[a] DD, Disk diffusion test.
[b] BD, Broth dilution test; resistance is defined as MIC > 2 μg/ml.
[c] ND, Not done.

TABLE 3. Differentiation of closely related species

Species	Hemolysis	Nitrate reduction	Colony size ≥ 5 mm	Acid from mannitol
S. hominis	−	+	−	−
S. haemolyticus	+	+	+	±
S. warneri	−	−	−	±

group 2 isolates, 14 of 96 (16%) were non-*S. epidermidis*. This was not statistically different from 22% in group 1 ($P > 0.05$ by χ^2). The species found in groups 1 and 2 were also similar (Table 2).

Among the *S. epidermidis* isolates from group 1, there was a large proportion resistant to methicillin (30 of 36 [83%]) and to nafcillin (28 of 36 [78%]). Of the six susceptible isolates (MIC ≤ 2 μg/ml), two were tolerant strains with MBCs of fourfold or greater than the MICs. There was a much smaller number, 5 of 36 (14%), resistant to cephalothin. The unreliable results of disk diffusion susceptibility tests in determining resistance to cephalothin among methicillin-resistant *S. epidermidis* were again demonstrated as there were two isolates which were cephalothin susceptible by disk diffusion tests but were resistant by broth dilution. Most methicillin-resistant *S. epidermidis* isolates were also resistant to erythromycin and clindamycin. There were no characteristics found among the *S. epidermidis* isolates to predict susceptibilities to antibiotics.

Among the non-*S. epidermidis* isolates, 11 were mannitol fermenters (of which 8 were methicillin resistant) and 13 were non-mannitol fermenters (of which none were methicillin resistant). The absence of mannitol fermentation appeared to be a characteristic of susceptible non-*S. epidermidis*, coagulase-negative staphylococci ($P < 0.001$ by χ^2).

The following tests were easy to perform and interpret and were very helpful in species identification: acid production from trehalose, maltose, mannitol, lactose, sucrose, arabinose, mannose, and xylitol; colony size after 5 days of growth; nitrate reduction; novobiocin resistance; and hemolysis of human blood. Phosphatase activity and anaerobic growth in thioglycolate varied and hence were difficult to interpret. Most species were readily identified, with the exceptions of *S. haemolyticus*, *S. warneri*, and *S. hominis*, which are all biochemically closely related. The latter three species were identified by the characteristics which most resemble those of the type species outlined in Table 3.

DISCUSSION

Coagulase-negative staphylococci, with the exception of *S. saprophyticus* biotype 3, appear to infect only patients with foreign devices such as prostheses and catheters. The route of entry for the organism is thought to be through the skin by direct extension.

Knowledge of the skin flora of normal humans has been well established, with *S. epidermidis*, *S. hominis*, *S. haemolyticus*, and *S. warneri* frequently found on the skin of arms and legs (7). It is reasonable to expect that the same species which colonize may later infect patients with intravascular accesses in their arms and legs. Unfortunately, such clinical information has not previously been obtained since most clinical laboratories do not fully identify the coagulase-negative staphylococci. Some investigators have tried to subdivide these organisms into biotypes and correlate biotypes with the sites of isolation (10), but no attempt was made to distinguish colonization from infection at these sites.

Coagulase-negative staphylococci have been the most common isolates from the blood cultures of the cancer patients we have treated. This may be related to the necessity for prolonged intravenous access for nutrition and therapy or to a compromised immune status such as leukopenia. The high level of resistance of the coagulase-negative staphylococcal population among our patients compared with that of a general hospital (10) may be related to the prolonged exposure to antibiotics of the patients we treat. Species identification of these isolates showed that a significant percentage were not *S. epidermidis*; 22% of those causing infection manifested by multiple episodes of bacteremia and 16% of those isolated from one blood culture bottle and thought to be skin contaminants. The most common isolates were *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. warneri* from both groups 1 and 2 organisms. These are the same species previously reported to colonize the human extremities (7). It is interesting that *S. saprophyticus*, previously documented to cause urinary tract infections, was not found in the blood cultures that we took.

All of the methicillin-resistant, non-*S. epidermidis*, coagulase-negative staphylococci fermented mannitol. Whether only the mannitol fermenters among these species of staphylococci are resistant to methicillin awaits confirmation by testing more clinical isolates. Exceptions to the above observation have been found

in a recent report (14) in which two isolates of *S. hominis* were resistant to methicillin but did not ferment mannitol. However, these two isolates were trehalose negative and would have been classified as *S. epidermidis* by the Kloos and Schleifer identification scheme. The high degree of resistance of these isolates to antibiotics is in accord with a previous report of these organisms from cardiac surgery patients (2).

Identification of blood isolates of coagulase-negative staphylococci currently contributes little to the management of cancer patients except to occasionally distinguish multiple-episode culture contamination by different species of coagulase-negative staphylococci from sustained bacteremia with the same species. Full identification of the isolates requires a minimum of 5 days, and the procedure is hindered by the fact that there is no single characteristic to distinguish the non-*S. epidermidis* species most commonly isolated: *S. haemolyticus*, *S. hominis*, and *S. warneri*. Nevertheless, species identification of these organisms may help us to learn more about the diversity, resistance pattern, epidemiology, and virulence (such as species difference in adherence to heart valves) of the coagulase-negative staphylococci which have long been thought of as *S. epidermidis*.

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