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# Antimicrobial Resistance in Nosocomial Isolates of *Staphylococcus haemolyticus*

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**_Staphylococcus haemolyticus_ is frequently cultured from hospitalized patients and is characterized by resistance to multiple antimicrobial agents. We found that _S. haemolyticus_ represented 70 of 524 (13%) coagulase-negative staphylococcal isolates identified by the clinical microbiology laboratories of two hospitals over 2 months. _S. haemolyticus_ isolates were recovered from wounds (44%), urine (26%), blood (10%), and other sources (20%). All _S. haemolyticus_ isolates were tested for susceptibility to six antimicrobial agents; 77% were resistant to three or more agents, and 41% were resistant to five or six agents. In addition, among 47 multiply resistant isolates, high MICs (≥6.25 μg/ml) of vancomycin (62% of isolates) and teicoplanin (91% of isolates) were found. DNA probes which were derived from _S. epidermidis_ or _S. aureus_ and which contained sequences associated with resistance to antimicrobial agents were used to detect specific genes in the total cellular and plasmid DNAs of 10 resistant _S. haemolyticus_ isolates. Resistance gene probes and the numbers of resistant isolates hybridizing were as follows: methicillin, 10 of 10; gentamicin, 9 of 10; erythromycin, 7 of 10; and trimethoprim, 0 of 10. Genes for resistance to methicillin were found only in chromosomal locations, genes for resistance to gentamicin were found in both chromosomal and plasmid locations, and genes for resistance to erythromycin were found in plasmid locations only. With the exception of trimethoprim resistance determinants, similar genes were found among concurrently isolated multiply resistant _S. epidermidis_ isolates from our hospitals. _S. haemolyticus_ is a potentially important nosocomial species which readily acquires antimicrobial resistance genes and which shares, to some extent, in a common gene pool with _S. epidermidis_.**

*Staphylococcus haemolyticus* has been reported to represent an average of 10% of clinical coagulase-negative staphylococcal isolates (5, 7, 9, 12, 15, 22, 24, 25, 27, 28). Many of these isolates have been found to be resistant to multiple antimicrobial agents. The importance of these organisms as human pathogens relative to that of other coagulase-negative staphylococci is uncertain, in part because species determinations of coagulase-negative staphylococci are not routinely done in most clinical microbiology laboratories. The mechanisms of antimicrobial resistance in *S. haemolyticus* and the extent to which it shares resistance genes with other staphylococci are also unknown. In a recent study of trimethoprim resistance among coagulase-negative staphylococci (6), we found that a specific trimethoprim resistance gene probe from a conjugative *S. aureus* plasmid hybridized with all trimethoprim-resistant *S. epidermidis* isolates but not with four *S. haemolyticus* isolates tested. The possibility of unique mechanisms of resistance in this poorly understood species led us to further investigate its characteristics.

In the present study, we determined the proportion of *S. haemolyticus* isolates among clinical isolates of coagulase-negative staphylococci in two hospitals and compared the antimicrobial resistance of *S. haemolyticus* with that of other coagulase-negative staphylococci collected. We then compared resistance genes in *S. haemolyticus* with resistance genes identified in isolates of *S. epidermidis* and *S. aureus* from our hospitals. In addition, the possibility of resistance transfer from *S. haemolyticus* to other staphylococci was investigated.

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## MATERIALS AND METHODS

**Identification and collection of isolates.** Isolates of coagulase-negative staphylococci recovered in the clinical microbiology laboratories of the Medical College of Virginia Hospitals and the McGuire Veterans Administration Hospital during January and February 1987 were collected. Sources of isolates were wound, urine, and blood specimens; isolates from sputum or cerebrospinal fluid were not collected. The isolates were identified as coagulase-negative staphylococci by colony morphology, Gram stain, catalase production, and their inability to coagulate rabbit plasma.

All coagulase-negative staphylococcal isolates collected were screened for trehalose utilization by inoculation onto purple broth base agar (Difco Laboratories, Detroit, Mich.) containing 10 mg of trehalose (Sigma Chemical Co., St. Louis, Mo.) per ml. All isolates were also tested for urease production by inoculation onto urease test agar (Difco). Isolates that were trehalose positive and urease negative were tested with the Staph-Ident rapid identification kit (Analytab Products, Plainview, N.Y.). Isolates that were not identified to the species level on initial Staph-Ident testing were evaluated further by observing colony morphology and hemolysis on bovine blood agar, by testing for nitrate reduction with inoculation into nitrate reduction broth in Durham tubes, and by testing fructose utilization with inoculation onto purple broth base agar containing 10 mg of fructose (Sigma) per ml.

**Case reviews.** Medical records of patients from whom *S. haemolyticus* isolates had been obtained during the study were retrieved. Records were reviewed to determine the body site from which the organisms were cultured, the purity of the cultures, and the results of concomitant cultures from similar and different sites. For each positive blood culture, the results of other blood cultures obtained on the same date

were recorded. Urine and peritoneal fluid leukocyte counts were recorded. Results of semiquantitative intravascular catheter tip cultures done by the method Maki et al. (19) were noted. Information on the clinical status of patients, including the location of patients (hospitalized or outpatient), the presence or absence of fever, and potential alternate sources of fever if present, was obtained.

**Antimicrobial susceptibility testing.** All isolates of coagulase-negative staphylococci from the Medical College of Virginia Hospitals laboratory were tested for antimicrobial susceptibility by agar dilution performed in accordance with the standards of the National Committee for Clinical Laboratory Standards (23). In the McGuire Veterans Administration Hospital laboratory, only isolates from blood specimens were tested for susceptibility with an automated testing system (Vitek Systems, Inc., Hazelwood, Mo.). To combine the susceptibility test results from the two clinical microbiology laboratories, we classified isolates as resistant to antimicrobial agents on the basis of attainable drug levels in serum by using the following breakpoints (micrograms per milliliter): methicillin, ≥10; gentamicin, >5; erythromycin, >2; tetracycline, >2; trimethoprim, >2; and chloramphenicol, >25. To confirm susceptibility test results from the two clinical microbiology laboratories, we screened all *S. haemolyticus* isolates identified for resistance to four antimicrobial agents by observing growth on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) to which the following amounts of the agents had been added per milliliter of agar: methicillin, 12.5 μg; gentamicin, 5 μg; erythromycin, 20 μg; and trimethoprim, 20 μg. In addition, microdilution MICs were determined for isolates selected for DNA probe analysis by standard methods in accordance with the protocol of the National Committee for Clinical Laboratory Standards (23).

**Preparation of DNA.** DNA was extracted from 10 multiply resistant *S. haemolyticus* isolates, 5 from each hospital. Of the 10 resistant *S. haemolyticus* isolates, 7 (5 from the Medical College of Virginia Hospitals and 2 from the McGuire Veterans Administration Hospital) were collected during the study period; the other 3 Veterans Administration Hospital isolates were collected in 1986. A total of 12 isolates collected during the study period, including 1 susceptible isolate of *S. haemolyticus*, 1 susceptible isolate of *S. epidermidis*, and 10 resistant isolates of *S. epidermidis*, served as controls. Each of the isolates chosen for investigation with DNA probes was distinct from all other isolates as determined by plasmid pattern analysis. DNA was recovered by two methods. *S. haemolyticus* total cellular DNA and that of the susceptible *S. epidermidis* isolate were extracted by a modification of the procedure of Marmur (21) in which lysostaphin (5 mg/ml) was used in place of lysozyme. It was determined during the study that chromosomal DNA from *S. haemolyticus* and *S. epidermidis* recovered from cell lysates by CsCl-ethidium bromide density gradient centrifugation yielded probe results identical to those obtained with DNA extracted by the modified Marmur procedure. Chromosomal DNA from the 10 resistant *S. epidermidis* isolates was therefore recovered by this density gradient method, as was plasmid DNA from all *S. haemolyticus* and *S. epidermidis* isolates. Each cell lysate used in this procedure was obtained from an overnight broth culture which was centrifuged, washed in a 0.01 M EDTA solution, washed in a 50% ethanol-acetone solution, and suspended in a solution of 0.01 M EDTA–low-salt buffer (0.1 M NaCl, 0.05 M EDTA [pH 6.9]) to which lysostaphin (Sigma) was added to a concentration of 25 μg/ml. A solution of 5 M NaCl–0.5 M EDTA–2% sodium dodecyl sulfate was added to achieve cell lysis. The samples were centrifuged, and 500 μg of RNase (Sigma) per ml and 500 μg of proteinase K (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) per ml were added to each supernatant. DNA precipitation occurred with the addition of 5 M NaCl and 87.5 μg of polyethylene glycol (molecular weight, 6,000; Sigma) per ml. DNA was suspended in low-salt buffer and added to CsCl.

Purified total and plasmid DNAs were digested by restriction endonuclease *Cla*I and with buffers and incubation conditions as specified by the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). This enzyme was found to digest DNA from all *S. haemolyticus* isolates effectively. In contrast, *Bgl*II and *Hin*dIII incompletely digested *S. haemolyticus* DNA, although *S. epidermidis* DNA was readily digested by both enzymes. Digested DNA was separated by electrophoresis through 0.7% agarose on a horizontal apparatus (Ann Arbor Plastics, Ann Arbor, Mich.) and was transferred to nylon membranes (GeneScreen Plus; Dupont, NEN Research Products, Boston, Mass.) by the method of Southern (32).

**DNA probes and hybridization.** The probe for the methicillin resistance gene was obtained as follows. An isogenic pair of methicillin-susceptible–methicillin-resistant *S. aureus* strains was produced by transforming strain RN450 with chromosomal DNA from the homogeneously methicillin-resistant isolate, COL, producing the transformant RN 450MR. Electrophoresis of genomic DNA from RN450 and RN450MR cleaved with *Bgl*II revealed a 3.5-kilobase (kb) fragment that was present in the methicillin-resistant strain but not in the isogenic methicillin-susceptible strain. As described by Beck et al. (3), that fragment was eluted from the gel and cloned on pUC9. A genomic library was made by cloning a *Sau*3A partial digest of COL genomic DNA into the lambda EMBL4 vector as described by Maniatis et al. (20) and the manufacturer (Promega Corp., Madison, Wis.). Lambda plaques were screened with the cloned 3.5-kb *Bgl*II fragment by hybridization, and positive clones were examined. One clone contained a 3.9-kb *Hin*dIII fragment overlapping the terminal 1.3 kb of the 3.5-kb probe fragment. This fragment was identical in size and restriction endonuclease cleavage sites to that determined by three groups of investigators (11, 31, 33) to encode the methicillin resistance structural gene, PBP2′. This entire *Hin*dIII fragment was used as the probe for the methicillin resistance gene and was designated pGO159. The 3.5-kb *Bgl*II probe and lambda library were kindly provided by John Kornblum, Public Health Research Laboratory, New York, N.Y. This probe has been shown to be both sensitive and specific for methicillin-resistant staphylococci (D. Galetto, J. Froggatt, J. Kornblum, and G. Archer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, A59, p. 10).

The gentamicin resistance gene probe was the entire plasmid pGO137, which contains a 2.5-kb *Hin*dIII fragment cloned from the staphylococcal conjugative plasmid pGO1 (1) and pBR322 vector sequences. Vector sequences did not hybridize with staphylococcal DNA on blots used in this study. On the basis of published data on Tn*4001*, which is probably similar to the pGO1-associated gene and flanking sequences (17), the *Hin*dIII fragment in this probe consisted of both a structural gene and a portion of insertion sequence (IS)-like elements at the 5′ and 3′ termini.

The probe for the erythromycin resistance gene (*ermC*) was a 940-base-pair *Hin*fI A fragment from the *S. aureus* plasmid pE194; this fragment contained the RNA methylase

structural gene (10) and was eluted from a 10% polyacrylamide gel as described by Maniatis et al. (20).

The trimethoprim resistance gene probe was the entire plasmid pGO18, which contains a 500-base-pair *Eco*RI-*Hin*dIII fragment cloned from pGO1 and pBR322 vector sequences (4). Probe DNA was labeled with [$^{32}$P]dCTP by nick translation in accordance with the protocol of the manufacturer (New England Nuclear Corp., Boston, Mass.). Hybridization of the probe with target DNA was performed at 42°C under conditions of high stringency. The hybridization solution consisted of 50% formamide, 1.0% sodium dodecyl sulfate, 1 M sodium chloride, 50% dextran sulfate, and denatured salmon sperm DNA. Membranes were washed twice after hybridization with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature, twice with 2× SSC–1.0% sodium dodecyl sulfate at 55°C, and twice with 0.1× SSC at room temperature. Autoradiographs were produced by timed exposure of the membranes to radiographic film. The nylon membranes onto which DNA was initially transferred were used repeatedly for rehybridization with all probes. We removed probe from the membranes before repeat hybridization by boiling the membranes for 30 min in a solution of 10 mM Tris–1 mM EDTA (pH 7.5 to 8.0)–1% sodium dodecyl sulfate.

**Filter mating.** The restriction-deficient *S. aureus* strain used as a recipient was RN2677 (2). The *S. epidermidis* recipient (SE42) contained a single cryptic plasmid but was susceptible to gentamicin, erythromycin, and trimethoprim. Isolates of staphylococci used as donors and recipients were cultured in brain heart infusion broth overnight at 37°C. Each pair of donor and recipient isolates was combined in a syringe, filtered onto a nitrocellulose paper filter, and incubated upright on nonselective agar plates. After 18 h at 37°C, the organisms were removed from the filters by vortexing and were plated onto appropriate selective agar media. To investigate the possibility of killing of recipient isolates by bacteriocins produced by donor isolates, we placed drops of filter-sterilized supernatants of overnight broth cultures of donors onto freshly inoculated lawns of recipients on Mueller-Hinton agar.

## RESULTS

**Identification of *S. haemolyticus* isolates.** A total of 524 coagulase-negative staphylococcal isolates were collected. Of these isolates, 78 were trehalose positive and urease negative, and 70 of these 78 (13% of the total coagulase-negative staphylococcal isolates) were identified as *S. haemolyticus*. The remaining eight trehalose-positive, urease-negative isolates were identified as *S. warneri* (five), *S. cohnii* (two), and *S. sciuri* (one). Over half of the coagulase-negative staphylococcal isolates not identified as *S. haemolyticus* were found to be trehalose negative and urease positive (287 of 454 isolates [63%]); the majority of these are likely to have been *S. epidermidis*.

**Sources of *S. haemolyticus* isolates.** The 70 isolates were cultured from a total of 60 patients: 51 patients were hospitalized, 8 were outpatients, and the status of 1 was not specified. The most common source of *S. haemolyticus* isolates during the study period was wounds, accounting for 44% of the total, followed by urine, blood, and other sources (Table 1).

**Case reviews.** The medical records of 47 of the 60 patients were available for review, providing data on 54 of the 70 *S. haemolyticus* isolates. Data from laboratory records combined with data from chart reviews revealed that 8 of the 70 *S. haemolyticus* isolates were from outpatients and that 62 of the 70 were from inpatients. The sources of all isolates are listed in Table 1. Four of the eight outpatient isolates were from urine, two were from urethral fluid, one was from peritoneal fluid, and one was from a suture wound. Cultures from hospitalized patients represented the remainder of the isolates in each category in Table 1.

TABLE 1. Sources of *S. haemolyticus* isolates

| Source | No. (%) of isolates |
|---|---|
| Wounds | 31 (44) |
| Urine | 18 (26) |
| Blood | 7 (10) |
| Urethral discharge | 3 (4) |
| Intravenous catheter tips | 3 (4) |
| Intravenous catheter-skin sites | 2 (3) |
| Thoracostomy tube drains | 2 (3) |
| Intra-abdominal catheter drains | 2 (3) |
| Peritoneal fluid | 2 (3) |

Analysis by specific sources from chart reviews showed that, for 22 specified wounds, *S. haemolyticus* was isolated from 6 burn wounds, 5 decubitus lesions, and 11 miscellaneous wounds. A total of 4 wound isolates were pure cultures, and the remaining 18 isolates were from mixed cultures of two to four organisms. None of the wounds were thought to have been sources of systemic infections at the time of culturing.

Of 15 specified urine isolates, 5 were from pure cultures and 10 were from mixed cultures of two to three organisms. All urine colony counts were <50,000 (mean, 25,000), and all urine leukocyte counts were <5 per high-power field. Only one patient with a urine isolate was febrile at the time of culturing, presumably from another source.

A review of blood isolates from separate patients showed that three of seven cultures were single cultures; one of the three patients was febrile, but from another obvious source. Four cultures were paired, each positive in only one of two cases, and none was clearly associated with fever.

Of four intravascular catheter tip isolates, two were from pure cultures and two were from mixed cultures. One of the two pure cultures grew with a colony count of >15 (90 colonies). The isolate was from a febrile 8-month-old infant who was being treated for meningitis at the time of the catheter culture. There was no concurrently obtained peripheral blood culture.

The single isolate that was most suggestively linked to infection was a peritoneal fluid isolate which grew in pure culture from an outpatient who was on chronic ambulatory peritoneal dialysis and who had fever, signs of peritonitis, a dialysate leukocyte count of 1,430 with 90% polymorphonuclear forms, and no other demonstrated cause of fever.

**Antimicrobial susceptibilities.** Table 2 shows the proportions of coagulase-negative staphylococci resistant to individual antimicrobial agents. *S. haemolyticus* isolates had a significantly higher frequency of resistance to each of the six agents tested, except for chloramphenicol. *S. haemolyticus* isolates were also resistant to significantly more antimicrobial agents than were the other coagulase-negative staphylococci (Table 3). The results of microdilution MIC determinations for the 10 *S. haemolyticus* isolates chosen for DNA probing are shown in Table 4. Of note were the high MICs of vancomycin for 4 of 10 isolates and of teicoplanin for 6 of 10 isolates (MIC, ≥6.25 μg/ml), as compared with the MICs for the first two *S. epidermidis* controls tested (MIC, ≤0.8 μg/ml

TABLE 2. Proportions of isolates resistant to individual antimicrobial agents

| Antimicrobial agent | Proportion (%) of isolates resistant | | |
|---|---|---|---|
| | *S. haemolyticus* | Other coagulase-negative staphylococci | *P* (chi square) |
| Methicillin | 56/70 (80) | 150/353 (43) | <0.001 |
| Gentamicin | 55/70 (79) | 119/353 (34) | <0.001 |
| Erythromycin | 55/70 (79) | 182/350 (52) | <0.001 |
| Tetracycline | 43/57 (75) | 174/344 (51) | <0.001 |
| Trimethoprim | 44/70 (63) | 129/350 (37) | <0.001 |
| Chloramphenicol | 6/57 (11) | 55/345 (16) | 0.4 |

TABLE 3. Numbers of isolates resistant to multiple antimicrobial agents

| No. of antimicrobial agents to which isolates were resistant | No. (%) of isolates resistant[a] | |
|---|---|---|
| | *S. haemolyticus* ($n$ = 70) | Other coagulase-negative staphylococci ($n$ = 354) |
| None to two | 16 (23) | 213 (60) |
| Three to four | 25 (36) | 76 (22) |
| Five to six | 29 (41) | 65 (18) |

[a] $P < 0.001$ (chi square).

for both drugs). Because of the comparatively high MICs found for these 10 *S. haemolyticus* isolates, an additional 50 *S. haemolyticus* isolates, including 37 multiply resistant and 13 susceptible isolates, were tested by broth microdilution for susceptibilities to these two antibiotics. These susceptibilities were compared with those of 16 multiply resistant *S. epidermidis* isolates previously collected from patients with prosthetic valve endocarditis (14). Of the combined total of 47 multiply resistant *S. haemolyticus* isolates, 29 (62%) had vancomycin MICs of ≥6.25 μg/ml. Of the same 47 isolates, 43 (91%) had teicoplanin MICs of ≥6.25 μg/ml. In contrast, only 2 of 16 (13%) multiply resistant *S. epidermidis* endocarditis isolates had vancomycin MICs of ≥6.25 μg/ml, and none had teicoplanin MICs of ≥6.25 μg/ml. The geometric mean MICs of the two antibiotics are shown in Table 5. The geometric mean MIC of vancomycin for multiply resistant *S. haemolyticus* was 5.7 μg/ml; that of teicoplanin was 14.7 μg/ml. These mean MICs are both significantly greater than the corresponding mean MICs for multiply resistant *S. epidermidis* isolates. No isolates were vancomycin resistant (MIC, ≥32 μg/ml) by the criteria of the National Committee for Clinical Laboratory Standards (23).

From case reviews, it was noted that of the eight outpatient *S. haemolyticus* isolates, only two were resistant to multiple antimicrobial agents. Both resistant isolates were cultured from individuals who had been discharged from the hospital within several weeks of their outpatient cultures. The six susceptible isolates from outpatients were cultured from six individuals; three had not been hospitalized for several months before their cultures were obtained, and records for the remaining three could not be retrieved.

**DNA probes.** Since total cellular and chromosomal DNA preparations always contained some plasmid DNA, hybridization of probes with chromosomal DNA could have been due to the presence of genes in either chromosomal or plasmid locations. Therefore, any probe hybridizing with a total cellular digest was interpreted as indicative of the presence of a chromosomal gene only if there was no hybridization with purified plasmid DNA.

The DNA probe data for the *S. haemolyticus* isolates are summarized in Table 6. No differences were noted in any hybridizing fragments from isolates collected in 1986 versus those collected in 1987. All target DNA was cleaved with *Cla*I.

The pGO159 probe for methicillin resistance hybridized with chromosomal DNA from all 10 methicillin-resistant isolates. There were two hybridizing fragments in each isolate, and the sizes of the two fragments varied between isolates. The smaller fragments ranged from 1.8 to 3.8 kb, and two pairs of isolates had smaller fragments of identical size. The larger fragments ranged from 3.0 to 8.1 kb, and only one pair of isolates had larger fragments of identical size.

The pGO137 probe for gentamicin resistance hybridized with a 2.5-kb chromosomal fragment from three gentamicin-resistant isolates. Each of the other six gentamicin-resistant isolates hybridized with a 2.5-kb plasmid fragment; one isolate also hybridized with a 4.5-kb plasmid fragment. This probe did not hybridize with either chromosomal or plasmid DNA from one gentamicin-resistant isolate.

The erythromycin resistance gene (*ermC*) probe hybridized with plasmid DNA from 7 of 10 erythromycin-resistant *S. haemolyticus* isolates and did not hybridze with any DNA from the remaining 3 erythromycin-resistant isolates. A single hybridizing 2.5-kb fragment was present in each of the positive isolates.

The pGO18 probe for trimethoprim resistance did not hybridize with any DNA from the 10 trimethoprim-resistant *S. haemolyticus* isolates.

The negative hybridization controls, one antimicrobial agent-susceptible *S. haemolyticus* isolate and one antimicrobial agent-susceptible *S. epidermidis* isolate, were both negative with all probes. The probe results for the 10 multiply-resistant *S. epidermidis* isolates were as follows. The pGO159 probe for methicillin resistance hybridized with DNA from all 10 methicillin-resistant *S. epidermidis* isolates. There were two hybridizing fragments from each isolate, 2.3 and approximately 10 kb in size. DNA from all 10 gentamicin-resistant *S. epidermidis* isolates hybridized with pGO137; the fragment in each isolate was 2.5 kb. The fragment was chromosomal in six instances and plasmid in the remaining four. DNA from all 10 erythromycin-resistant *S. epidermidis* isolates hybridized with a 2.5-kb plasmid *ermC* fragment. Only 4 of the 10 multiply resistant *S. epidermidis* isolates were not inhibited by trimethoprim; for 6 of the isolates the trimethoprim MIC was 8.0 μg/ml. DNA from all four resistant isolates hybridized with pGO18: two isolates with a 1.2-kb plasmid fragment, one isolate with a >15-kb plasmid fragment, and one isolate with a 2.0-kb chromosomal fragment. Of the six moderately susceptible isolates, two were probe negative and four hybridized with a 1.8-kb chromosomal fragment.

**Antimicrobial resistance transfer.** Resistance to gentamicin, erythromycin, tetracycline, trimethoprim, and chloramphenicol did not transfer on filter membranes from any *S. haemolyticus* donor to a restriction-deficient *S. aureus* recipient. Gentamicin resistance, previously found to be carried on conjugative plasmids in many *S. epidermidis* isolates from our hospitals (1), did not transfer from any *S. haemolyticus* donor to a plasmid-free *S. epidermidis* recipient on filter membranes. In the test for bacteriocin production by the donors, no inhibition of recipient growth was observed when filter-sterilized supernatant was dropped onto freshly inoculated recipient cultures.

TABLE 4. Antimicrobial susceptibilities of *S. haemolyticus* isolates investigated by hybridization with DNA probes

| Isolate no. | MIC (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Methicillin | Gentamicin | Erythromycin | Tetracycline | Trimethoprim | Chloramphenicol | Vancomycin | Teicoplanin |
| 1 | ≥12.5 | 12.5 | ≥100 | 3.1 | 156 | 6.3 | 6.3 | 12.5 |
| 2 | ≥12.5 | 6.3 | ≥100 | 6.3 | 312 | 1.6 | 12.5 | 12.5 |
| 3 | ≥12.5 | 12.5 | ≥100 | 3.1 | 312 | 50.0 | 6.3 | 12.5 |
| 4 | ≥12.5 | 12.5 | ≥100 | 6.3 | 625 | 12.5 | 3.1 | 12.5 |
| 5 | ≥12.5 | 25.0 | 25 | 0.3 | ≥625 | 50.0 | 0.3 | 3.1 |
| 6 | ≥12.5 | 50.0 | ≥100 | 3.1 | 312 | 12.5 | 12.5 | 12.5 |
| 7 | ≥12.5 | 25.0 | ≥100 | 3.1 | 625 | 12.5 | 1.6 | 3.1 |
| 8 | ≥12.5 | 3.1 | ≥100 | 1.6 | 156 | 0.4 | 1.6 | 1.6 |
| 9 | ≥12.5 | 6.3 | ≥100 | 1.6 | 625 | 3.1 | 0.8 | 1.6 |
| 10 | ≥12.5 | 25.0 | ≥100 | 0.8 | 156 | 1.6 | 1.6 | 6.3 |

## DISCUSSION

*S. haemolyticus* isolates accounted for 13% of all coagulase-negative staphylococcal clinical isolates during the study period. This result is consistent with previously reported data from 10 studies that identified species in collections of clinical isolates of coagulase-negative staphylococci: *S. haemolyticus* represented an average of 10% of the isolates (range, 2 to 18% for 43 to 336 coagulase-negative staphylococcal isolates) (5, 7, 9, 12, 15, 22, 24, 25, 27, 28). Eighty percent of our isolates were from wounds, urine, and blood. There was no conclusive evidence in the patient records studied that the isolates were associated with an active infection in any case, with the exception of one patient with peritoneal dialysis-associated peritonitis. *S. haemolyticus* has been reported elsewhere to be associated with peritonitis in patients undergoing peritoneal dialysis (30). The five pure cultures of *S. haemolyticus* from urine may have reflected the presence of infection (8), although not clinically obvious; however, the low colony counts and the lack of pyuria in these cases make the diagnosis questionable. One intravascular catheter was colonized with a heavy, pure growth of *S. haemolyticus*.

*S. haemolyticus* isolates were multiply resistant to antimicrobial agents more frequently than were other coagulase-negative staphylococcal isolates. Moderate susceptibility to vancomycin and teicoplanin (MIC, ≥6.25 μg/ml) was found in a high percentage of isolates. Similarly high vancomycin MICs for *S. haemolyticus* have recently been described (30).

The DNA probe data indicate that the genetic basis of methicillin resistance in the *S. haemolyticus* isolates tested is similar to that previously observed in *S. epidermidis* isolates in our hospitals (Galetto et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). However, the various chromosomal fragment sizes found only in the *S. haemolyticus* isolates suggest that there is considerable restriction site polymorphism within the *S. haemolyticus* methicillin resistance gene complex (*mec*), in contrast to the apparent uniformity of the *S. epidermidis mec* genes.

Gentamicin resistance in *S. epidermidis* isolates collected from our hospitals from 1976 to 1984 was shown to be associated with a gene located on conjugative plasmids in 72% of isolates (1). The location of gentamicin resistance genes among the 28% of isolates that did not transfer resistance on filter membranes was not determined in that study. In the present study, 3 of 10 gentamicin-resistant *S. haemolyticus* isolates clearly had chromosomal DNA that hybridized with pGO137 (derived from the gentamicin resistance genes prevalent on our resident conjugative plasmids), and 6 of the 10 had plasmid DNA that hybridized with the probe but did not transfer resistance on filter membranes. Furthermore, 6 of 10 gentamicin-resistant *S. epidermidis* controls had chromosomal resistance genes that hybridized with the plasmid-derived probe. This result suggests that the gentamicin resistance determinant may reside on a transposon, as has been described for Tn*4001* and Tn*3851*, both of which have been found in Australian *S. aureus* strains (16, 18, 35). Both of these transposons encode an enzyme [aminoglycoside 2″-phosphotransferase/aminoglycoside 6′-acetyltransferase; APH(2″)/AAC(6′)I] (29) identical to that encoded by our probe. We have recently found a transposon (Tn*4031*) encoding gentamicin resistance in the chromosome of an *S. epidermidis* isolate from our hospital (W. Thomas and G. Archer, submitted for publication).

Tn*4001* contains the gene encoding gentamicin resistance flanked by directly repeated IS-like elements (18). These IS-like elements are apparently responsible for the mobility of Tn*4001* and may also have independent mobility themselves (17). Gentamicin resistance genes on conjugative plasmids are not able to transpose (Thomas and Archer, submitted), presumably because the IS-like elements are truncated at one or both ends (16). Because our pGO137

TABLE 5. Geometric mean MICs of vancomycin and teicoplanin

| Isolates | Geometric mean MIC (μg/ml) | | P (t test) |
|---|---|---|---|
| | Vancomycin | Teicoplanin | |
| Resistant *S. haemolyticus* (n = 47) | 5.7 | 14.7 | <0.001 |
| Susceptible *S. haemolyticus* (n = 13) | 3.3 | 4.1 | 0.2 |
| Resistant *S. epidermidis* (n = 16) | 2.8 | 0.54 | <0.001 |
| P (adjusted t test) (resistant isolates only) | <0.001 | <0.001 | |

TABLE 6. DNA probes for resistance determinants in *S. haemolyticus*

| Resistance phenotype | Probe[a] | No. of isolates[b] hybridizing with: Plasmid DNA | Total cellular or chromosomal DNA |
|---|---|---|---|
| Methicillin | pGO159 | 0 | 10 |
| Gentamicin | pGO137 [APH(2″)/AAC(6′)I] | 6 | 3 |
| Erythromycin | pE194 (*ermC*) | 7 | 0 |
| Trimethoprim | pGO18 (DHFR S1) | 0 | 0 |

[a] APH(2″)/AAC(6′)I, Aminoglycoside 2″-phosphotransferase/aminoglycoside 6′-acetyltransferase; DHFR S1, dihydrofolate reductase type S1.
[b] A total of 10 isolates were examined.

probe may contain, in addition to the resistance gene, several hundred base pairs of DNA that include portions of the flanking IS-like elements, it is possible that hybridization signals represent only the independently mobile IS-like elements rather than the gentamicin resistance genes themselves. We feel that this is unlikely, however, on the basis of the high intensity of the signals and the presence of a single hybridizing fragment in each isolate.

Erythromycin resistance in the majority of *S. haemolyticus* isolates also appeared to be encoded by genes similar to those in *S. epidermidis*. The size of our hybridizing plasmid fragments, 2.5 kb, was similar to the size of the constitutive macrolide-lincosamide-streptogramin B plasmid, pNE131, originally recovered from *S. epidermidis* (26). This plasmid was recently shown to be predominant among resistant coagulase-negative staphylococcal isolates (including *S. haemolyticus*) from clinical sources; undigested plasmid DNA from those isolates hybridized with pE194 (two fragments of 1.6 and 2.4 kb) (13, 34). The lack of hybridization of DNA from three erythromycin-resistant *S. haemolyticus* isolates with the erythromycin resistance probe suggests that there may be additional genes associated with erythromycin resistance in these organisms.

We found no hybridization between the probe for trimethoprim resistance and *S. haemolyticus* DNA, indicating the existence of a resistance gene different from that recently found to be prevalent in *S. epidermidis* and *S. aureus* in our hospitals (6).

Because susceptible isolates of *S. haemolyticus* are found in outpatient clinical specimens and in some specimens from hospitalized patients, it is unlikely that the antimicrobial resistance of these organisms is intrinsic. The finding that identical genes for resistance to methicillin, erythromycin, and gentamicin were found in *S. haemolyticus* and *S. epidermidis* isolates recovered during the same time period in our hospitals suggests that these organisms share a common gene pool. However, the failure to demonstrate resistance transfer between *S. haemolyticus* and other staphylococci by filter matings, the presence of distinct resistance determinants for trimethoprim in *S. haemolyticus*, and the decreased susceptibility to vancomycin and teicoplanin in *S. haemolyticus* further suggest that some resistance genes may have evolved separately under the selective pressure of antimicrobial use in hospitals.

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