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Low-Level Colonization of Hospitalized Patients with Methicillin-Resistant Coagulase-Negative Staphylococci and Emergence of the Organisms during Surgical Antimicrobial Prophylaxis

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By use of techniques that have been developed to detect small numbers of methicillin-resistant staphylococci, we cultured samples from the nares and subclavian and inguinal areas of 29 patients before and after cardiac surgery and 10 patients before and after coronary angioplasty. Methicillin-resistant coagulase-negative staphylococci were recovered before the surgical or angioplasty procedure from 74% of patients. The quantitative recovery of methicillin-resistant isolates before cardiac surgery or coronary angioplasty was compared with the number of methicillin-resistant staphylococci detected at the same site 3 days after the procedure. In cardiac surgery patients (who received antibiotic prophylaxis), 17 of the 28 sites (61%) in which low-level colonization with methicillin-resistant strains was detected preoperatively contained high levels of methicillin-resistant staphylococci postoperatively. In contrast, coronary angioplasty patients (who did not receive antibiotic prophylaxis) did not have any of the 14 sites containing low levels of methicillin-resistant strains before angioplasty emerge to harbor high levels of methicillin-resistant staphylococci after angioplasty. Methicillin-resistant coagulase-negative staphylococci from each site in which high levels of methicillin-resistant staphylococci emerged postoperatively were paired with preoperative isolates from the same site. Identical antibiograms and plasmid profile patterns were demonstrated for seven of the pre- and postoperative isolate pairs, suggesting that the high levels of methicillin-resistant coagulase-negative staphylococci detected on the skin or in the nares after cardiac surgery were derived from methicillin-resistant organisms present at the site preoperatively in much smaller numbers.

Two factors have hindered the epidemiologic study of methicillin-resistant (MR) coagulase-negative staphylococci as colonizing flora of hospitalized patients. First, routine skin culture techniques sample only a fraction of the skin's quantitatively complex flora (9, 11, 16), potentially missing small subpopulations of antibiotic-resistant organisms. Second, organisms that possess the MR genotype may fail to express resistance phenotypically unless they are encouraged to do so by certain laboratory manipulations (growth in salt-supplemented media, incubation at a lower temperature, use of a large inoculum, etc.), further impairing recognition of their presence (1, 5, 7, 14). We adopted culture techniques which ensured that a reasonable fraction of the colonizing flora of a patient would be sampled and which encouraged the phenotypic expression of the MR genotype. We used these techniques to reexplore the epidemiology of MR coagulase-negative staphylococci in cardiac surgery and coronary angioplasty patients.

MATERIALS AND METHODS

Patient enrollment, demographic data, and acquisition of cultures. The study protocol was approved by the Saint Thomas Hospital Institutional Review Board. Patients scheduled for elective cardiac surgery or coronary angioplasty from November 1985 to February 1986 were eligible for participation in this study. On the evening before the elective surgical or angioplasty procedure, written informed consent was obtained, charts were reviewed, and patients were interviewed with regard to the possible risk factors of prior colonization with MR coagulase-negative staphylococci (hospitalization or antibiotic usage within the last 3 months or time spent in the intensive care unit). After the patients were enrolled in the study, samples from the nares and right subclavian and left inguinal areas were obtained for the initial culturing procedure. All patients subsequently showered with chlorhexidine, povidone-iodine, or nonmedicated soap. A second set of samples for culturing was obtained from the same three sites 8 to 14 h later, 1 to 2 h before the surgical or angioplasty procedure. The surgical incision and angioplasty catheterization sites were prepared antiseptically with povidone-iodine. A third set of samples for culturing (same three sites) was obtained at 72 h postoperatively from all cardiac surgery patients and from 6 of the 10 coronary angioplasty patients; four coronary angioplasty patients were discharged at 48 h, and samples for culturing were obtained at that time.

Processing of cultures. Sampling of the subclavian and inguinal sites was done by using a modification of the glass cylinder method described by Williamson and Kligman (17). A sterile plastic cylinder was placed firmly over the skin, thus defining an 8.0-cm^2 area for culture. Broth-moistened rayon swabs (Culturette; Marion Scientific, Div. Marion Laboratories, Kansas City, Mo.) were vigorously rubbed across the template-defined area (subclavian or inguinal) or twisted several times in both anterior nares. The swabs were promptly transported to the laboratory and agitated for 15 s in 2.0 ml of tryptic soy broth (Difco Laboratories, Detroit, Mich.) containing antiseptic neutralizers (0.3% lecithin, 2%

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Tween 80). Results of preliminary studies in our institution and elsewhere (10) have indicated that these neutralizers are effective in inactivating the small amount of antiseptic (chlorhexidine or povidone-iodine) that may be left as a residue on the skin of a patient following a shower or surgical scrub. Portions of 100 μl of the freshly inoculated broth were then spread onto staphylococcal medium 110 (S110) (Difco) and S110 agar containing 6 μg of nafcillin per ml (S110N6). Agar plates were prepared fresh at least twice weekly and were kept refrigerated prior to use. The residual inoculated tryptic soy broth was incubated at 32°C overnight, after which 100-μl portions (undiluted and diluted 10^{-2}) were again plated on S110N6 agar. After 72 h of incubation at 32°C, plates were examined, colonies were counted, and one to three representative colonies were selected from each plate.

Organisms that grew on nafcillin-containing medium were identified as coagulase-negative staphylococci on the basis of Gram stain and catalase and coagulase testing. One representative isolate from each site (nares and subclavian and inguinal areas) was identified to the species level by using the Staph-Ident system (Analytab Products, Plainview, N.Y.) (8) and underwent disk diffusion testing (12) of susceptibility to penicillin G, oxacillin, cephalothin, erythromycin, clindamycin, chloramphenicol, gentamicin, tetracycline, vancomycin, ticarcillin-clavulanic acid, and trimethoprim-sulfamethoxazole. The ability of a strain to produce slime was determined by the presence or absence of an opaque film on glass culture tubes following 48 h of incubation of the isolate at 32°C in tryptic soy broth.

Comparison of hospital courses. All 29 cardiac surgery patients received perioperative cephalosporin prophylaxis (cefazolin or cefamandole) for 72 h; this period began at the induction of anesthesia and extended through postoperative day 3. Some patients also received a single dose of gentamicin at the induction of anesthesia. None of the 10 coronary angioplasty patients received antimicrobial agents during their hospitalization.

All cardiac surgery patients spent at least 48 h in the intensive care unit postoperatively and were subsequently transferred to the intermediate care unit. Of the 10 coronary angioplasty patients, 2 went to the intensive care unit postprocedure, and the other 8 went directly to the intermediate care unit. Because Saint Thomas Hospital has nonstratified intensive and intermediate care units, both cardiac surgery and coronary angioplasty patients likely were exposed to a similar microbial flora during their hospitalization.

Definitions. A coagulase-negative staphylococcal isolate was considered to be MR if it grew on S110N6 agar within 72 h and if susceptibility testing with a 1-μg oxacillin disk was confirmatory. Two isolates grew on S110N6 agar but were susceptible to oxacillin by the Kirby-Bauer test. For both isolates the methicillin MIC was in excess of 128 μg/ml by the macrodilution methods described by the National Committee for Clinical Laboratory Standards (12a), and both isolates were also considered to be MR.

S110N6 plates were defined as primary or overnight broth plates, depending on whether they were inoculated from the freshly agitated broth or from broth that had been incubated overnight.

Each site on each patient (nares and subclavian and inguinal areas) was classified into one of three categories based on our ability to detect MR coagulase-negative staphylococci at that site: (i) no MR coagulase-negative staphylococci present; (ii) low levels of MR coagulase-negative staphylococci present; and (iii) high levels of MR coagulase-negative staphylococci present. Since two samples were obtained from each site preoperatively, the one that yielded the highest number of staphylococcal colonies on the S110 plate was used for site classification. The number of phenotypically MR coagulase-negative staphylococci obtained from each site was compared with the total (susceptible and resistant) number of coagulase-negative staphylococci recovered from the site. For a high-level site, MR coagulase-negative staphylococci grew on the S110N6 primary plate and made up more than 1% of the total staphylococcal flora at the site (i.e., growth on the S110N6 primary plate was greater than 1% of the growth on the S110 plate). Low-level sites yielded MR coagulase-negative staphylococci on overnight broth plates only or on both overnight broth and primary S110N6 plates. When growth was present on the S110N6 primary plate, it represented less than or equal to 1% of the S110 plate growth.

Postoperative high-level sites were designated as emerged (new high-level sites) if they yielded only low levels of growth of MR coagulase-negative staphylococci on culturing of samples obtained preoperatively but yielded greater than 1% MR coagulase-negative staphylococci (as a fraction of the total staphylococcal flora) from the same site postoperatively. In addition, to minimize the risk of overinterpreting small differences in the recovery of MR coagulase-negative staphylococci secondary to sampling variation, if there was some low-level growth on the S110N6 primary plate preoperatively, at least a 20-fold increase in the S110N6/S110 growth ratio was required postoperatively for a site to be designated as emerged.

Plasmid studies. The lysis of organisms and isolation of DNA for analysis by agarose gel electrophoresis were accomplished by the methods described by Forbes and Schaberg (6). Each isolate was grown overnight in 5 ml of tryptic soy broth and was processed for initial plasmid profile pattern analysis. Electrophoresis of crude DNA preparations was performed through a 0.7% agarose gel. Purified plasmid DNA was obtained from selected isolates by performing cesium chloride density gradient centrifugation on large-scale (400- to 800-ml) lysis preparations. Restriction endonuclease digestion of the purified plasmid DNA with *Hin*dIII (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was performed according to the instructions of the manufacturer, followed by electrophoresis through a 1.2% agarose gel. DNA was stained with ethidium bromide (Sigma Chemical Co., St. Louis, Mo.) and photographed by UV transillumination.

One to three colonies of MR coagulase-negative staphylococci obtained preoperatively and postoperatively from each emerged site were selected for plasmid analysis. In addition, a comprehensive comparison of the plasmid profile patterns of 120 isolates was performed. To facilitate the recognition of similar profile patterns among isolates of MR coagulase-negative staphylococci, strains that had similar antibiograms and that belonged to the same species were grouped together for electrophoresis through the same gel.

RESULTS

Demographic data: comparability of surgery and angioplasty groups. A total of 29 cardiac surgery and 10 coronary angioplasty patients were enrolled in the study. These groups were comparable in age, underlying disease (coronary artery disease), recent antibiotic use (20%), hospitalization within the past 3 months (60%), or hospitalization in our intensive care unit before enrollment in the study (20%).

TABLE 1. Quantitative range of MR coagulase-negative staphylococci recovered from 117 sites

Category	R range (%)[a]	No. of sites
None detected	0	53
Low level	Overnight broth only	23
	$R \leq 0.1$	6
	$0.1 < R \leq 0.5$	9
	$0.5 < R \leq 1$	4
High level	$1 < R \leq 10$	7
	$10 < R \leq 40$	7
	$40 < R \leq 70$	4
	$70 < R \leq 100$	4

[a] R indicates the ratio of colonies that grew on the S110N6 primary plate/colonies that grew on the S110 plate. Isolates not recovered by primary plating but only by the use of overnight broth enhancement techniques are indicated by overnight broth only. There were a total of 42 sites (36%) from which low levels of organisms were recovered. There were a total of 22 sites (19%) from which high levels of organisms were recovered. A total of 64 sites (55%) yielded MR coagulase-negative staphylococci.

The median interval between the time of admission into the hospital and the time that samples for the initial culture were obtained was 24 h for patients in both groups.

Preprocedure detection of MR coagulase-negative staphylococci. Of the 117 sites (3 sites per patient, 39 patients) from which samples were obtained before the surgical or angioplasty procedure, 64 (55%) yielded MR coagulase-negative staphylococci. There was a wide range in the number of phenotypically MR organisms recovered as a proportion of the total coagulase-negative staphylococci present at a site (Table 1); this represented a continuum from growth on overnight broth plates only to heavy growth on primary plates. High-level sites always exhibited growth on the S110N6 primary plate (usually more than 10 colonies). If a site was classified as high level by the first culture (obtained during the evening before the cardiac surgery or coronary angioplasty), this was always confirmed by repeat culture (during the morning of the procedure). These sites harbored enough MR coagulase-negative staphylococci that they likely would have been recognized by direct plating techniques as well.

In contrast, only a minority of low-level sites yielded MR coagulase-negative staphylococci on the S110N6 primary plate, and when present, only a few colonies were recovered (usually one to three). More often, the presence of MR coagulase-negative staphylococci at low-level sites was recognized only by allowing the inoculated broth to be incubated overnight before it was plated on S110N6 agar (Fig. 1). Recognition of low-level sites was aided by obtaining repeat samples for culturing; in the second culture 9 additional low-level sites were detected, supplementing the 33 that were detected during the initial culture.

MR coagulase-negative staphylococci were recovered from at least one site in 74% of the patients (72% presurgery, 80% preangioplasty). Sites that harbored MR coagulase-negative staphylococci were found with comparable frequencies in the patients admitted for surgery (44 of 87; 51%) and angioplasty (20 of 30; 67%). The inguinal area was most frequently colonized, yielding MR coagulase-negative staphylococci in 25 patients (65%). The nares and subclavian area harbored MR coagulase-negative staphylococci at a slightly lower frequency. Each area was colonized in approximately 50% of patients.

The prevalence of colonization with MR coagulase-negative staphylococci was not influenced by whether a patient had been hospitalized or received antibiotic therapy within the 3 months before enrollment in the study. Among the 8 patients who were exposed to antibiotics before samples were obtained for culturing, 6 (75%) yielded MR coagulase-negative staphylococci versus 23 of 31 (74%) patients who were not exposed to antibiotics. MR coagulase-negative

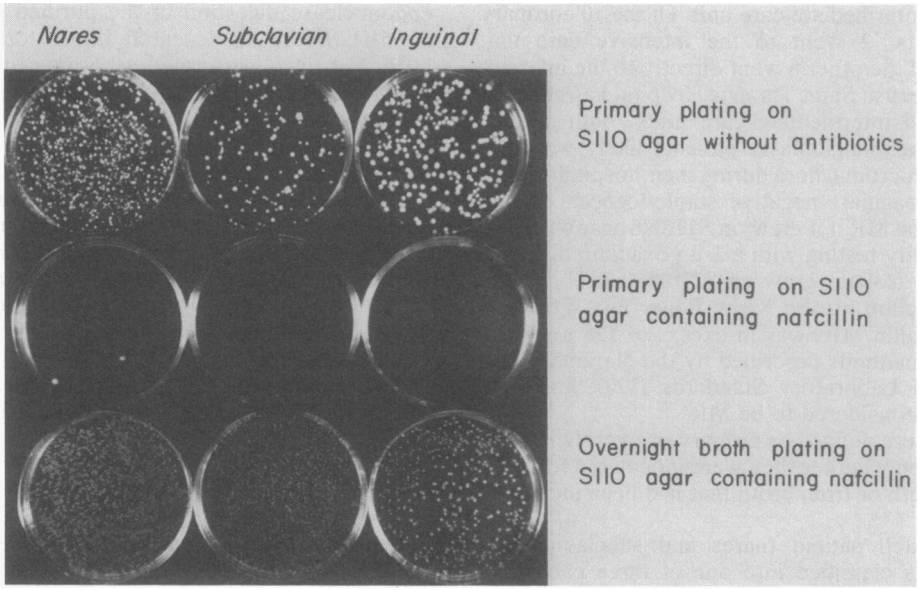

FIG. 1. Cultures from a patient in which the utility of incubating the original specimen overnight in broth was demonstrated. Primary plating demonstrated that many staphylococci could be recovered from each site; only a small proportion of the staphylococci were phenotypically MR. Overnight incubation of broth prior to plating on nafcillin-containing medium allowed the recovery of many MR coagulase-negative staphylococci. The low-level colonization of this patient with MR coagulase-negative staphylococci at the subclavian and inguinal sites would have been missed if the overnight broth technique had not been used.

staphylococci were isolated from 19 of 23 patients (83%) who had been hospitalized within the previous 3 months versus 10 of 16 patients (63%) who had not been hospitalized ($P > 0.15$; chi-square test). These risk factors, however, did influence the quantitative recovery of MR coagulase-negative staphylococci from a site. Among the eight patients who received antibiotic therapy during the 3 months before samples were obtained for culturing, high-level colonization with MR coagulase-negative staphylococci was demonstrated in at least one site in five patients (63%). In contrast, a high-level colonization site was observed in only 6 of 31 patients (19%) who had not received recent antibiotic therapy ($P < 0.05$; chi-square test). The association between recent hospitalization and high-level colonization was less pronounced; 9 of 23 patients (39%) who had been hospitalized during the previous 3 months versus 2 of 16 patients (13%) who had not been hospitalized yielded high levels of MR coagulase-negative staphylococci from at least one site ($P = 0.07$; Fisher exact test).

Of the MR staphylococcal isolates recovered during the preoperative period, 86% were *Staphylococcus epidermidis*. Other species found were *Staphylococcus haemolyticus* (12%) and *Staphylococcus capitus* (2%). Susceptibility testing of the low-level-MR coagulase-negative staphylococci recovered from the initial two culture sets identified the resistance of 20% of the isolates to gentamicin, 34% to trimethoprim-sulfamethoxazole, 5% to chloramphenicol, and 20% to clindamycin. Resistance to erythromycin was more common; it was found in 70% of strains obtained preoperatively. All isolates were susceptible to vancomycin.

Postprocedure detection of MR coagulase-negative staphylococci. Pre- and postprocedure differences in the quantitative recovery of MR coagulase-negative staphylococci at a site are represented in Fig. 2. Among cardiac surgery patients, there was a strong trend toward an increase in the quantitative recovery of MR coagulase-negative staphylococci postoperatively relative to that preoperatively. Of the 43 sites which did not yield MR coagulase-negative staphylococci preoperatively, 36 (84%) harbored MR coagulase-negative staphylococci postoperatively, usually at low levels. Similarly, 17 of 28 (61%) sites that yielded low levels of MR coagulase-negative staphylococci preoperatively emerged to high-level status postoperatively. The inguinal area was the most common site of emergence, accounting for nine of the emerged sites. The other eight emerged sites were divided evenly between the nares and the subclavian area. Emergence occurred with equal frequency among patients who received cefazolin or cefamandole, with or without gentamicin as perioperative antibiotic prophylaxis. All 29 cardiac surgery patients were colonized with MR coagulase-negative staphylococci by postoperative day 3.

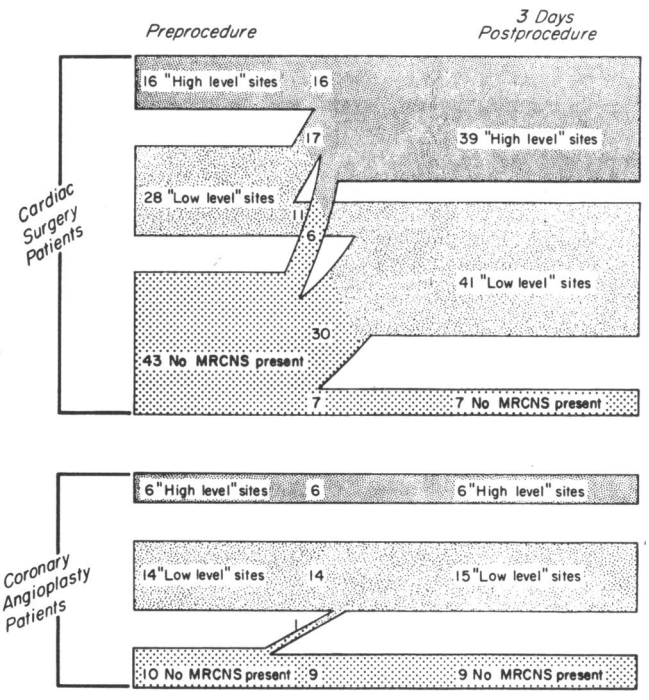

FIG. 2. Relationship of pre- to postprocedure recovery of MR coagulase-negative staphylococci (MRCNS) in 29 cardiac surgery and 10 coronary angioplasty patients. Samples from 117 sites (3 sites per patient; 39 patients) were cultured and designated as high level, low level, or no MR coagulase-negative staphylococci present based on the quantitative recovery of MR coagulase-negative staphylococci from the site. Numbers indicate numbers of sites.

The quantitative recovery of MR coagulase-negative staphylococci in coronary angioplasty patients varied minimally before and after the angioplasty procedure. No angioplasty patient had a site in which high-level status emerged.

Comparison of MR coagulase-negative staphylococci from emerged sites. Postoperative MR coagulase-negative staphylococci from all 17 of the emerged sites in 12 cardiac surgery patients were compared with low-level-MR coagulase-negative staphylococci recovered from the same site preoperatively. Analysis of pre- and postoperative MR coagulase-negative staphylococci from 10 emerged sites in seven patients showed distinctive differences in antibiograms and plasmid profile patterns, suggesting that they are unrelated strains. However, postoperative MR coagulase-negative staphylococci from seven emerged sites in five patients had the same plasmid profile pattern and antibiogram as those shown by the preoperative low-level isolate obtained from samples of the same site cultured 4 days earlier (Table 2). Endonuclease restriction of plasmid DNA from pre- and postoperative pairs from three of the emerged sites (patients 1, 3, and 4) was performed (Fig. 3), and in each instance plasmid identity was confirmed. All of these isolate pairs were determined to be *S. epidermidis*, and all were susceptible to gentamicin. Patient 5 had three emerged sites, with the same organism found pre- and postoperatively in all sites. One site pair (patient 2) had no plasmids seen, despite adequate lysis, but identical antibiograms and growth characteristics (slime production, percentage of organisms phenotypically expressing MR) were demonstrated.

Comprehensive comparison of plasmid profile patterns. Although multiple strains of MR coagulase-negative staphylococci could, on occasion, be recovered from a single patient, a colonized individual often yielded a distinctive, patient-specific strain (identical plasmid profile, antibiogram) from multiple culture sites or from the same site on multiple occasions. For example, 10 of the 14 (70%) patients in whom MR coagulase-negative staphylococci could be recovered at all three sites before surgery or angioplasty had two or more sites colonized with a unique (e.g., patient-restricted), recognizable strain of MR coagulase-negative staphylococci. Similarly, we frequently recovered the same strain from a site during all three cultures.

In contrast, there was minimal similarity among strains of MR coagulase-negative staphylococci recovered from different patients. First, 20 distinct phenotypes (as defined by differences in species and antibiograms) were recognized among the MR coagulase-negative staphylococcal isolates

TABLE 2. Comparison of selected MR coagulase-negative staphylococcal isolates recovered pre- and postoperatively from emerged sites

Patient and recovery site	Time of culture	Antibiogram[a]		Slime production	% Isolates that expressed methicillin resistance[b]
		Resistant	Susceptible		
1					
Nares	Preoperatively	Sxt, Ery	Gm, Chl, CC	Absent	0.1
Nares	Postoperatively	Sxt, Ery	Gm, Chl, CC	Absent	5
2					
Inguinal	Preoperatively	Ery	Sxt, Gm, Chl, CC	Present	OVNB only
Inguinal	Postoperatively	Ery	Sxt, Gm, Chl, CC	Present	25
3					
Inguinal	Preoperatively		Sxt, Ery, Gm, Chl, CC	Present	0.4
Inguinal	Postoperatively		Sxt, Ery, Gm, Chl, CC	Present	44
4					
Inguinal	Preoperatively	Sxt, Ery	Gm, Chl, CC	Present	0.5
Inguinal	Postoperatively	Sxt, Ery	Gm, Chl, CC	Present	100
5					
Nares	Preoperatively	Ery	Sxt, Gm, Chl, CC	Present	0.4
Nares	Postoperatively	Ery	Sxt, Gm, Chl, CC	Present	20
Subclavian	Preoperatively	Ery	Sxt, Gm, Chl, CC	Present	OVNB only
Subclavian	Postoperatively	Ery	Sxt, Gm, Chl, CC	Present	50
Inguinal	Preoperatively	Ery	Sxt, Gm, Chl, CC	Present	0.6
Inguinal	Postoperatively	Ery	Sxt, Gm, Chl, CC	Present	67

[a] Antibiograms to non-beta-lactam antibiotics. Abbreviations: Sxt, Trimethoprim-sulfamethoxazole; Ery, erythromycin; Gm, gentamicin; Chl, chloramphenicol; CC, clindamycin. All isolates were susceptible to vancomycin.

[b] Expressed as the ratio of colonies that grew on the S110N6 primary plate/colonies that grew on the S110 plate. Preoperative MR isolates not recovered by primary plating but only by the use of overnight broth enhancement techniques are indicated by OVNB only.

evaluated. Second, plasmid profile patterns of strains that demonstrated a common phenotype, yet that were recovered from different patients, generally showed marked dissimilarities. The only exception to this rule was one *S. haemolyticus* strain which was recovered from three patients preoperatively and from an additional patient postoperatively. Two of the three patients who were colonized with this strain preoperatively had been hospitalized in the intensive care unit before samples for their first culture were obtained, and one patient who had two sites colonized with this strain at high levels had received parenteral beta-lactam antimicrobial agents. The strain found postoperatively in the single patient, who was found to be newly colonized with this strain, was recovered from the overnight broth culture only (e.g., low-level colonization). In none of the patients did this strain emerge to achieve high-level colonization of a site postoperatively. No similarities in plasmid profiles among strains of MR *S. epidermidis* recovered from different patients was noted.

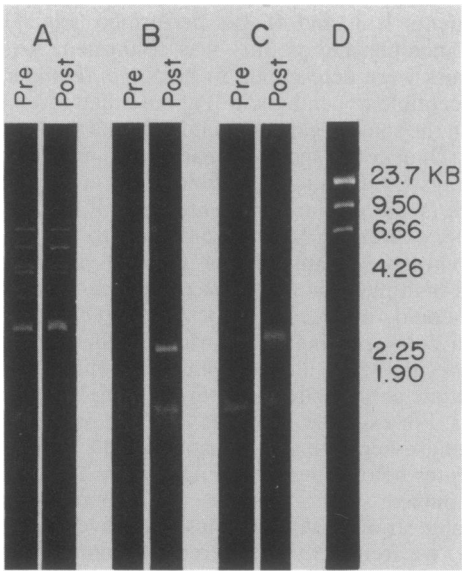

FIG. 3. Purified plasmid DNA from preoperative (Pre) strains and postoperative (Post) emerged strains of MR coagulase-negative staphylococci after cleavage with *Hin*dIII. Lane A, Patient 1; lane B, patient 3; lane C, patient 4; lane D, lambda phage DNA. Strains obtained from each patient pre- and postoperatively had identical plasmid restriction patterns.

DISCUSSION

There were three major findings from the results of this study. First, the use of culture methods designed to facilitate the recovery of small numbers of MR coagulase-negative staphylococci can enhance the detection of these organisms as colonizing flora of hospitalized patients. Second, use of these methods demonstrates that nearly three-fourths of patients scheduled for elective cardiac surgery or coronary angioplasty in our institution were colonized with MR coagulase-negative staphylococci. Third, MR coagulase-negative staphylococci that colonize cardiac surgery patients preoperatively at low levels are capable of emerging to achieve a high level of colonization during the postoperative period. The demonstration of identical plasmid profile patterns between pre- and postoperative MR coagulase-negative staphylococci at seven emerged sites in five patients shows that emergence of an endogenous MR subpopulation is a mechanism for achieving the observed postoperative

increase in the number of coagulase-negative staphylococci that colonize the skin of cardiac surgery patients.

These observations differ from the results of earlier investigations into the epidemiology of MR coagulase-negative staphylococci (2, 3). In previous studies, the preoperative detection of MR coagulase-negative staphylococci on the skin of surgical patients has been uncommon (2, 3). Indeed, it was largely because of the inability to find MR coagulase-negative staphylococci preoperatively that nosocomial acquisition was proposed as a theory to explain the presence of MR coagulase-negative staphylococci postoperatively. Archer and Tenenbaum (3), in their study of MR coagulase-negative staphylococci in cardiac surgery patients, commented that "the absence of resistant isolates on preoperative skin and the progressive increase in the MR phenotype for 5 days postoperatively suggests that the hospital is the source of the isolates." Our observation that MR coagulase-negative staphylococci can frequently be found on the skin of hospitalized patients preprocedure and that these strains can quantitatively amplify their numbers coincident with surgical prophylaxis has led us to advance emergence as another possible mechanism to account for the observed postoperative increase in MR coagulase-negative staphylococci that colonize the skin of cardiac surgery patients.

The fundamental difference between emergence and nosocomial acquisition hinges on the preoperative detection of MR coagulase-negative staphylococci. The high recovery of MR coagulase-negative staphylococci from our patients was a result of the enhanced sensitivity of our culture methods. Several considerations were instrumental in our recovery of MR coagulase-negative staphylococci. First, to standardize our technique and minimize the risk that an MR subpopulation would be missed because of inadequate sample size, we employed a modification of a previously described semiquantitative skin culture technique (16, 17). This technique has been estimated to yield from 3 to 20% of the total flora (as determined from skin biopsy specimens) at a skin site (16). Second, we obtained duplicate samples from each site for culturing to increase further the amount of patient flora sampled. Third, antiseptic neutralizers were added to our broth media and may have facilitated the recovery of staphylococcal organisms from a recently prepped or showered skin surface. Most importantly, however, to optimize the likelihood of recognizing an MR organism, we adjusted the culture methods to encourage the phenotypic expression of the MR genotype. Although salt supplementation of the media and incubation at a lower than standard temperature can enhance the phenotypic expression of MR (1, 14), these techniques may still not be sufficient to obtain 100% phenotypic expression of resistance (5, 7, 14). An additional measure that facilitated the detection of MR in staphylococci was the testing of a large inoculum. Although this is easily performed as a part of susceptibility testing once an organism has been isolated, the incorporation of this technique into screening cultures for MR coagulase-negative staphylococci is not employed routinely. To increase the number of staphylococci tested, an overnight broth step was used in which a swab containing a sample from the skin or nares was inoculated into nutrient broth and incubated overnight before it was plated onto selective (antibiotic-containing) medium. This maneuver should enable a single MR bacterium that is present to multiply, improving the likelihood that some of the progeny MR organisms will phenotypically express resistance and be recognized when challenged with antibiotic-containing media. The fact that 23 of 64 (36%) sites that contained MR coagulase-negative staphylococci preoperatively were detected only by growth on overnight broth plates reflects the importance of this step. The efficacy of similar enhancement techniques relative to that of direct plating methods in the recovery of MR *S. aureus* as skin-colonizing flora has recently been reported (B. P. Cookson, M. Webster, and I. Phillips, Letter, Lancet i:696, 1987). These investigators determined that 71 of 210 (34%) skin sites that harbored MR *S. aureus* would have been missed if enhancement methods had not been employed.

Furthermore, it is likely that culture methods that are more sensitive than those employed in our study might yield MR coagulase-negative staphylococci from an even higher proportion of sites. Our median staphylococcal inoculation into broth from an 8.0-cm^2 area of the subclavian area was 400 organisms (or 50/cm^2) and 7,000 organisms (or 875/cm^2) for the inguinal area. In contrast, quantitative measurements of skin flora yield values in the range of 4,400/cm^2 for relatively clean sites, such as the breasts, and up to 400,000/cm^2 for dirty sites, such as the axillae (16). At best, we recovered only a small fraction of the staphylococcal flora from each site. By use of better culture methods, we could potentially find more sites that were positive for MR coagulase-negative staphylococci or discover several distinct subpopulations of MR coagulase-negative staphylococci at the same site.

Although these observations enhance our understanding of the epidemiology of MR coagulase-negative staphylococci in hospitals, it is important to recognize the limitations of this study. First, our data were not adequate to determine the original source of the MR coagulase-negative staphylococci, whether it was the community or the hospital. It is possible that the low-level presence of MR coagulase-negative staphylococci detected preoperatively reflects organisms that were acquired nosocomially from a previous hospitalization or even from early in the hospitalization during which the present study was performed. Indeed, our recovery of one distinctive *S. haemolyticus* strain from four patients suggests the possibility of a common source of acquisition, perhaps nosocomial, for this strain. A better understanding of the epidemiology of MR coagulase-negative staphylococci in the community requires a study group that consists of individuals who have not had recent hospital exposure.

Second, infection due to MR coagulase-negative staphylococci did not occur in any of our study patients, and we cannot comment as to whether the colonizing MR coagulase-negative staphylococci that emerged under antibiotic pressure are important in the generation of the disease. Indeed, at present we have a very limited understanding of the origin of isolates of MR coagulase-negative staphylococci that infect patients. Although the demonstration of identical plasmid profile patterns among isolates recovered from different neonates (13) and several patients with prosthetic valve endocarditis (4) suggests a common nosocomial source for some infections, results of most clinical studies of MR coagulase-negative staphylococci have provided no clues as to the source of the infecting strain. It appears probable, however, that colonizing isolates will prove to be important in producing illness that originates from skin flora that gain access to a usually sterile site (intravascular catheter-related sepsis, surgical wound infections, etc.), regardless of the mechanism of colonization.

Finally, we do not have data that are sufficient to assess the relative contribution of emergence and nosocomial acquisition to the epidemiology of MR coagulase-negative staphylococci in hospitals. Indeed, the epidemiology of MR

coagulase-negative staphylococci may vary in different patient populations. It could be that emergence is a major mechanism for achieving heavy colonization with MR coagulase-negative staphylococci in cardiac surgery patients, who possess a quantitatively, qualitatively, and anatomically complex flora (9, 11, 16) from which an MR subpopulation might be selected. In contrast, nosocomial acquisition alone may explain the epidemiology of MR coagulase-negative staphylococci in neonatal nurseries, as the skin of infants is sterile in utero and receptive to colonization following birth. Also, it is possible that the epidemiology of MR coagulase-negative staphylococci has changed with time. The experience with MR *S. aureus* in Detroit, Mich. (15), has demonstrated that what was once regarded as only a nosocomial pathogen can cause disease outside of the hospital as well, so that community-derived strains now account for a substantial proportion of MR *S. aureus* infections. MR coagulase-negative staphylococci may have disseminated in a similar fashion so that although nosocomial acquisition occurs, it is no longer adequate to explain all factors that influence the complex epidemiology of MR coagulase-negative staphylococci. The relative contribution of acquisition versus emergence in both the hospital and community setting will be difficult to determine. It is likely that both mechanisms play a role.

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