JOURNAL OF CLINICAL MICROBIOLOGY, July 1988, p. 1257–1262
0095-1137/88/071257-06$02.00/0

Vol. 26, No. 7

Prospective Study of Microbial Colonization of the Nose and Skin and Infection of the Vascular Access Site in Hemodialysis Patients

LISA G. KAPLOWITZ,[1] JANE A. COMSTOCK,[2] DOUGLAS M. LANDWEHR,[3]† HARRY P. DALTON,[4] AND C. GLEN MAYHALL[1,2]*

Hospital Epidemiology Unit,[2] Division of Infectious Diseases,[1] and Division of Nephrology,[3] Department of Medicine, and Department of Pathology,[4] Medical College of Virginia and the Medical College of Virginia Hospitals, Virginia Commonwealth University, Richmond, Virginia 23298-0049

Received 30 November 1987/Accepted 21 March 1988

We conducted a prospective study of nasal and skin floras in 71 patients receiving chronic hemodialysis. We wished to determine whether a sterile skin preparation technique was more effective than a clean technique in removing microorganisms from the skin of the vascular access site. We also examined the effect of administration of antibiotics and status of patient hygiene on microbial flora. The presence of *Staphylococcus aureus* in the nose had a low predictive value for the simultaneous presence of the microorganism on the skin. The status of skin colonization can be accurately assessed only by culture of the skin. Sterile technique was no more effective at removing microorganisms from skin than was clean technique. Antibiotics significantly affected nasal flora but not skin flora. *S. aureus* was significantly more likely to remain on the skin after application of an antiseptic in patients with poor hygiene than in patients with good hygiene ($P = 0.002$). Patients with poor hygiene also had a significantly higher concentration of *S. aureus* on the skin of the vascular access site after application of antiseptic than patients with good hygiene ($P = 0.005$). We found no evidence to support a change from clean to sterile technique for skin preparation, but improvement in personal hygiene may be an effective strategy for prevention of vascular access infections.

Staphylococcus aureus carriage in the nose and on the skin has been shown to be more common in patients receiving chronic hemodialysis than in the general population (2–4, 8). *S. aureus* has also been found to be a major pathogen in this population, especially as the causative agent of access site infections (1, 2, 12, 13). In 1979, an outbreak of *S. aureus* access site infections occurred in the hemodialysis unit of the Medical College of Virginia Hospitals. This outbreak resulted in the substitution of a sterile skin preparation regimen for a clean regimen for preparation of the skin prior to the placement of dialysis needles. In 1983, D. M. Landwehr conducted a survey of hemodialysis units in Virginia and surrounding states to determine the type of predialysis skin preparation used. All units surveyed, aside from those affiliated with the Medical College of Virginia, used a clean skin preparation regimen prior to the placement of dialysis needles. Therefore, we planned a prospective randomized study to evaluate the effect of the two skin preparation regimens on nasal and skin colonization and access site infection in patients receiving chronic hemodialysis.

The first objective of the present study was to determine whether a sterile skin preparation technique was more effective at removing skin flora from the access site than a clean preparation technique and whether the types of organisms remaining on the skin after each skin preparation technique differed. The second objective was to study changes in nasal and skin flora over time in this population. Our third objective was to determine the relationship of flora in the nose to flora on the skin of the access site. A fourth objective was to evaluate potential risk factors for nasal and skin colonization including antibiotic administration and patient hygiene. Finally, we wished to study the relationship between nose and skin colonization and access site infection.

MATERIALS AND METHODS

Patients enrolled were those receiving chronic, staff-assisted hemodialysis in the dialysis unit of the Medical College of Virginia Hospitals. All study patients received dialysis via an internal fistula or graft; patients with temporary access devices such as indwelling catheters were excluded from the study. Any patient enrolled in the study who required dialysis temporarily via a catheter device at any time during the study year was excluded from the study until the catheter was removed and an internal fistula or graft could again be used for dialysis access. Study entry began on 1 March 1984, and data collection was completed on 7 March 1985. Patients could enter or exit the study at any time during the year. Reasons for exiting the study prior to study termination included death, renal transplantation, transfer to home or self-care dialysis, and failure of the graft or fistula access.

Patients were randomized via a computer-generated table of random numbers to receive a clean or sterile skin preparation regimen throughout the course of the study. The nurses and physicians working in the hemodialysis unit were aware of which skin preparation regimen each patient was receiving. The study physicians responsible for monitoring infections (L.G.K. and C.G.M.) were unaware of the assignment of skin preparation regimens until data collection and entry were completed.

Skin preparation regimens. (i) Clean technique. The patient scrubbed the access arm with a disposable soft brush containing an iodophor just prior to being positioned in the dialysis chair for cannulation. A clean barrier was placed under the patient's arm. Two 10-ml syringes that were to be used of flush the dialysis tubing were each filled with normal saline. Xylocaine (1 ml) was drawn up into a sterile 3-ml

* Corresponding author.
† Present address: Allegheny General Hospital, Pittsburgh, PA 15212.

syringe. The nurse donned nonsterile gloves and selected the cannulation sites by palpation. Povidone-iodine solution (Operand; Redi-Products, Prichard, W.V.) was applied to the skin over the access site and allowed to dry for 1 min. The arterial and venous sites were injected with xylocaine. Needles for dialysis were inserted by a no-touch aseptic technique with Band-Aids placed over needle access sites after the needles were in place.

(ii) Sterile technique. The patient scrubbed the access arm with a disposable soft brush containing an iodophor just prior to being positioned in the dialysis chair for cannulation. All materials used to cannulate the access site except for containers of fluids were placed on a sterile barrier next to the patient. A sterile barrier was placed under the patient's arm. After donning sterile gloves, the nurse picked up the two 10-ml syringes that were to be used to flush the dialysis tubing and filled each with normal saline. Next, the nurse drew up 1 ml of xylocaine into a sterile 3-ml syringe. To maintain the sterile field, the nurse, when filling the syringes, grasped the containers of saline and xylocaine with sterile sponges (4 by 3 in. [10 by 7.5 cm]) which were then discarded by dropping them into a trash can. The cannulation sites were selected by palpation, and povidone-iodine solution (Operand) was applied to the skin over the access site and allowed to dry for 1 min. The nurse removed the gloves and placed a tourniquet on the patient's arm. Then the nurse donned a second pair of sterile gloves and unfolded and placed sterile sponges (4 by 3 in.) above and below the prepared area to extend the sterile field. The arterial and venous sites were injected with xylocaine. The fistula needles were then inserted, and the insertion sites were covered with Band-Aids. A sterile technique for dialysis needle insertion had been used routinely in the dialysis unit at the Medical College of Virginia Hospitals for 4 years prior to initiation of this study.

Culture techniques. Cultures were taken from the anterior nares and the skin overlying the access site every 2 weeks.

(i) Anterior nares. Cultures were obtained from both nares with a single sterile cotton swab (Medi-Pak; Whittaker General Medical, Richmond, Va.). The swab was plated immediately.

(ii) Needle insertion sites. Before skin preparation, cultures were obtained from an area (3 by 3 cm) around each insertion site with a sterile cotton swab (Medi-Pak), using the same swab for both needle insertion sites. A sterile template was used to define the culture area. The area enclosed by the template was swabbed first from top to bottom and then from side to side, and the swab was plated immediately. After skin preparation and placement of the dialysis needles, an area (3 by 3 cm) around each needle insertion site was swabbed using a sterile template. Separate swabs were used for each needle insertion site, and each was plated immediately.

Microbiologic techniques. The anterior nares and skin specimens were inoculated onto tryptic soy agar containing 5% sheep blood (Difco Laboratories, Detroit, Mich.). Specimens from skin taken after application of antiseptic were inoculated onto FDA agar (Difco) containing 1% Tween 80, 0.3% asolectin, and 0.3% sodium thiosulfate for inactivation of antiseptic carried over to the agar by the swab (14). Incubation was at 35°C for 48 h. All organisms isolated were identified by standard methods as outlined in the *Manual of Clinical Microbiology* (9). Agar dilution antimicrobial susceptibility tests were performed by the recommendations of the National Committee for Clinical Laboratory Standards (10).

A semiquantitative technique was used to quantitate the colonies per swab. This consisted of counting the number of colonies per plate after incubation and assigning the following scale: light growth, 1 to 15 colonies; moderate growth, 16 to 30 colonies; heavy growth, greater than 30 colonies.

Epidemiologic data. Epidemiologic data were recorded for each patient at the time of study entry and monthly thereafter. These included data on administration of antibiotics and assessment of patient hygiene. Hygiene status for more than 90% of the patients was determined by one physician (D.M.L.). The following scale was used: good hygiene, the patient had clean skin and clothing; intermediate hygiene, the skin was moderately encrusted with dirt, and the clothing was moderately soiled; poor hygiene; the skin was heavily encrusted with dirt and scale, and the clothing was soiled.

Definition of vascular access infection. Infection was diagnosed when (i) purulent fluid was recovered from the access site, or (ii) cultures taken from the access site during surgery yielded a pathogen, or (iii) bacteremia occurred with no identifiable source and with no evidence of endocarditis.

Statistical analysis. Data were entered into an IBM 3081 mainframe computer and analyzed by the Statistical Analysis System (SAS Institute, Inc., Cary, N.C.).

Categorical data were analyzed by the likelihood ratio chi-square test or a two-tailed Fisher's exact test. For continuous variables, a t test for test of equality of means was used. When the data were continuous but nonnormal, we used the Wilcoxon rank-sum test. Beta-binomial regression was used to compare proportional responses measured on each person between the clean and sterile skin preparation groups (15). Matched-pair partial correlation coefficients were calculated to determine the relationship between two variables while adjusting for the repeated measures on individuals (6). The significance test using this correlation is a generalization of McNemar's test for the case without repeated measures when the variables are measured as yes/no.

RESULTS

Seventy-one patients participated in the study for a total of 676 dialysis months. Epidemiologic data for the clean and sterile skin preparation groups at the time of entry into the study are shown in Table 1. The clean and sterile skin preparation groups were comparable for all epidemiologic characteristics except for months of hemodialysis prior to entry into the study. Patients receiving a sterile skin preparation had a mean of 29 months of hemodialysis prior to study entry, while those receiving a clean skin preparation had a mean of 19.5 months; this difference was significant ($P = 0.01$).

Culture data were analyzed by combining culture results from each site during each month. Although arterial and venous sites were cultured separately after skin preparation, culture results from the two sites were combined for analysis. The sterile skin preparation technique was no more effective at removing skin flora from the access site than the clean skin preparation technique (Table 2). Except for *S. aureus*, there were no significant differences in the percentage of a given type of microorganism remaining on the skin after the clean skin preparation regimen compared with the sterile skin preparation regimen. For *S. aureus*, the results were the opposite of what might have been predicted. The clean technique appeared to be more effective than the sterile technique at removing this microorganism.

Fifty-nine patients had 4 or more months of cultures of the nose and skin over the access site (before skin preparation),

TABLE 1. Epidemiologic characteristics of patients

Characteristic	Clean skin prepn (n = 37)	Sterile skin prepn (n = 34)	P value
Age (yr) (mean [range])	52 (18–88)	53 (25–78)	0.6[a]
Sex (no. male/no. female)	20/17	22/12	0.4[b]
Karnofsky scale (mean [range])	82 (50–100)	81 (40–99)	0.7[a]
Mo of hemodialysis (mean [range])	19.5 (0–85)	29 (1–116)	0.01[a]
Hygiene (no. of patients) (good/intermediate/poor)	23/11/3	19/9/6	0.5[b]
Illicit drug use (no. of patients) (yes/suspicious/no)	1/2/34	0/1/33	0.4[b]
Diabetes (no. of patients) (insulin requiring)	12 (4)	7 (3)	0.3[b]
Access type (no. of patients) (fistula/Dacron graft/saphenous vein)	17/25/0	14/21/1	0.4[b]

[a] t test.
[b] Likelihood ratio chi square.

with an average of 10 months of cultures for each patient. Of the 59 patients, 29 (49%) had *S. aureus* present in the nose and 25 (42%) had *S. aureus* present on the skin over the access site at some time during the study year. However, in none of these 59 patients was at least one culture site positive for *S. aureus* during every culture month.

Of the 59 patients, 41 (69%) had gram-negative bacilli in the nose and 27 (46%) had gram-negative bacilli present on the skin over the access site before skin preparation at some time during the study year. In five patients (8.5%), gram-negative bacilli were present in at least one culture from the nose during every culture month, but in no patient were gram-negative bacilli recovered from skin cultures in every culture month. Only in the five patients with at least one nose culture positive for gram-negative bacilli in every culture month was at least one culture site positive in every month for gram-negative bacilli.

The relationship between flora present in the nose and flora present on the skin prior to skin preparation is shown in Table 3. The presence of *S. aureus* and *Micrococcus* species in the nose was significantly related to the simultaneous presence of these species on the skin of the access site. Also, the presence of gram-negative bacilli in the nose was significantly related to the simultaneous presence of gram-negative bacilli on the skin of the access site. Heavy growth of these microorganisms in the nose was correlated with heavy growth of the same microorganisms on the skin, with P = 0.014 for *S. aureus*, P = 0.0001 for *Micrococcus* species, P = 0.002 for *Citrobacter freundii*, and P = 0.003 for all gram-negative bacilli (data not shown).

The predictive value of the presence of *S. aureus* in the nose for the simultaneous presence of *S. aureus* on the skin was 0.2. The predictive value for *Micrococcus* species was 0.81, and that for gram-negative bacilli was 0.15. The predictive value for the simultaneous presence of each of these microorganisms in the nose when present on the skin was as follows: *S. aureus*, 0.60; *Micrococcus* species, 0.33; and gram-negative bacilli, 0.53.

Administration of antibiotics resulted in some significant changes in nasal flora but had no impact on skin flora. Persons receiving antibiotics were more likely to develop nasal colonization with *Klebsiella pneumoniae* and with all gram-negative bacilli (P = 0.001 and 0.04, respectively) and less likely to develop nasal colonization with *S. aureus* (P = 0.009) in the subsequent month.

Skin flora at the access site was correlated with the level of patient hygiene. For this analysis, patients with intermediate and poor hygiene were combined and compared with those with good hygiene. *S. aureus* was present in 2 of 386 skin cultures from patients with good hygiene compared with 8 of 225 skin cultures from patients with poor hygiene for cultures taken after, but not before, skin preparation (P = 0.002). Further, when *S. aureus* was present after skin preparation, it occurred with significantly heavier growth in patients with poor hygiene (P = 0.005). There was no correlation between level of hygiene and *S. aureus* colonization in the nose. There was no significant difference in the presence of coagulase-negative staphylococci before or after skin preparation between patients with good and poor hygiene. However, when coagulase-negative staphylococci were present on the skin of the access site before skin preparation, they were significantly more likely to be present in heavy growth in patients with poor hygiene (P = 0.02).

Table 4 shows the correlation between access site infec-

TABLE 2. Effect of skin preparation technique on removal of flora

Microorganism	No. of positive skin cultures		P value[a]
	Clean technique (before/after) (% remaining)	Sterile technique (before/after) (% remaining)	
Staphylococcus aureus	32/2 (6)	17/6 (35)	0.04
Coagulase-negative staphylococci	306/109 (36)	259/89 (34)	0.95
Micrococcus species	122/11 (9)	96/5 (5)	0.42
Gram-positive microorganisms	319/118 (37)	271/99 (37)	0.89
Klebsiella pneumoniae	2/0 (0)	4/0 (0)	ND[b]
Enterobacter aerogenes	0/0	2/0 (0)	ND
Proteus mirabilis	2/0 (0)	0/0	ND
Citrobacter freundii	1/0 (0)	2/0 (0)	ND
Acinetobacter calcoaceticus	25/2 (8)	11/0 (0)	ND
Gram-negative microorganisms	31/2 (6)	22/0 (0)	ND
All microorganisms	319/119 (37)	272/99 (36)	0.99

[a] Beta-binomial analysis.
[b] ND, Not done, numbers too small for statistical analysis.

TABLE 3. Relationship between presence of a microorganism in the nose and on the skin of the access site before skin preparation

Microorganism	No. of paired cultures				P value[a]
	Absent nose, absent skin	Absent nose, present skin	Present nose, absent skin	Present nose, present skin	
S. aureus	440	20	117	29	0.005
Micrococcus species	370	146	17	73	<0.001
Gram-negative bacilli	400	25	153	28	0.004

[a] Matched-pair partial correlations.

TABLE 5. Colonization with *S. aureus* and access site infection[a]

Colonization site	Access site infection (n = 6) (colonization absent/present)	No access site infection (n = 64) (colonization absent/present)	P value[b]
Nose	1/5	33/31	0.08
Skin (prior to prepn)	1/5	42/22	0.02

[a] 70 patients with surveillance cultures done at appropriate times (before infection in patients with access site infections and any time during the study in noninfected patients).

[b] Likelihood ratio chi square.

tions and prior skin or nasal colonization with the organism causing the infection. Seven patients had access site infections caused by *S. aureus*. Of these, six had surveillance cultures done prior to the onset of infection. All six patients were colonized with *S. aureus* 1 to 10 weeks prior to the diagnosis of access site infection. Five patients had skin colonization, and four had nasal colonization. Antimicrobial susceptibility test results were available for three of the five isolates of *S. aureus* that colonized the access site prior to infection. All had an antibiotic susceptibility pattern similar to that of the infecting strain.

Table 5 compares *S. aureus* colonization prior to infection in patients with staphylococcal access site infections with staphylococcal colonization in the rest of the study population. Nasal colonization was not significantly associated with access site infection, but skin colonization with *S. aureus* prior to skin preparation was significantly related to the occurrence of infection.

Density of colonization with *S. aureus* in patients with and without *S. aureus* access site infections was compared. There was no significant difference in the density of *S. aureus* colonization between those with and those without access site infections.

DISCUSSION

Randomization of patients to clean or sterile technique groups resulted in two groups of patients with only one significant difference. The sterile skin preparation group received hemodialysis for a significantly higher mean number of months prior to study entry. However, it would appear that this was not an important difference. In a parallel study, mean number of months receiving dialysis prior to entry into the study was unassociated with occurrence of vascular access infection (L. G. Kaplowitz, J. A. Comstock, D. M. Landwehr, H. P. Dalton, and C. G. Mayhall, submitted for publication). The sterile skin preparation technique was no more effective at removing skin flora from the access site than the clean preparation technique. Also, except for *S. aureus*, there was no significant difference in the types of organisms remaining on the skin after use of either of the two regimens. The significant relationship between use of the sterile skin preparation regimen and persistence of *S. aureus* on the skin after preparation is unexplained. However, that observation would not support the use of the sterile skin preparation in place of the widely used clean preparation technique. Failure to show a difference in quantity and type of skin floras between the sterile and clean skin preparation groups supported our epidemiologic observation of no difference in infection rates between these two groups (Kaplowitz et al., submitted).

In the recent report of Yu and associates (16), *S. aureus* was found in 52% of nasal cultures but in only 6% of simultaneously obtained skin cultures taken from their hemodialysis patients. These investigators concluded that culture of the anterior nares was the more sensitive indicator of *S. aureus* carriage. However, with more frequent culturing, we were able to culture *S. aureus* from the nose of 49% and the skin of 42% of the study patients. Therefore, in contrast to the study of Yu and associates (16), a similar percentage of patients in the present study had *S. aureus* colonization of the nose and of the skin at some time during the study year. With a larger number of cultures, Goldblum and co-workers (2, 3) also observed that a much higher percentage (about 25%) of skin cultures were positive for *S. aureus*. We and Goldblum and co-workers (2, 3) also noted a significant correlation between the simultaneous presence of *S. aureus* in the nose and on the skin. However, our data and those of Goldblum and co-workers showed that the presence of the organism in the nose is not highly predictive for the simul-

TABLE 4. Correlation between causative microorganisms of access site infections and microorganisms recovered from skin or nose or both in the 10 weeks prior to infection

Causative microorganism	Site of colonization prior to infection (same organism)	Interval between colonization and infection
Staphylococcus aureus	Skin (after prepn)	6 wk
Staphylococcus aureus	No surveillance cultures available prior to infection	
Staphylococcus aureus	Nasal and skin (before and after prepn)	9 days
Staphylococcus aureus	Nasal and skin (before prepn)	26 days (nose) and 10 days (skin)
Staphylococcus aureus	Skin (before prepn)	10 wk
Staphylococcus aureus	Nasal	9 days
Staphylococcus aureus	Nasal and skin (before and after prepn)	25 days
	Nasal	11 days
Coagulase-negative staphylococci	Nasal and skin (before prepn)	6 wk
Streptococcus salivarius	No colonization (with same organism)	

taneous presence of the organism on the skin. It would appear that the status of skin colonization can be accurately assessed only by culture of the skin.

Of additional interest were the parallels between the microbiology of *S. aureus* and the microbiology of gram-negative bacilli in our hemodialysis patient population. Thus, the percentages of patients with nasal and skin carriage of *S. aureus* and gram-negative bacilli were similar; other parallels included the significant relationship between nasal and simultaneous skin colonization, the significant association between heavy growth in the nose and heavy growth on the skin, and the low predictive power of nasal colonization for simultaneous skin colonization.

Few studies of the microbial flora in the nose and on the skin of hemodialysis patients have examined the presence or absence of gram-negative bacilli. Noble and associates (11) recovered multiple species of gram-negative bacilli from the nose and skin of hemodialysis patients and renal transplant patients, but they did not study the relationship between nose and skin colonization. In the first of two studies published by Goldblum and co-workers (2), they recovered very few gram-negative bacilli from the skin of either hemodialysis patients or normal controls and suggested that failure to culture gram-negative rods from skin in their patients might have been due to the arid climate of New Mexico where the studies were performed. In the second study (3), they recovered gram-negative bacilli from 25% of skin cultures taken from hemodialysis patients, but they did not study nasal colonization with gram-negative bacilli.

Given the parallels between *S. aureus* and gram-negative bacillary colonization of hemodialysis patients, it is interesting to note the absence of access site infections caused by gram-negative bacilli in our study and the low incidence of vascular access infections caused by gram-negative bacilli reported in the literature (1, 7, 12, 13). This might be due to a lower degree of adherence of gram-negative bacilli to vascular endothelium. In an in vitro study, Gould and colleagues (5) showed that most gram-negative bacilli adhered less well to cardiac valvular endothelium than did gram-positive cocci.

We observed that antibiotics given for intercurrent infections appeared to predispose patients to nasal colonization with gram-negative bacilli as a group and with *K. pneumoniae* in particular. On the other hand, antibiotic administration seemed to diminish the risk of colonization of the nose with *S. aureus*. Antibiotic therapy had no effect on the flora over the access site. Again, given the dissociation between the effect of antibiotics on floras of the nose and skin, culture of nasal flora may not accurately reflect the status of skin colonization. Further, the lack of effect of antibiotics on skin flora is consistent with our observation that administration of antibiotics had no effect on the incidence of vascular access site infection (Kaplowitz et al., submitted). To our knowledge, this study is the first to assess the impact of antibiotic administration on the floras of the nose and skin of hemodialysis patients.

S. aureus skin colonization prior to skin preparation was significantly associated with subsequent *S. aureus* access site infection. These data suggest that skin colonization with *S. aureus* is a risk factor for the development of staphylococcal access site infection. Therefore, it would appear that effective removal of *S. aureus* from the skin of the access site would be critical to prevention of vascular access site infection.

Of particular interest was our observation of a significant relationship between the presence of *S. aureus* on the skin after skin preparation and poor personal hygiene. The relationship was strengthened by the observation that, when *S. aureus* remained on the skin after skin preparation, it was present in significantly heavier growth in persons with poor hygiene. We were unable to find published studies of hemodialysis patients that examined skin flora after preparation of the skin with an antiseptic. The significant relationship between the presence and density of *S. aureus* colonization after skin preparation and the status of patient hygiene support infection control measures based on improvement in the hygienic practices of our patients. In our hemodialysis patients, this approach would appear to be preferable to periodic nasal cultures and antibiotic prophylaxis as proposed by Yu and associates (16). These researchers were able to significantly reduce the rate of dialysis access site, skin, and soft tissue infections in hemodialysis patients by prophylaxis with rifampin given every 3 months when nasal cultures were positive for *S. aureus*. However, there are several potential difficulties with routine antibiotic prophylaxis, including the need for repeated cultures, untoward reactions to antibiotics, development of antibiotic resistance, problems ensuring patient compliance, and cost.

In summary, the sterile skin preparation technique was no more effective at removing skin flora than the clean technique and is not recommended. We showed that *S. aureus* skin colonization is a risk factor for the subsequent development of staphylococcal access site infections. Patients with poor hygiene were shown to have a significantly increased incidence of skin colonization of the access site with *S. aureus* after skin preparation. This corroborates our findings of poor hygiene as a major risk factor for the development of access site infection (Kaplowitz et al., submitted). Improvement in patient hygiene in populations such as ours could result in a significant decrease in the incidence of access site infections without the potential problems related to prophylactic antibiotic administration. A study of the impact of improved patient hygiene on access site infection rates is planned for the near future.

ACKNOWLEDGMENTS

We thank Gaye O. Hall for technical assistance, Vernon Chinchilli for assistance with the statistical analysis, Patricia Wood for administrative assistance, the medical staff and nursing staff of the hemodialysis unit for their cooperation during conduct of the study, and Julie Rhodes for preparation of the manuscript.

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