

The Prevalence of Resistant Bacterial Colonization in Chronic Hemodialysis Patients

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Key Words

Bacterial colonization · Chronic kidney disease · Dialysis · Methicillin-resistant *Staphylococcus aureus* · Bacteria, resistant · Vancomycin-resistant *Enterococcus*

Abstract

Background: Hospitalized dialysis patients are at increased risk for colonization and infection with resistant bacterial strains. **Methods:** We performed a cross-sectional analysis of the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) colonization in 198 hemodialysis outpatients, 75 of whom had longitudinal screening data from prior hospitalization. Nasal specimens for MRSA, perirectal specimens for VRE, and permanent catheter exit site specimens were collected. **Results:** MRSA colonization was present in 5.6% and VRE colonization in 3.14%. Univariate analyses revealed that prior exposure (defined as infection/colonization) with MRSA, hospitalization, and low serum albumin were associated with MRSA colonization. VRE colonization was associated with hospitalization, prior VRE or MRSA exposure, low serum albumin, and low ferritin. Multivariate analyses revealed MRSA colonization was predicted by prior MRSA exposure and VRE coloni-

zation was predicted by prior VRE exposure and number of hospitalizations. Among the 75 participants with longitudinal screening data, MRSA colonization was associated with prior MRSA history, and VRE colonization was associated with prior MRSA or VRE. **Conclusions:** Generally low rates of MRSA and VRE colonization were observed in hemodialysis outpatients. Prior hospital screening was predictive of future outpatient colonization and may be useful in risk assessment.

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Introduction

Infection remains a major cause of morbidity, and the second leading cause of mortality, in patients with end-stage renal disease (ESRD). The prevalence of clinical infections with antibiotic-resistant bacteria has been increasing. The percentage of US outpatient dialysis facilities reporting patients with methicillin-resistant *Staphylococcus aureus* (MRSA) increased from 40 to 76% between 1995 and 2002 [1]. Over that same time period the percentage of units reporting patients with vancomycin-resistant *Enterococcus* (VRE) increased from 12 to

30% [1]. Several of the initial reports of clinical infection due to VRE and vancomycin-intermediate *S. aureus* were reported in patients on dialysis [2].

Patients on maintenance hemodialysis are at increased risk for colonization with resistant bacteria since they often receive prolonged courses of antibiotics, are frequently hospitalized, have frequent contact with healthcare, and thus have multiple opportunities for horizontal transmission of resistant microorganisms. VRE colonization rates ranging from 1.1 to 33% have been reported [3–8]. Colonization with VRE or MRSA increases the risk for clinical infections with these organisms and transmission of resistant bacteria between patients in chronic health care settings is clearly documented [9, 10]. Once a patient acquires MRSA or VRE, antibiotic usage and concurrent illnesses appear to impact the degree to which these resistant bacteria are shed, and whether they are detectable [11]. *S. aureus* nasal carriage is frequently persistent, and perirectal VRE carriage can persist for weeks to months [10, 12, 13].

We undertook a cross-sectional study to determine the rates of resistant bacterial colonization in three large, urban, outpatient dialysis centers in North Carolina to assess for risk factors associated with MRSA and VRE colonization. In addition, we compared the results of outpatient screening with those in an existing hospital infection control database used at our institution to track MRSA and VRE colonization/clinical infection rates. This allowed us to consider the utility of using an inpatient database to predict future outpatient culture results.

Materials and Methods

Patients

Participants were recruited at three outpatient hemodialysis centers owned and operated by the Wake Forest University School of Medicine (WFUSM) in Winston-Salem, N.C. All dialysis patients >18 years of age and capable of independently providing informed consent were invited to participate. Subjects were oriented about study requirements, including obtaining specimens for culture from the nares, perirectal area, and catheter exit site (if present). Cultures were collected from participants on a subsequent dialysis treatment day. Demographic and medical data were collected from clinical records and through brief patient interviews. The study was approved by the WFUSM Institutional Review Board. All participants provided written informed consent at the time of enrollment.

Sample Collection

Nasal cultures for MRSA were collected using a BBL™ Cultureswab™ Liquid Stuart Medium swab (BD Diagnostic Services;

Sparks, Md., USA) that was rotated within the nares. Perirectal cultures were collected by swabbing over the anus with a separate liquid Stuart Medium swab. If the patient had a tunneled permanent dialysis catheter, a specimen was collected from the catheter skin exit site.

Isolation of MRSA

Nasal and catheter exit specimens were inoculated on a blood agar plate and perirectal specimens on a colistin and nalidixic acid (CNA) plate to isolate MRSA. After 24 h of incubation at 35°C, plates were examined for medium to large, creamy white to yellow colonies. Candidate colonies were tested with a Staph-aurex® agglutination kit. If agglutination was positive, a catalase test and a Gram stain were performed. Catalase-positive, Gram-positive cocci were considered a positive result for *S. aureus*. A 0.5 Mcfarland standard of the colony was suspended into a 1-ml tube of normal saline and inoculated onto a plate containing 6 µg of oxacillin and a blood agar plate. Both plates were incubated at 30–35°C for 24 h. Pure growth on the oxacillin and check plates was indicative of MRSA.

VRE Isolation

To isolate VRE, perirectal specimens were plated onto Enterococcosel plates and incubated at 30–35°C for 24 h. Any colonies that were gray to black on the Enterococcosel plate were inoculated to a CNA plate and incubated for 24 h. On day 2, Gram stain, catalase and Pyr/Esculin tests were performed on any small to medium, gray colonies that were with or without alpha hemolysis. Gram-positive cocci that were catalase negative and Pyr/Esculin positive are indicative of *Enterococcus*. A 0.5 Mcfarland standard of the colony was suspended into a 1-ml tube of normal saline and inoculated to a Mueller-Hinton agar plate and a blood agar. A 30-µg vancomycin-containing disc was placed on the Mueller-Hinton plate to check for antibiotic resistance. The plates were incubated for 24 h at 30–35°C. If the zone of inhibition was less than or equal to 14 mm and the plates had pure growth, the organism was considered to be vancomycin-resistant enterococci.

Data Collection

At the time consent was obtained the investigator verbally administered a brief questionnaire asking about history of diabetes, cause of ESRD, and history of transplantation. Dialysis center records were examined to determine the subjects' hemodialysis start date, vascular access type, HIV/hepatitis C status, the number of hospitalizations in the prior year, and outpatient administration of intravenous vancomycin in the prior year. Monthly laboratory data was collected and the most recent results, prior to the date the cultures were obtained, were recorded.

The North Carolina Baptist Hospital (NCBH) initiated a routine MRSA and VRE screening protocol for all dialysis inpatients in 2002 and maintains a database of these nasal MRSA and perirectal VRE culture results. In addition, clinical culture results revealing VRE or MRSA are also recorded on this database and used to identify patients who require isolation precautions in accordance with the NCBH infection control policy. We screened the NCBH database to identify a history of prior MRSA/VRE screenings or infections, while hemodialysis patients were hospitalized. Existing MRSA and VRE data was available in 75 and 71 patients, respectively, and prior MRSA or VRE positivity was defined as a positive screening culture or clinical culture with MRSA or VRE.

Statistical Analysis

Basic descriptive statistics, including frequencies and percentages, were calculated for patient data. Characteristics of participants and nonparticipants were compared using t tests (for continuous variables) and χ^2 tests (for categorical variables). For univariate statistical analyses, Fisher's exact test was used to assess the relationship between MRSA or VRE colonization and categorical measures. Logistic regression was used when the independent measures were continuous. Forward stepwise logistic regression was performed using the variables that were significant in the univariate analysis. In a forward stepwise regression, the most significant variable is put into the model first, then followed by the next most significant; if both are significant, both remain, and the next variable is added, with the process done iteratively until the added measures are not significant. These analyses were repeated limiting the cohort to the 75 subjects with prior MRSA screening/infection data and the 71 with prior VRE screening/infection data.

Results

Of 443 chronic hemodialysis patients treated in the three facilities, 212 consented to participate and 93% (198) completed the protocol. We excluded 14 participants who initially consented, but subsequently missed dialysis on the day samples were collected or withdrew consent. Demographic characteristics of the participants are summarized in table 1. Participants were similar to nonparticipants with respect to gender, race, time on

chronic dialysis, cause of ESRD, and age (all p values >0.05). Nasal swabs were collected from 197 patients and perirectal swabs from 193. Five patients had nasal cultures, but refused perirectal cultures. The culture result from one nasal swab was not available. Of the 197 participants, 11 (5.6%) tested positive for MRSA nasal colo-

Table 1. Participant characteristics

Characteristics	n (%)
Race	
White	54 (27.3)
Black	142 (71.7)
Other	2 (1.0)
Cause of ESRD	
Diabetes	88 (45.1)
Other	107 (54.9)
Dialysis access	
AVF	116 (58.9)
Goretex® graft	41 (20.8)
Catheter	40 (20.3)
Gender	
Female	94 (47.5)
HIV status	
Positive	3 (1.5)
Hepatitis C status	
Positive	13 (6.6)
Time on dialysis, years	4.384.0
Age, years	61.9815.0

Data missing for cause of ESRD in 3 participants and dialysis access type missing in 1 participant.

Table 2. Univariate analysis in all 198 subjects

Variable	MRSA p value	Odds ratio (95% CI)	VRE p value	Odds ratio (95% CI)
Gender (male:female)	0.76	NS	1.000	NS
Race (black:nonblack)	0.08	0.30 (0.09, 1.02)	0.67	NS
Cause ESRD (diabetes:other)	0.068	0.26 (0.05, 1.22)	1.000	NS
Access (AVF vs. graft vs. catheter)	0.83	NS	0.53	NS
Dialysis unit	0.57	NS	0.24	NS
VRE history ^a	0.087	NS	<0.0001	not estimable ^e
MRSA history ^a	<0.0001	6.8 (1.5, 30.5)	<0.0001	not estimable ^e
Hospitalizations ^b	0.0162	1.4 (1.1, 1.8)	0.0002	2.2 (1.5, 3.3)
Hemoglobin	0.16	NS	0.63	NS
Serum albumin concentration ^c	0.017	0.85 (0.74, 0.97)	0.026	0.83 (0.70, 0.98)
Kt/V	0.77	NS	0.39	NS
Serum ferritin ^d	0.74	NS	0.033	0.71 (0.52, 0.97)

^a No prior infection/colonization:previous infection/colonization.

^b Each hospital visit increased the odds that a patient will be colonized.

^c Higher serum albumin levels less likely to be colonized; rate expressed as risk per 0.1 mg/dl change.

^d Higher level indicates reduced risk of colonization; risk expressed per 100 mg/dl change.

^e Not estimable in the case of no events; odds ratio estimates cannot be obtained.

nization, 6 (3.1%) tested positive for VRE perirectal colonization and 0% of tunneled catheter exit sites tested positive for MRSA colonization (n = 39).

Table 2 contains the results of the univariate analysis in all 197 subjects. Prior MRSA infection or colonization was the most significant predictor of current MRSA colonization. Current nasal MRSA colonization was detected in 29% of those with a history of MRSA compared to 2.9% without a history of prior MRSA ($p < 0.0001$). There also appeared to be a trend toward an increased risk of current MRSA colonization in subjects with a history of prior VRE ($p = 0.087$). Number of hospitalizations in the preceding 12 months was also predictive of current MRSA colonization: subjects with current positive MRSA nasal cultures had been hospitalized an average of 3.2 times versus 1.7 times in the group without current MRSA colonization ($p = 0.0162$). Lower serum albumin concentrations were present in the current MRSA colonization group (3.6 vs. 3.9 mg/dl; $p = 0.017$). Subjects with diabetes as the cause of ESRD tended to have lower rates of current MRSA nasal colonization, 2.3 compared to 8.4%, respectively ($p = 0.068$). A trend toward lower rates of current MRSA nasal colonization was also observed in African Americans with 3.5% compared to 10.9% in non-African Americans ($p = 0.08$). There was no difference in the risk for MRSA colonization between men and women or between patients in the three different dialysis centers. Rates of colonization did not differ by access type, hemoglobin concentration, Kt/V, or serum ferritin concentration.

Outpatient use of vancomycin over the prior year was assessed and 165 of study subjects received no doses. Of these 165 with no vancomycin exposure, 10 had MRSA colonization (6.1% of the sample) compared to 3.1% among the 32 participants who received vancomycin ($p = 0.50$). Of the 161 subjects with no vancomycin exposure (6 were missing VRE data), 4 had VRE colonization (2.5% of the sample), while 2 (6.7%) of the other 30 subjects had MRSA colonization ($p = 0.24$). We also considered the number of vancomycin doses to be continuous and fit models using the actual number of doses of vancomycin and the cumulative vancomycin dose. The results were also not significant (data not shown).

A logistic regression analysis was performed for all variables having a p value < 0.1 in univariate analysis. Current MRSA colonization was placed in the model first, followed singularly by the addition of number of hospitalizations, serum albumin concentration, race, cause of ESRD and prior MRSA infection/colonization. Only prior MRSA infection/colonization remained sig-

nificant in the model. Participants without prior MRSA were unlikely to be positive for MRSA colonization when compared to subjects with a previous history of MRSA [odds ratio of 0.02 (95% CI 0.0004–0.1); $p < 0.0001$].

The results of univariate analysis for current VRE colonization are summarized in table 2. A positive perirectal swab for VRE was associated with prior history of both VRE and MRSA infection/colonization. In subjects with a history of VRE, 27.8% had positive perirectal cultures for VRE versus 0.5% without a history of prior VRE infection/colonization ($p < 0.0001$). In subjects with a history of prior MRSA infection/colonization, 25% had current VRE colonization while only 0.5% without known MRSA tested positive for VRE ($p < 0.0001$). Lower serum albumin concentration was also associated with VRE colonization, 3.5 versus 3.9 mg/dl ($p = 0.026$). As with MRSA, hospitalizations predicted VRE colonization as subjects who were positive for VRE had an average of 5.8 hospitalizations in the last year compared to 1.6 hospitalization in those screening negative for VRE ($p = 0.0002$).

Serum ferritin concentration was a significant predictor of VRE colonization in the univariate analysis. The average ferritin in the 6 patients who tested positive for VRE was 342 mg/dl (range 44–639) compared to 801 mg/dl (range 8–4,015) in the 185 patients who screened negative. Hemoglobin concentrations were similar between both groups (12.0 and 12.3 mg/dl; $p = 0.63$). Neither race nor gender was predictive of VRE colonization. No correlation between dialysis adequacy and VRE colonization was detected.

VRE colonization was fit in a logistic regression model with the five variables found to be significant in the univariate analysis. Only prior VRE infection/colonization and number of recent hospitalizations remained in the final reduced model, containing only significant measures. Subjects without a prior history of VRE infection/colonization had an odds ratio of 0.04 (95% CI 0.004–0.46) compared to those with a prior history of VRE ($p = 0.01$). Each additional hospital visit increased the odds of current VRE colonization by 82% (OR 1.82, 95% CI 1.15–2.89; $p = 0.01$).

The NCBH infection control database was searched for each study participant, recording prior MRSA or VRE screening and clinical culture results. Many subjects did not have prior culture results in this database because they had not been hospitalized in this academic medical center or failed to meet the screening criteria for MRSA or VRE during their admissions. We were able to identify 75 subjects with prior MRSA data (21 positive) and

71 with prior VRE data (18 positive). All of these subjects were tested for nasal MRSA in the current study, but current VRE screening was refused by 6 of 75 subjects with prior MRSA data and 2 of 71 subjects with prior VRE data. We repeated the statistical analysis in these participants to test the usefulness of such a database in predicting whether an individual will subsequently test positive for VRE or MRSA in the outpatient dialysis setting.

Twelve percent of the 75 subjects with prior MRSA data (n = 9) tested positive for current nasal MRSA colonization (table 3). Univariate analysis of the relationship between MRSA colonization and predictive variables is summarized in table 4. As in the overall cohort, prior MRSA positivity was predictive of current colonization (p = 0.012). Serum albumin concentration also remained a significant predictor (p = 0.042), and race was a predictor in this subgroup with 7.0% of African Americans and 27.8% of non-African Americans testing positive for MRSA (p = 0.032). If the model allowed for inclusion of prior MRSA positivity, only that variable remained in the model. When logistic regression analysis was performed on predictive variables for MRSA (excluding prior MRSA positivity), only race remained in the stepwise model; no other variables were significant. African American patients had an OR of 0.25 (95% CI 0.05–0.85).

Five of the 71 subjects (7.25%) with prior VRE screening/infection data tested positive for VRE on perirectal

culture (table 5). Univariate analysis of this group is shown in table 4. The results for previous VRE and MRSA positivity were similar: 28% (5/18) with prior colonization/infection had current VRE colonization versus 0% (0/51) without prior colonization/infection. For the 5 patients with current VRE colonization, all were in the hospital at some point (mean of 6.0 ± 3.1 hospitalizations vs. 2.3 ± 1.7 hospitalizations in the 64 non-VRE patients) and mean serum albumin concentration was 3.4 ± 0.2 mg/dl in VRE-colonized subjects versus 3.9 ± 0.4 mg/dl in non-VRE patients. When logistic regression analysis

Table 3. Current colonization in 75 subjects with prior MRSA data

History of MRSA	Current MRSA colonization		Current VRE colonization ^a	
	yes	no	yes	no
Yes	6 (28.6)	15 (71.4)	5 (27.8)	13 (72.2)
No	3 (5.6)	51 (94.4)	0	51 (100)

Values represent frequency with the percentage in parentheses.

^a Current VRE results available in 69 subjects (6 declined perirectal swab).

Table 4. Univariate analysis – limited to subjects previously screened for colonization with resistant organisms

Variable	MRSA p value	Odds ratio (95% CI)	VRE p value	Odds ratio (95% CI)
Gender (male:female)	0.74	NS	1.000	NS
Race (black:nonblack)	0.032	0.20 (0.04, 0.83)	0.30	NS
Cause ESRD (diabetes:other)	0.14	NS	1.000	NS
Access (AVF vs. graft vs. catheter)	0.90	NS	0.47	NS
Dialysis unit	0.33	NS	0.25	NS
VRE history ^a	0.36	NS	<0.0001	not estimable ^d
MRSA history ^a	0.012	0.15 (0.03, 0.66)	<0.0001	not estimable ^d
Hospitalizations ^b	0.90	NS	0.005	2.2 (1.3, 3.8)
Hemoglobin	0.12	NS	0.81	NS
Serum albumin concentration ^c	0.042	0.83 (0.69, 0.99)	0.023	0.79 (0.64, 0.97)
Kt/V	0.65	NS	0.57	NS
Serum ferritin	0.40	NS	0.19	NS

^a No prior infection/colonization:previous infection/colonization.

^b Each hospital visit increased the odds that a patient will be colonized.

^c Higher serum albumin levels less likely to be colonized; rate expressed as risk per 0.1 mg/dl change.

^d Not estimable in the case of no events; odds ratio estimates cannot be obtained.

was performed using predictive variables for VRE (excluding prior VRE positivity), only documented hospital visits remained significant in the full model, $p = 0.005$ with OR 2.17 (each additional hospital visit increased the likelihood of observing VRE colonization by 117%; 95% CI 1.26–3.72).

Discussion

Chronic dialysis patients are at increased risk for colonization and infection with resistant bacteria [1]. Little contemporary data on nasal MRSA colonization exist in chronic hemodialysis patients. The rates of nasal MRSA and intestinal VRE colonization was assessed in three inner city dialysis facilities. The prevalence of resistant bacterial colonization, although higher than in healthy cohorts, was relatively low. The 5.6% frequency of MRSA nasal colonization was encouraging, given that 12.1% of the cohort previously had MRSA detected during hospitalization. Previous studies of VRE colonization rates in the US chronic hemodialysis population detected rates of 8–10% [4, 6]. A recent South American study reported a 14.4% rate [7]. One reason for the lower prevalence of VRE colonization in our report may relate to the single outpatient VRE screening that was performed, as opposed to serial screenings as in other analyses. It is possible that additional dialysis patients would have been found to have VRE colonization with repeat cultures. However, in a subset of 51 subjects with prior negative VRE screening during hospitalization, none subsequently tested positive for VRE in this outpatient study.

While development of resistant bacterial colonization has been linked to antibiotic exposure, we explored additional risk factors for MRSA and VRE colonization in hemodialysis patients. Kt/V was not significantly associated with either MRSA or VRE colonization, nor was the type of dialysis access. In our cohort, the use of vancomycin in our dialysis centers did not predict VRE colonization. We were unable, however, to assess the impact of other antibiotics not administered in the outpatient dialysis units.

S. aureus colonization at dialysis catheter exit sites has been associated with increased risk for future bloodstream infection with that organism [14]. Topical antibiotics reduce this risk, although they may promote bacterial resistance with chronic exposure [15]. It is noteworthy that none of the 39 catheter exit sites were found to be colonized with MRSA. This suggests that routine catheter care techniques (which include use of antibiotic oint-

Table 5. Current colonization in 71 subjects with prior VRE data

History of VRE	Current MRSA colonization		Current VRE colonization ^a	
	yes	no	yes	no
Yes	3 (16.7)	15 (83.3)	5 (27.8)	13 (72.2)
No	4 (7.6)	49 (92.5)	0	51 (100)

Values represent frequency with the percentage in parentheses.

^a Current VRE results available in 69 subjects (2 declined perirectal swab).

ment at the exit site) are effective at preventing MRSA colonization at tunneled hemodialysis catheter exit sites.

Low serum albumin concentrations were associated with both nasal MRSA colonization and perirectal VRE colonization. Poorer nutritional status or protein malnutrition may increase susceptibility to colonization with these pathogens. Alternatively, low serum albumin concentration may reflect inflammation, immunosuppressed status, or additional comorbidities which may predispose to hospitalization or antibiotic treatment. The lower ferritin concentrations observed in the subjects colonized with VRE suggest that inflammation alone may not predispose to VRE colonization.

Although a previous report suggested that diabetes increases the risk of MRSA nasal carriage, we detected a trend toward lower rates of MRSA in subjects with diabetic ESRD [16]. A trend was also observed toward lower rates of MRSA colonization in African American patients. Ethnic differences were significant when analyzing the group with prior MRSA data from the Academic Medical Center's infection control database. African Americans had lower odds of MRSA colonization, even when controlling for other significant risk factors from the univariate analysis. Nasal *S. aureus* carriage becomes persistent in only a subset of carriers. Inherited factors may influence an individual's risk for persistent nasal MRSA colonization.

The number of recent hospitalizations (in the previous year) was a significant predictor of both MRSA and VRE colonization. In a logistic regression model, hospitalizations predicted VRE perirectal colonization, but not MRSA nasal colonization. It is clear from this, and other studies, that hospitalization places patients at increased risk for acquisition of resistant microorganism coloniza-

tion. This may occur through horizontal transmission in a population of patients treated with broad-spectrum antibiotics.

The strongest predictor of current MRSA or VRE colonization was past history of MRSA or VRE colonization or infection. The hospital infection control database revealed that prior negative screenings for MRSA or VRE were powerful predictors for the lack of colonization in the outpatient dialysis setting. Screening dialysis patients when they are hospitalized may prove to be an adequate method for monitoring chronic hemodialysis outpatients for MRSA and VRE colonization.

A weakness of this report is that participants were screened once for bacterial colonization, as serial cultures may have revealed higher rates of both VRE and MRSA. One report demonstrated a sensitivity of 58% when perirectal swabs were used to detect asymptomatic VRE colonization [17]. In that study, higher densities of VRE in the stool were associated with better detection of VRE using the perirectal swab technique. We believe that the swab technique is likely to detect patients who are densely colonized and most likely to be vectors for the colonization of others. Additionally, both the CDC and the Society for Healthcare Epidemiology of America recommend this method for screening hospitalized patients for VRE colonization [18, 19].

In summary, the rate of MRSA nasal colonization that was detected in a contemporary analysis of chronic hemodialysis outpatients was lower than previously reported in the US dialysis population. This improvement was observed despite an overall increase in the number of MRSA infections in dialysis patients during the preceding decade. Rates of VRE colonization were also lower than in recent reports. These colonization rates suggest that current infection control practices that are employed in outpatient hemodialysis units are effective at limiting MRSA and VRE colonization in the high-risk ESRD population. Previous colonization with VRE or MRSA and the number of recent hospitalizations were significant predictors of colonization. Finally, an inpatient infection control database was an effective tool for predicting outpatient colonization with MRSA and VRE. Further research should be directed at establishing the natural history of MRSA and VRE colonization in outpatients on hemodialysis. Particular attention should be paid to the factors leading to persistent and/or recurrent MRSA or VRE colonization.

Acknowledgment

This research was supported in part by a grant from the National Kidney Foundation of North Carolina.

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