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Prevalence of Nontuberculous Mycobacteria in Water Supplies of Hemodialysis Centers

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Infection of hemodialysis patients with nontuberculous mycobacteria (NTM) has been associated with water used in reprocessing hemodialyzers. This study was conducted to determine the prevalence of NTM and other bacteria in water samples collected over a 13-week period from 115 randomly selected dialysis centers in the United States. Total viable counts were determined by membrane filter assays; increased recovery of NTM was obtained by dosing a portion of each water sample with 1% formaldehyde (HCHO) before filtering. NTM were widely distributed and occurred with a high frequency in water supplies in dialysis centers. NTM were detected in water from 95 centers (83%), and 50% of all samples examined contained NTM. The results of this study support recommendations to use 4% HCHO or a chemical germicidal equivalent for disinfecting dialyzers that are to be reused.

Over the past 20 years, the art and science of maintenance hemodialysis have been perfected to the extent that currently about 100,000 patients are dialyzed in approximately 1,500 centers in the United States. Concomitant with the growth in hemodialysis has been the increasing practice of reprocessing disposable hemodialyzers (artificial kidneys) for multiple use on the same patient. Since early 1980, the number of centers practicing reuse of dialyzers has increased remarkably (1, 6); 63% of the centers licensed by the Health Care Financing Administration now reuse disposable hemodialyzers. This represents approximately 68% of the patient population.

Most centers with dialyzer reuse programs have used aqueous formaldehyde (HCHO) as the chemical germicide to disinfect dialyzers between patient treatments (5). For many years it was assumed that the primary microbiologic challenge associated with reprocessing dialyzers consisted of gram-negative bacteria (GNB) in the water used to rinse dialyzers and prepare disinfectant storage solutions. In 1982 (25) Petersen suggested that nontuberculous mycobacteria (NTM) may constitute part of the normal microbial flora of water used in dialysis centers and pose potential hazards, based on an earlier investigation of peritonitis cases in which rapidly growing NTM were isolated from patient peritoneal fluids and from water in the peritoneal dialysis machines (4). Because these NTM isolates were extraordinarily resistant to HCHO and other chemical germicides (4, 11), we questioned the efficacy of a 2% concentration of aqueous HCHO as a chemical germicide in reprocessing procedures and described its use as a marginal disinfection procedure that might theoretically fail.

Subsequently, we investigated a major outbreak of infections with rapidly growing NTM involving 27 patients in a center that reprocessed hemodialyzers (7). The probable source of NTM in that outbreak was the water used to rinse dialyzers and prepare 2% HCHO storage solutions; NTM also were isolated from the blood compartments of reprocessed dialyzers stored between patient reuse. Additional studies (5–7) showed that the most resistant NTM in our collection of strains from these outbreak investigations were inactivated by 4% aqueous HCHO after 24 h of exposure at 22 to 25°C. We therefore recommended that centers using HCHO as the chemical germicide for reprocessing hemodialyzers use at least 4% aqueous HCHO (5, 15, 24). We also recommended (15) that it should be assumed that NTM are present in water used for reprocessing procedures and that NTM, rather than the broad group of GNB, be considered the probable worst-case microbiologic challenge.

The purpose of this study was to provide a broad assessment of the frequency with which NTM, particularly the rapidly growing NTM, occur in water used in dialysis centers for preparing dialysis solutions and for dialyzer reprocessing procedures.

MATERIALS AND METHODS

Dialysis centers. In 1984, a random sample of 10% of 1,171 centers in the National Listing of Providers Furnishing Kidney Dialysis and Transplant Services (1983, Health Care Financing Administration, Baltimore, Md.) was invited to participate in the study. These included 46 centers from the Atlantic Region (Connecticut, Florida, Georgia, Massachusetts, Maryland, Maine, North Carolina, New Jersey, New York, Pennsylvania, Rhode Island, South Carolina, Virginia, and West Virginia); 44 centers from the Central Region (Alabama, Arkansas, Indiana, Illinois, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Missouri, Ohio, South Dakota, Texas, and Wisconsin); 8 centers from the Mountain Region (Arizona, Colorado, New Mexico, Utah, and Wyoming); and 17 centers from the Pacific Region (California). Participating centers were asked to complete a questionnaire that included information on water treatment systems, disinfection procedures, and microbiologic monitoring.

Sample collection. For collection of water samples, a package containing sterile 500-ml sample bottles (one bottle designated for collecting a sample of incoming city water contained 0.1 ml of 1% sodium thiosulfate to neutralize free chlorine), a DPD kit (Hach Co., Loveland, Colo.) for mea-

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TABLE 1. Comparative levels of microorganisms in water supplies in dialysis centers by geographic region

Geographic region	Log_{10} total no. of viable microorganisms/100 ml in sample[a]:		
	A	B	C
Atlantic	3.24 ± 1.38 (46)	4.41 ± 1.08 (46)	4.52 ± 1.46 (22)
Central	3.55 ± 1.25 (44)	4.67 ± 1.02 (46)	4.24 ± 1.63 (21)
Mountain	3.13 ± 0.77 (8)	4.32 ± 1.16 (8)	4.88 ± 0.54 (4)
Pacific	3.55 ± 1.00 (17)	4.08 ± 0.98 (17)	4.15 ± 1.47 (17)
Total	3.40 ± 1.24 (115)	4.45 ± 1.05 (115)	4.35 ± 1.47 (64)

[a] Values are expressed as means ± standard deviations. Values in parentheses indicate number of centers sampled.

TABLE 2. Comparative levels of NTM in water supplies in dialysis centers by geographic region

Geographic region (no. of centers sampled)	Log_{10} no. of NTM/100 ml in sample[a]:		P
	A	B	
Atlantic (46)	1.77 ± 1.60	1.17 ± 1.74	Not significant
Central (44)	1.84 ± 1.79	0.81 ± 1.55	<0.01
Mountain (8)	1.68 ± 1.20	—[b]	0.001
Pacific (17)	2.29 ± 1.40	1.70 ± 1.70	Not significant
Total (115)	1.87 ± 1.62	1.03 ± 1.63	0.001

[a] Values are expressed as means ± standard deviations.
[b] —, No NTM detected.

suring free and combined chlorine, and detailed instructions for collection and shipment of water samples to the Centers for Disease Control was mailed to a designated staff member at each center. Sites of sample collection included municipal water supplied to the dialysis center, water produced by the center's water treatment system and used to prepare dialysis fluids, and water used to rinse dialyzers and prepare chemical germicidal solutions for disinfecting dialyzers between reuses on the same patient. Samples were shipped by air express on wet ice and processed at the Centers for Disease Control within 24 h of collection at the dialysis center.

Microbiologic assay procedures and datum analysis. Water samples were received from 115 centers, 55% of which reused dialyzers. On receipt, each sample was checked for temperature and pH, and endotoxin levels were determined by the *Limulus* amebocyte lysate assay procedure (10). The rationale and methodology for the microbiologic membrane filter assay procedure used in this study are detailed elsewhere (9). Briefly, each water sample was divided into two portions. The first portion was assayed to determine the total number of viable organisms present. Filters were placed on Standard Methods Agar (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 30°C. Total and differential colony counts based on colony morphology and pigmentation were done after incubation for 3 and 6 days. The second portion was exposed to HCHO (final concentration, 1%) for 5 min before filtering to reduce background levels of GNB and increase selectivity for NTM. Filters were placed on Middlebrook-Cohn 7H10 agar (BBL) and incubated at 30°C. Total and differential colony counts were done after incubation for 6 and 14 days.

Gram and acid-fast (Ziehl-Neelsen) stains were done on subcultures of each colony type on Standard Methods Agar and 7H10 agar plates. For analysis and presentation of data, the higher count obtained on Standard Methods Agar or 7H10 agar was selected as the number of total viable microorganisms or NTM (acid-fast counts) for each sample assayed. Student's t test was used to determine statistical significance. NTM isolates were biochemically characterized by conventional procedures (28).

RESULTS

A comparison of total viable counts of microorganisms in sample A (municipal water supply entering the center's water treatment system) and sample B (water from the center's water treatment system used to prepare patient dialysis fluids) showed that overall mean levels were significantly higher ($P < 0.001$) in sample B than in sample A (Table 1). Higher counts of microorganisms in sample B were obtained in 92 of 115 (80%) centers. Overall mean levels also were significantly higher ($P < 0.001$) in sample C (water used to rinse dialyzers and prepare disinfectant solutions for dialyzer storage between patient reuse) than in sample A; higher viable counts were found in 48 of 64 (75%) centers. There were no statistically significant differences in mean viable counts between sample B and sample C or in mean viable counts amoung the four geographic subregions for each sample type. Results of assays for bacterial endotoxin, which are reported elsewhere (8), showed that 14 of 64 centers (22%) with dialyzer reuse programs had endotoxin levels in water used to rinse dialyzers and prepare disinfectant solutions for dialyzer storage (sample C) in excess of the 1 ng/ml recommended in Association for the Advancement of Medical Instrumentation and Centers for Disease Control Guidelines (2, 6).

In 95 of 115 centers (83%), NTM were detected in at least one of the water samples collected from each center. In centers with dialyzer reuse procedures, NTM were detected in at least one of the water samples collected from 62 of 64 (97%) centers. Overall mean levels of NTM were significantly higher ($P = 0.001$) in sample A ($n = 115$) than in sample B (Table 2). However, no statistically significant differences in mean NTM levels in sample A or sample B were observed among the four geographic subregions. In centers with dialyzer reuse procedures (Table 3), although mean levels of NTM were slightly higher in sample A than in sample C, the differences were not statistically significant. No significant differences in mean levels of NTM in water from these centers were observed among the four geographic subregions.

Overall, approximately 550 NTM isolates were obtained. Biochemical tests of selected isolates showed representatives in each of the following groups: *Mycobacterium fortuitum*, *M. chelonae*, *M. chelonae*-like organisms, *M. scrofulaceum*, *M. gordonae*, and the *M. avium* and *M. terrae*

TABLE 3. Comparative levels of NTM in water supplies in dialysis centers with dialyzer reuse programs

Geographic region (no. of centers sampled)	Log_{10} no. of NTM/100 ml in sample[a]:	
	A	C
Atlantic (22)	1.82 ± 1.59	1.47 ± 1.72
Central (21)	1.98 ± 1.99	1.70 ± 1.77
Mountain (8)	1.16 ± 0.90	1.39 ± 2.78
Pacific (17)	2.29 ± 1.40	1.33 ± 1.77
Total (64)	1.96 ± 1.65	1.50 ± 1.78

[a] See Table 2, footnote *a*.

complexes of NTM organisms. Rapidly growing NTM accounted for approximately 65% of the total NTM isolates.

To assess possible correlations between observed levels of NTM in water supplies in dialysis centers and the types of water treatment systems used, we examined data from centers that responded to a survey questionnaire (n = 91). The data (not presented) showed no apparent correlations between the levels of NTM in treated water or water used to rinse dialyzers and prepare disinfectant storage solutions and the type or combination of types of water treatment (e.g., softening, reverse osmosis [RO], deionization, carbon filtration, ultrafiltration) or storage components used in the centers.

DISCUSSION

There are a high prevalence and wide geographic distribution of NTM in water supplies in dialysis centers. These organisms are now clearly recognized as significant pathogens in a variety of clinical disorders (29). In particular, *M. fortuitum*, *M. chelonae*, and *M. chelonae*-like organisms have been associated with episodes of septicemia and peritonitis in hemodialysis and peritoneal dialysis patients (3, 4, 7, 22, 25, 26).

Historically, microbiologic assays done on water used in dialysis procedures were designed to assess the effect of water treatment, storage, and disinfection strategies on levels of GNB to reduce the risk of bacteremia and pyrogenic reactions in dialysis patients (2, 6, 18). Results from this study show significantly higher levels of bacteria and bacterial endotoxin (8) in effluent from water treatment systems in dialysis centers than in incoming municipal water supplies. These results support our assumption that GNB are the dominant population in most water environments. They further support our observation that the potential health hazards of microbial contaminants in water associated with dialysis procedures are correlated with a variety of factors: the presence of high levels of GNB and/or bacterial endotoxin in water used to prepare dialysis fluids, rinse dialyzers, and prepare disinfectant storage solutions; the efficacy of various components of water treatment systems in reducing or amplifying levels of GNB contaminants; and the adequacy of disinfection procedures in preventing colonization of these components (8, 14–19, 21, 23).

NTM have now been isolated from a variety of natural aqueous environments and are known to grow well in treated water and dialysis fluids, colonizing the interior surfaces of distribution pipes, dialysis machines, and components of water treatment systems such as water softener and deionizer resins and RO membranes (12, 20, 27). Indeed, the data from this study show that levels of NTM are higher in treated water than in community water supplied to the dialysis centers. Although GNB are able to multiply more rapidly in water and reach higher concentrations than NTM (11), they are relatively more susceptible to disinfectants commonly used in dialysis centers. NTM, particularly strains in the *M. fortuitum* complex, have demonstrated extreme resistance to both HCHO and chlorine disinfectants and thus may become the dominant population in water treated with such chemicals (4–7, 11, 13, 20).

In this study the use of a chemical dosing technique and a longer incubation period for 7H10 agar plates resulted in increased overall recovery of NTM (9); an incubation period of 14 rather than 21 or more days also tended to favor more frequent isolation of rapidly growing than of slowly growing NTM. With regard to the detection of NTM in dialysis center environments, however, the mechanics of microbiologic assay procedures most commonly used by private or hospital laboratories are such (e.g., incubation time of 24 to 48 h, nonselectivity of media for NTM, minimal volume of fluid typically applied by standardized loop or swab application) that NTM would not usually be demonstrated. Moreover, no data are yet available to suggest what level of NTM would constitute unacceptable contamination. Consequently, any disinfection strategy should be conservative and assume a worst-case condition.

The results of the study reported here clearly show that in designing disinfection strategies, one must consider NTM as part of the normal microbiologic flora of water used in dialysis centers. This fact is important in the practice of reusing disposable dialyzers because water is used for rinsing the dialyzers during the reprocessing procedures and as a diluent for preparing the chemical germicides subsequently placed in a dialyzer until it is next used on the same patient. The high prevalence and wide geographic distribution of NTM in water supplies in dialysis centers lend further support to our recommendations of several years ago (15, 24) that it is prudent for dialysis centers to assume the presence of NTM in water supplies as a worst-case condition and therefore to use a minimum of 4% HCHO at 20°C or a chemical germicide of equivalent efficacy for disinfecting dialyzers that are to be reused.

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