APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1988, p. 1756–1760
0099-2240/88/071756-05$02.00/0

Vol. 54, No. 7

Efficacy of Chemical Dosing Methods for Isolating Nontuberculous Mycobacteria from Water Supplies of Dialysis Centers

LORETTA A. CARSON,* LUCY B. CUSICK, LEE A. BLAND, AND MARTIN S. FAVERO

Nosocomial Infections Laboratory Branch, Hospital Infections Program, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Received 23 November 1987/Accepted 29 April 1988

Investigations of nontuberculous mycobacterium (NTM) infections associated with various environmental sources have been hampered by the lack of adequate techniques for selective isolation of these organisms from environmental fluids. This study compared chemical dosing techniques for recovery of NTM from water samples collected from 115 randomly selected dialysis centers. Cell suspensions of NTM group II and IV isolates and gram-negative bacteria were exposed to solutions containing sodium hypochlorite (0.2 μg/ml of free available chlorine), formaldehyde (1, 0.75, or 0.5%), oxalic acid (1.25%), cetylpyridinium chloride (25 μg/ml), or cetyltrimethylammonium bromide (100 μg/ml). Results of standard membrane filtration assays with laboratory test strains and water samples from dialysis centers showed that 5 min of exposure to 1% formaldehyde effectively reduced gram-negative bacterial populations and allowed increased recovery of NTM in environmental fluids containing mixed microbial populations.

Awareness of clinical infections with nontuberculous mycobacteria (NTM) has increased markedly, and reports of infection outbreaks involving environmental fluids as probable sources of these organisms have become more common (3, 4, 6–8, 11, 20, 22). Infections occurring in patients in hemodialysis and peritoneal dialysis centers (3, 4, 6) typically have involved the *Mycobacterium fortuitum* complex of organisms, also known as rapid growers or Runyon group IV organisms (31).

Environmental investigations of NTM have been hampered largely by the lack of adequate techniques for detecting these organisms in fluids that also contain high levels of naturally occurring gram-negative water bacteria (GNB). However, in one outbreak of infections with *M. chelonae* and *M. chelonae*-like organisms (MCLO) in hemodialysis patients (6), these organisms, as well as *M. fortuitum* and group II NTM (*M. gordonae* and *M. scrofulaceum*), were found as essentially pure populations of several hundred microorganisms per milliliter in water used to rinse dialyzers and prepare disinfectant solution for storing dialyzers before reuse on patients. Levels of chlorine or chloramines in the municipal water supply were apparently sufficient to inhibit growth of GNB but insufficient to prevent the inherently germicide-resistant NTM from increasing. An unidentified rapid grower (probable MCLO), *M. gordonae*, *M. scrofulaceum*, and strains of slow-growing, pink-pigmented gram-negative bacteria (SGP) also were found in formaldehyde (HCHO)-containing disinfectant solutions (1 to 3% aqueous HCHO) from blood compartments of dialyzers processed for reuse. These findings suggested the possibility that chlorine or HCHO disinfectants could effectively be used in microbiologic assay procedures to dose environmental fluids, thereby reducing background levels of GNB and increasing the isolation rate of NTM present in the sample.

We undertook a national study in a random sample of 115 dialysis centers (5) in 1984 to determine the prevalence of rapidly growing NTM in potable and treated water supplies used in dialysis centers. This paper reports the results of initial experiments for that study which were designed to assess the comparative efficacies of chlorine, HCHO, and other chemical agents suitable for use with the membrane filtration assay procedure (1) to effect a substantial reduction in GNB populations and allow optimal recovery and quantitation of NTM in water supplies from dialysis centers. Data are also presented showing the efficacy of the dosing technique selected (exposure to 1% aqueous HCHO for 5 min) for reducing background levels of naturally occurring GNB and increasing recovery of NTM and SGP in dosed and nondosed samples of water from dialysis centers.

MATERIALS AND METHODS

Preparation of test organisms. Rapidly growing strains in the *M. fortuitum* complex included *M. fortuitum* (5 strains), *M. chelonae* (9 strains), and MCLO (10 strains). Slowly growing group II isolates of *M. gordonae* (three strains) and *M. scrofulaceum* (five strains) from a dialysis center, which had shown high resistance to HCHO and chlorine disinfectants, also were tested. GNB commonly found in association with water treatment and pipe distribution systems in hemodialysis centers included *Acinetobacter anitratus*, *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Achromobacter xylosoxidans*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, *P. cepacia*, *P. diminuta*, *P. maltophilia*, *P. palleronii*, *P. paucimobilis*, *P. pickettii*, *P. putida*, *P. stutzeri*, and *P. thomasii*.

NTM were cultured on Middlebrook and Cohn 7H10 agar with OADC supplement (BBL Microbiology Systems, Cockeysville, Md.) at 30°C for 72 h (*M. fortuitum*, *M. chelonae*, and MCLO strains) or 14 days (*M. gordonae* and *M. scrofulaceum* strains), and GNB were cultured on standard methods agar (SMA; BBL) at 30°C for 24 h. Cells were harvested, washed, and suspended to approximately 10^7 CFU/ml in autoclaved water from a reverse osmosis system. After 7 days at 25°C, cell suspensions were diluted 1:100 in sterile reverse osmosis water and kept at 25°C for 4 to 5 weeks, and these water-adapted cell suspensions were used as sources of inocula for chemical dosing tests.

Chemical dosing tests. The chemicals tested and the concentrations used were selected on the basis of reports in the literature and our own laboratory and field experience of

* Corresponding author.

TABLE 1. Survival of NTM and GNB exposed to various chemical disinfectants

Organism(s) (no. of solutions tested)	$\log_{10}$ no. of organisms exposed	Mean % survival after 10-min exposure to:				
		Oxalic acid (1.25%)	HCHO (1.0%)	Chlorine (0.2 μg/ml)	CPC (25 μg/ml)	CTAB[a] (100 μg/ml)
Group IV NTM						
M. fortuitum (5)	1.91–2.66	53.4	85.2	92.7	94.8	79.7
M. chelonae (9)	1.16–2.52	64.8	99.2	93.7	95.4	85.2
MCLO (10)	1.70–2.45	5.6	78.0	84.3	81.1	76.5
M. gordonae (3)	1.66–2.36	54.1	60.8	84.0	96.3	70.0
M. scrofulaceum (5)	2.32–3.14	73.7	98.7	84.2	97.6	81.8
Mixture of 15 strains of GNB[b]	2.90	8.8	96.9	58.6	99.5	99.2

[a] CTAB, Cetyltrimethylammonium bromide.
[b] See Materials and Methods for a list of the strains.

their overall effectiveness in reducing background levels of GNB and allowing recovery of NTM. Given the logistics of processing 20 to 30 water samples per day, an exposure time of 10 min was selected as a probable near-maximum allowable time for chemical dosing of each sample.

Stock cell suspensions of test organisms were diluted in sterile reverse osmosis water to 10^2 to 10^3 CFU/ml, and appropriate amounts of undiluted chemicals or stock solutions prepared in reverse osmosis water were added at ambient room temperature (ca. 22°C) to attain the following final concentrations of chemicals: (i) oxalic acid (Sigma Chemical Co., St. Louis, Mo.), 1.25%; (ii) HCHO, (Mallinckrodt, Inc., St. Louis, Mo.), 0.5, 0.75, and 1.0%; (iii) sodium hypochlorite (James August Co., Mars, Pa.), 0.2 μg/ml of free Cl_2 as determined by the DPD test (Hach Co., Loveland, Colo.); (iv) cetylpyridinium chloride (CPC; Sigma), 25 μg/ml; (v) cetyltrimethylammonium bromide (Eastman Kodak Co., Rochester, N.Y.), 100 μg/ml.

Samples from test and control (no disinfectant) solutions were assayed by the membrane filter procedure (1), and sample dilutions were prepared in American Public Health Association phosphate-buffered distilled water (BDW; pH 7.2). Filters from test disinfectant solutions were rinsed with approximately 400 ml of BDW to remove residual disinfectant before plating on SMA (GNB strains) or 7H10-OADC (NTM strains); filters from control solutions also were rinsed with 400 ml of BDW. Plates were incubated at 30°C, and colony counts were recorded after 72 h and 7 and 14 days.

A technique for direct acid-fast staining of colonies on membrane filters (15, 16) was evaluated by using pure and mixed cultures of NTM and GNB incubated on 7H10 agar. Gradations in uptake of stain among NTM strains and variations in the degree of colony discoloration among GNB made differentiation of acid-fast character difficult, particularly on filters containing comparatively low numbers of NTM and high numbers of GNB. This technique for quantitating NTM was not pursued further.

Field study. Water samples were collected from the following sites from 115 hemodialysis centers over a 13-week period: (i) sample A, municipal water supply to the dialysis center; (ii) sample B, water produced by the center water treatment system that was used to prepare dialysis fluids; (iii) sample C, water used to rinse dialyzers and prepare the chemical germicides for disinfecting dialyzers before reuse on the same patient.

For microbiologic assay, each water sample was divided into two portions. Because of the total number of samples to be processed each day and the further time constraints involved in processing undosed and chemically dosed portions for each sample, it was necessary to restrict the number of incubation media used. Previous investigations conducted in our laboratory (6, 10, 18) had shown SMA to be a suitable recovery medium, not only for GNB and SGP but also for rapid-growing NTM from water supplies in dialysis centers. Although GNB also appeared to grow well on 7H10-OADC, quantitative data comparing recovery of GNB on SMA and 7H10-OADC were not collected. For the untreated sample portion, therefore, sample dilutions in BDW (10^2 to 10^{-3}) were filtered and rinsed with 400 ml of BDW, and filters were placed on SMA and incubated at 30°C. Total and differential colony counts based on colony morphology and pigmentation were done after incubation for 72 h or 6 days; Gram and acid-fast (Ziehl-Neelsen) stains were done on subcultures of each colony type to determine overall levels of GNB and relative levels of NTM and SGP. The second sample portion was treated by exposure to 1% aqueous HCHO for 5 min to reduce background levels of GNB so that presumed lower levels of NTM and SGP could more readily be detected. After exposure, sample dilutions in BDW (10^2 to 10^{-2}) were filtered and rinsed with 400 ml of BDW and plated on 7H10-OADC agar. Again, although studies in our laboratory had shown that rapidly growing NTM from untreated water supplies could be recovered in comparable numbers on SMA or 7H10-OADC, the latter was used as the plating medium for dosed samples because it was felt that the more enriched medium might enhance recovery of NTM and SGP stressed by exposure to a disinfectant. Differential colony counts and stains were done as described above after incubation of 7H10 plates for 6 or 14 days at 30°C.

RESULTS

Overall, sodium hypochlorite (0.2 μg/ml of free chlorine) appeared to be the most effective both in reducing GNB populations likely to be present as background contaminants and in allowing good recovery of NTM in test samples (Table 1). However, because materials to be sent to participating dialysis centers were to include instructions for collecting type A samples in containers with sodium thiosulfate (to neutralize chlorine present in municipal water supplies to the centers), the use of sodium hypochlorite as the chemical dosing agent for that study was essentially precluded.

Oxalic acid solutions (1.25%) were highly effective in reducing GNB populations. However, some strains in each group of *M. fortuitum*, *M. chelonae*, and MCLO tested also showed greater than 100-fold reductions in viable counts, so that oxalic acid was considered unacceptable for use as a decontaminating agent in the study of water supplies in dialysis centers.

TABLE 2. Comparative resistances of NTM and GNB to aqueous HCHO solutions

Organisms and HCHO concn (%)	$\log_{10}$ initial titer (CFU/ml)	$\log_{10}$ reduction in CFU/ml at:		
		5 min	10 min	15 min
NTM[a]				
1.0	2.20	0.34	0.38	0.50
0.75	2.20	0.30	0.34	0.41
0.5	2.20	0.21	0.24	0.30
GNB[b]				
1.0	2.12	1.40	1.52	1.82
0.75	2.12	1.34	1.45	1.57
0.5	2.12	1.30	1.34	1.47

[a] Mixture of *M. fortuitum* (four strains), *M. chelonae* (six strains), MCLO (six strains), *M. gordonae* (three strains), and *M. scrofulaceum* (three strains).
[b] Mixture of 15 strains; see Materials and Methods for a list of the strains.

The quaternary ammonium compounds CPC and cetyltrimethylammonium bromide achieved less than 10-fold reductions in GNB populations in the 10-min exposure time. Additional studies with CPC showed that, even at concentrations of 500 and 1,000 μg/ml, 30- and 15-min exposure times, respectively, were required to obtain greater than 100-fold reductions in viable counts of GNB. Moreover, at these higher concentrations of CPC, up to 100-fold reductions in viable counts of some *M. chelonae* and MCLO strains also were observed.

Of the five chemicals tested, only HCHO appeared to meet the minimal criteria required for selection as the dosing agent to be used in the national study: suitability for use in a membrane filtration procedure, applicability for all types of samples collected from the dialysis centers, and reduction in background GNB levels sufficient to allow selective isolation and high recovery of NTM. Additional tests with HCHO solutions were done to determine the most effective combination of HCHO concentration and exposure time for dosing of water supplies from dialysis centers. A 5-min exposure of 1% HCHO slightly improved the recovery of NTM while still effecting reductions in viable counts of GNB comparable to those obtained with a 10-min exposure (Table 2).

Data from microbiologic assays of water samples collected from 115 hemodialysis centers are presented in Tables 3–5. The data in Table 3, which were adjusted to exclude HCHO-resistant SGP, as well as acid-fast organisms, show the comparative efficacy of chemical dosing for reducing background levels of GNB. Exposure of water samples to 1% HCHO for 5 min achieved mean log reductions in viable counts of GNB up to approximately 10-fold times greater than those obtained in undosed samples.

TABLE 3. Efficacy of 1% aqueous HCHO for reducing background levels of GNB in water from dialysis centers[a]

Types[b] (no.) of samples	Range (mean) of $\log_{10}$ CFU/ml with:	
	No chemical dosing	Fluid exposure to 1% aqueous HCHO for 5 min
A (113)	0.00–5.92 (3.04)	0.04–5.38 (1.80)
B (115)	0.00–7.39 (4.22)	0.00–7.16 (3.77)
C (63)	0.00–7.39 (4.11)	0.00–6.16 (3.44)

[a] Comparative levels of background GNB were determined by subtracting viable counts of acid-fast and SGP colonies from total viable counts obtained for undosed and HCHO-dosed samples.
[b] For descriptions of sample types, see Materials and Methods.

TABLE 4. Effect of chemical dosing on levels of specific types of microorganisms in water supplies from dialysis centers

Organisms and types[a] (no.) of samples	Range (mean) of $\log_{10}$ CFU/ml with:	
	No chemical dosing	Fluid exposure to 1% aqueous HCHO for 5 min
Acid-fast bacteria		
A (115)	0.04–5.85 (2.39)	0.04–5.26 (2.51)
B (115)	1.00–5.70 (3.35)	0.04–4.99 (2.89)
C (64)	1.52–5.54 (3.11)	0.04–5.37 (2.97)
SGP		
A (115)	0.04–4.67 (2.22)	0.04–4.57 (2.49)
B (115)	0.30–4.96 (3.07)	0.04–6.00 (3.17)
C (64)	0.30–4.69 (2.89)	1.00–6.58 (3.26)

[a] For descriptions of sample types, see Materials and Methods.

Chemical dosing did not appear to have an adverse effect on mean levels of acid-fast bacteria recovered in water supplies from the dialysis centers (Table 4); with SGP, mean levels recovered at all three sample sites were slightly higher in chemically dosed samples than in undosed samples. Although chemical dosing substantially increased the number of samples in which acid-fast organisms and SGP were recovered (Table 5), some NTM and SGP were recovered only in undosed samples. Overall, the data suggested that, for maximum recovery of NTM or SGP, both dosed and undosed samples should be processed.

DISCUSSION

Current methods for isolating NTM from environmental fluids (11, 14–16, 20, 21, 25, 31–33) are, in most cases, adaptations of procedures developed to detect tubercle bacilli in sputum and body tissues and fluids (11–13, 23, 26, 30–32, 35, 36). These procedures typically involve a variety of steps, including homogenization, decontamination of the sample with alkali, acids, or quaternary ammonium compounds up to 24 h, neutralization of the chemical, concentration of microorganisms in the sample by means of centrifugation or membrane filtration, and plating of the sediment or filter on nonselective and antibiotic-containing media. When membrane filtration is used as the concentrating technique, enumeration of NTM is usually done by means of acid-fast stains of colonies developed on the membrane

TABLE 5. Effect of chemical dosing[a] on recovery efficiency for specific types of microorganisms from water supplies in dialysis centers

Organisms and types[b] (no.) of samples	% of samples positive in:		
	Both dosed and undosed samples	Dosed samples only	Undosed samples only
Acid-fast bacteria			
A (115)	54.8	24.4	18.3
B (115)	55.3	26.3	7.9
C (64)	70.0	13.3	10.0
SGP			
A (115)	66.2	18.2	10.4
B (115)	55.1	30.6	5.1
C (64)	48.9	36.2	4.3

[a] Fluids were exposed to 1% aqueous HCHO for 5 min before filtering.
[b] For descriptions of sample types, see Materials and Methods.

filters (15, 16, 21, 29). Many of these procedures tend to be time consuming or impractical for examination of either large numbers or large volumes of environmental fluid samples. Moreover, the decontaminating agent itself or specific antibiotics used in selective media in many instances affect the viability and recovery of one or more groups of NTM (11-16, 20, 25, 26, 29, 32, 34, 36).

The studies reported here show that single-step chemical dosing of potable or treated water with aqueous HCHO, combined with the membrane filtration procedure, can be used to enhance isolation of NTM from samples containing mixed microbial populations. Data from microbiologic assays of water samples collected from 115 hemodialysis centers showed that chemical dosing with 1% aqueous HCHO for 5 min effectively reduced mean levels of background GNB, with only a negligible effect on mean levels of NTM recovered, so that the actual number of samples in which NTM were detected was substantially increased. Maximum recovery of NTM, however, was obtained by plating both chemically dosed and undosed samples. Unfortunately, because of the unexpected loss of some isolates on subculture and storage, recovery rates of specific NTM groups could not be accurately compared.

Chemical dosing also resulted in increases in both the levels of SGP detected and the number of samples in which SGP were recovered. Although the extent of their relationship to disease has not been clearly demonstrated, SGP have been associated with infections (28) and have been recovered from a variety of clinical specimens from patients with debilitating chronic disease (19). These organisms, which have been tentatively characterized as *P. mesophilica* and related taxons (2, 19), appear to be a heterogeneous group of organisms with regard to physiologic and resistance characteristics. That some strains of SGP appear to be more susceptible to 1% aqueous HCHO, as evidenced by their isolation only from undosed samples, may provide an additional parameter for subgrouping these organisms.

Finally, the results of laboratory studies presented here substantiate an ever-increasing number of reports that rapidly growing and certain other NTM are highly resistant to chlorine and HCHO disinfectants (3–6, 9–11, 15–18, 21, 22, 24, 27, 32). Mixed populations of GNB in field samples from dialysis centers showed somewhat greater resistance to 1% aqueous HCHO (mean reduction, <10-fold) when compared with GNB strains used in laboratory tests (mean reduction, 18-fold). This may reflect a greater variety of innately resistant organisms in natural environments, or it may be related to basic differences in the resistance characteristics of naturally occurring organisms versus subcultured strains from laboratory media (albeit water adapted), as observed in *P. aeruginosa*, *P. alcaligenes*, *P. cepacia*, and other organisms (10, 18, 27).

LITERATURE CITED

1. **American Public Health Association.** 1984. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, New York.
2. **Austin, B., and M. Goodfellow.** 1979. *Pseudomonas mesophilica*, a new species of pink bacteria isolated from leaf surfaces. Int. J. Sys. Bacteriol. **29**:373–378.
3. **Azadian, B. S., A. Beck, J. R. Curtis, L. E. Cherrington, P. E. Gower, M. Phillips, J. B. Eastwood, and J. Nicholls.** 1981. Disseminated infection with *Mycobacterium chelonei* in a haemodialysis patient. Tubercle **62**:281–284.
4. **Band, J. D., J. I. Ward, D. W. Fraser, N. J. Petersen, P. W. Hayes, V. A. Silcox, R. C. Good, P. R. Ostroy, and J. Kennedy.** 1981. Peritonitis due to a *Mycobacterium chelonei*-like organism associated with intermittent chronic peritoneal dialysis. J. Infect. Dis. **145**:9–17.
5. **Bland, L. A., M. J. Alter, M. S. Favero, L. A. Carson, and L. B. Cusick.** 1985. Hemodialyzer reuse: practices in the United States and implication for infection control. Trans. Am. Soc. Artif. Intern. Organs **XXXI**:556–559.
6. **Bolan, G., A. L. Reingold, L. A. Carson, V. A. Silcox, C. L. Woodley, P. S. Hayes, A. W. Hightower, L. McFarland, J. W. Brown III, N. J. Petersen, M. S. Favero, R. C. Good, and C. V. Broome.** 1985. Infections with *Mycobacterium chelonei* in patients receiving dialysis and using processed hemodialyzers. J. Infect. Dis. **152**:1013–1019.
7. **Borghans, J. G. A., and J. L. Stanford.** 1973. *Mycobacterium chelonei* in abscesses after injection of diphtheria-pertussis-tetanus-polio vaccine. Am. Rev. Respir. Dis. **107**:1–8.
8. **Brown, T. H.** 1985. The rapidly growing mycobacteria—*Mycobacterium fortuitum* and *Mycobacterium chelonei*. Infect. Control **6**:283–288.
9. **Carson, L. A., M. S. Favero, W. W. Bond, and N. J. Petersen.** 1972. Factors affecting comparative resistance of naturally occurring and subcultured *Pseudomonas aeruginosa* to disinfectants. Appl. Microbiol. **23**:863–869.
10. **Carson, L. A., N. J. Petersen, M. S. Favero, and S. M. Aguero.** 1978. Growth characteristics of atypical mycobacteria in water and their comparative resistance to disinfectants. Appl. Environ. Microbiol. **36**:839–846.
11. **Collins, C. H., J. M. Grange, and M. D. Yates.** 1984. A review: mycobacteria in water. J. Appl. Bacteriol. **57**:193–211.
12. **Corper, H. J., and N. Uyei.** 1930. Oxalic acid as a reagent for isolating tubercle bacilli and a study of the growth of acid-fast nonpathogens on different mediums with their reaction to chemical reagents. J. Lab. Clin. Med. **15**:348–369.
13. **Damato, J. J., M. T. Collins, M. V. Rothlauf, and J. K. McClatchy.** 1983. Detection of mycobacteria by radiometric and standard plate procedures. J. Clin. Microbiol. **17**:1066–1073.
14. **du Moulin, G. C., and K. D. Stottmeier.** 1978. Use of cetylpyridinium chloride in the decontamination of water for culture of mycobacteria. Appl. Environ. Microbiol. **36**:771–773.
15. **Engelbrecht, R. S., C. N. Haas, J. A. Shular, D. L. Dunn, D. Roy, A. Lalchandani, B. F. Severin, and S. Farooq.** 1979. Acid-fast bacteria and yeasts as indicators of disinfection efficiency. U.S. Environmental Protection Agency, technology series no. EPA-IAG-D6-0432. Washington, D.C.
16. **Engelbrecht, R. S., B. F. Severin, M. T. Masarik, S. Farooq, S. H. Lee, C. N. Haas, and A. Lalchandani.** 1977. New microbial indicators of disinfection efficiency. U.S. Environmental Protection Agency, technology series no. EPA-IAG-D4-0432. Washington, D.C.
17. **Favero, M. S.** 1983. Distinguishing between high-level disinfection, reprocessing, and sterilization, p. 19–23. *In* Reuse of disposables: implications for quality health care and cost containment. Association for the Advancement of Medical Instrumentation, Arlington, Va.
18. **Favero, M. S., N. J. Petersen, L. A. Carson, W. W. Bond, and S. H. Hindman.** 1975. Gram-negative water bacteria in hemodialysis systems. Health Lab. Sci. **12**:321–334.
19. **Gilardi, G. L., and Y. C. Faur.** 1984. *Pseudomonas mesophilica* and an unamed taxon, clinical isolates of pink-pigmented oxidative bacteria. J. Clin. Microbiol. **20**:626–629.
20. **Gruft, H. J., O. Falkinham, and B. C. Parker.** 1981. Recent experience in the epidemiology of disease caused by atypical mycobacteria. Rev. Infect. Dis. **2**:990–996.
21. **Haas, C. N., M. M. Meyer, and M. S. Paller.** 1983. The ecology of acid-fast organisms in water supply, treatment, and distribution systems. J. Am. Water Works Assoc. **75**:139–144.
22. **Hayes, P. S., D. L. McGiboney, J. D. Band, and J. C. Feeley.** 1982. Resistance of *Mycobacterium chelonei*-like organisms to formaldehyde. Appl. Environ. Microbiol. **43**:722–724.
23. **Matajack, M. L., M. L. Bissett, D. Schifferle, and R. M. Wood.** 1973. Evaluation of a selective medium for mycobacteria. Am. J. Clin. Pathol. **59**:391–397.
24. **Park, U. K., and W. S. Brewer.** 1976. The recovery of *Mycobacterium marinum* from swimming pool water and its resis-

tance to chlorine. J. Environ. Health **38**:390–392.
25. **Powell, B. L., Jr., and J. E. Steadham.** 1981. Improved technique for isolation of *Mycobacterium kansasii* from water. J. Clin. Microbiol. **13**:969–975.
26. **Rothlauf, M. V., G. L. Brown, and E. B. Blair.** 1981. Isolation of mycobacteria from undecontaminated specimens with selective 7H10 medium. J. Clin. Microbiol. **13**:76–79.
27. **Russell, A. D., S. A. Hammond, and J. R. Morgan.** 1986. Bacterial resistance to antiseptics and disinfectants. J. Hosp. Infect. **7**:213–225.
28. **Smith, S. M., R. H. K. Eng, and C. Forrester.** 1985. *Pseudomonas mesophilica* infections in humans. J. Clin. Microbiol. **21**: 314–317.
29. **Smithwick, R. W., and C. B. Stratigos.** 1981. Acid-fast microscopy on polycarbonate membrane filter sputum sediments. J. Clin. Microbiol. **13**:1109–1113.
30. **Smithwick, R. W., C. B. Stratigos, and H. L. David.** 1975. Use of cetylpyridinium chloride and sodium chloride for the decontamination of sputum specimens that are transported to the laboratory for the isolation of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **1**:411–413.
31. **Sommers, H. M., and R. C. Good.** 1985. *Mycobacterium*, p. 216–248. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
32. **Songer, J. G.** 1981. Methods for selective isolation of mycobacteria from the environment. Can. J. Microbiol. **27**:1–7.
33. **Steadham, J. E.** 1980. High-catalase strains of *Mycobacterium kanasasii* isolated from water in Texas. J. Clin. Microbiol. **11**: 496–498.
34. **Swenson, J. M., C. Thornsberry, and V. A. Silcox.** 1982. Rapidly growing mycobacteria: testing of susceptibility of 34 antimicrobial agents by broth microdilution. Antimicrob. Agents Chemother. **22**:186–192.
35. **Tazir, M., and H. L. David.** 1979. Evaluation of the chloride and bromide salts of cetylpyridinium for the transportation of sputum in tuberculosis bacteriology. Tubercle **60**:31–36.
36. **Thoen, C. O., W. D. Richards, and J. L. Jarnigin.** 1974. Comparison of six methods for isolating mycobacteria from swine lymph nodes. Appl. Microbiol. **27**:448–451.