APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1978, p. 839-846
0099-2240/78/0036-0839$02.00/0

Vol. 36, No. 6
Printed in U.S.A.

Growth Characteristics of Atypical Mycobacteria in Water and Their Comparative Resistance to Disinfectants

LORETTA A. CARSON,* NORMAN J. PETERSEN, MARTIN S. FAVERO, AND SONIA M. AGUERO

Hepatitis Laboratories Division, Bureau of Epidemiology, Center for Disease Control, Phoenix, Arizona 85014

Received for publication 2 October 1978

With the increasing significance of group IV atypical mycobacteria as etiological agents in a variety of infections, studies were conducted to determine their growth capabilities in water and their comparative resistance to disinfectants used to decontaminate hospital equipment. Isolates of *Mycobaterium chelonei* (TM strains) from peritoneal fluids of patients and peritoneal dialysis machines were able to multiply in commercial distilled water, with generation times at 25°C ranging from 8 to 15 h. Levels of 10^5 to 10^6 cells per ml were attained, and these stationary-phase populations declined only slightly over a 1-year period. Results of studies to determine resistance to disinfectants showed the following. (i) TM strains of *M. chelonei* cultured in commercial distilled water showed survivors in 2% aqueous formaldehyde (HCHO) solutions up to 24 h; in 8% HCHO, only a 2-log reduction in viable counts was observed over a 2-h sampling period. Reference ATCC strains of *M. chelonei* and *M. fortuitum* were rapidly inactivated, with no survivors after 2 h of exposure to 2% HCHO or 15 min of exposure to 8% HCHO. (ii) In 2% alkaline glutaraldehyde, TM strains survived 60 min, whereas ATCC strains showed no survivors after 2 min of contact time. (iii) All *M. chelonei* and *M. fortuitum* strains survived 60 min of exposure to concentrations of 0.3 and 0.7 μg of free chlorine per ml at pH 7.

Until the last decade, mycobacteria other than the tubercle bacilli have only sporadically been reported as etiological agents in human infections (18, 34). However, they have been demonstrated as part of the normal flora in sputum and feces of healthy animals and humans (8, 17, 29, 34), and their widespread distribution in nature has been documented by isolations from soil sources (8, 29), wastewater effluents (12, 13), and a variety of municipal, laboratory, and other freshwater sources (2, 11, 17, 18, 32).

The infective potential of the atypical mycobacteria has increasingly been recognized, and Barksdale and Kim (3) recently described the range of human infections now known to be caused by these organisms. Opportunistic pathogens in the *Mycobacterium fortuitum* complex (29, 34; formerly characterized as Runyon group IV strains) produce primarily chronic, localized injection and abrasion abscesses or corneal infections, as well as pulmonary complications, lymphadenitis, and occasional disseminated disease in compromised patients. There have been further reports of problems ascribed to their presence as contaminants in hemodialysis fluids (19, 21) and in pharmaceutical preparations and disinfectant solutions (4, 7, 19, 24, 30, 33). Although it has been suggested that growth requirements of the atypical mycobacteria are not complex (8), studies on their ability to multiply in water environments have been scarce (4, 22, 23). Moreover, relatively little information is available on resistance characteristics of these "rapid growers" to physical and chemical agents, because studies reported have largely been directed toward clinically significant pathogens in Runyon groups I to III (9, 10, 26, 31, 34).

Organisms identified as *M. chelonei* strains were isolated from the peritoneal fluids of patients experiencing episodes of peritonitis in a hospital peritoneal dialysis center. Samples of dialysate and other fluids from dialysis machine reservoirs, sumps, and fluid pathways also yielded *M. chelonei* strains even after disinfection of machines with a 2% formaldehyde solution. Studies were undertaken. (i) to determine the growth capabilities of these organisms in water environments and (ii) to examine their resistance to disinfectants commonly used in hospitals for decontamination of water treatment systems and machines in hemodialysis and peritoneal dialysis centers or for sterilization of patient care materials.

MATERIALS AND METHODS

Organisms. The following strains of mycobacteria and gram-negative bacteria were used for growth or disinfectant studies: (i) 12 isolates (TM strains), in-

cluding 8 from patient peritoneal fluids and 4 from peritoneal dialysis machines in a hospital dialysis unit, presumptively identified as *M. chelonei* by the Mycobacteriology Branch, Center for Disease Control Atlanta, Ga.; (ii) isolates from a human hand wound (*M. chelonei* strain M77-385) and human abscess (*M. fortuitum* strain M77-382), also received from the Center for Disease Control, Atlanta, Ga.; (iii) reference strains of *M. chelonei* (no. 14472) and *M. fortuitum* (no. 6841) obtained from the American Type Culture Collection; (iv) strains of *Pseudomonas cepacia* and *P. aeruginosa* isolated in pure culture in distilled water from hospital mist therapy units (5, 6, 14) and maintained for 8 years solely by periodic transfer in sterile distilled water; (v) strains of *Klebsiella* and *Escherichia coli* isolated from the effluent of a sewage treatment plant; and (vi) a mixed microbial flora in water from a soft-water storage tank.

Preparation of cell suspensions. Mycobacterial strains were initially subcultured on heart infusion agar (Difco) at 37°C for 72 h. Cells were washed three times in commercial sterile distilled water ([CDW] Cutter Laboratories), resuspended, and diluted to approximately 10^5 cells per ml in 100 ml of CDW in screw-capped milk dilution bottles. These suspensions in CDW were held for 5 days at 25°C to allow for metabolic depletion of trace nutrients carried over in washed cells and for stabilization of cells stressed in a distilled-water environment. Viable cell counts were determined by the membrane filter technique (0.45 μm, Millipore Corp.); filters were incubated on Standard Methods (SM) agar and on Middlebrook and Cohn 7H10 agar base with OADC enrichment (Baltimore Biological Laboratory). Colony counts on filters incubated at 37°C for 2, 3, and 7 days were comparable on the two media, and SM agar was routinely used thereafter. Other experiments in which cells were exposed to 2, 5, 10, and 20 min of ultrasonic energy (model A-300, Branson Instruments, Stamford, Conn.) to effect dispersal of cells before filtering and plating on SM agar showed no increases in viable cell counts compared with cells dispersed by mechanical agitation, and cultures were routinely filtered after a 10-s agitation on a Vortex mixer. The 5-day CDW suspensions were diluted in CDW to approximately 10^2 cells per ml, and after 7 days at 25°C assays showed population levels of 10^5 to 10^6 cells per ml. These cultures were thereafter maintained in CDW and used as stock cultures.

To obtain stable populations of waterborne fecal coliforms without carry-over of excess nutrients from standard laboratory growth media, isolates of *Klebsiella* and *E. coli* from filtered samples of sewage effluent incubated on FC agar (Difco) were streaked onto water agar (CDW with 0.9% Ionagar no. 2 [Colab Laboratories]) and incubated at 37°C for 72 h. Bacterial growth was harvested in CDW and transferred to approximately 500 ml of CDW in sterile, 1-liter bottles held at ambient room temperature. Assays with membrane filters on SM agar showed initial viable counts of 10^5 to 10^6 cells per ml, with no significant decreases in population levels after 12 and 21 days.

Growth and resistance studies. For determination of mycobacterial growth patterns, cells from the CDW stock cultures were diluted decimally to obtain populations of 10^1 to 10^2 cells per ml in 100 ml of CDW in screw-capped milk dilution bottles. Cultures were incubated in stationary positions at 25°C.

Cells from CDW stock cultures of mycobacterial strains and strains of *P. cepacia* and *P. aeruginosa* were transferred to fresh CDW and incubated 5 to 7 days at 25°C to obtain cultures for disinfectant resistance tests. For studies with glutaraldehyde, 2% (vol/vol) alkaline solutions of Cidex (CS-250, Arbrook, Inc.) were prepared as directed by the manufacturer. One milliliter each of cultures in CDW or softened water containing the mixed microbial flora was added at 25°C to 50 ml of 2% activated glutaraldehyde (AG) solution in 125-ml Erlenmeyer flasks covered with aluminum foil. In one series of tests, AG solution was added to CDW cultures to attain 0.2% final AG concentrations. For formaldehyde (HCHO) tests, CDW cultures were diluted 1:2 in CDW, and sufficient 37% HCHO (certified ACS, Fisher Scientific Co.) was added at 25°C to obtain concentrations ranging from 0.5 to 10% (vol/vol) HCHO in a total volume of 100 ml per flask. After preliminary tests showed that sodium bisulfite was not consistently effective in neutralizing AG or HCHO, aliquots of test samples in approximately 50 ml of APHA phosphate-buffered distilled water (1) (pH 7) were passed through membrane filters and then rinsed three times with 50-ml portions of phosphate-buffered distilled water to remove final traces of these disinfectants before plating on SM agar.

For chlorine resistance tests, desired levels of free chlorine were obtained by adding approximately 1 ml of concentrated sodium hypochlorite solution (reagent grade; Matheson, Coleman and Bell Co.), appropriately diluted in chlorine demand-free CDW, to each flask containing 74 ml of CDW test culture adjusted to pH 7 with APHA phosphate buffer. Free chlorine assays were performed spectrophotometrically using the *N*, *N*-diethyl-*p*-phenylenediamine (DPD) method (1), and calibrations for spectrophotometric assay were conducted with a standardized solution of free chlorine (Hach Chemical Co.). To determine levels of free chlorine in test flasks, a premeasured amount of commercial powdered DPD reagent (Hach Chemical Co.) was added to each 5-ml test sample, and percent transmittance at 530 nm was measured immediately after the addition of the chlorine reagent and again either at 5 min or at 30 and 60 min. The level of free chlorine exposure for each set of tests was considered to be the mean value of all chlorine determinations in that set. Fluids for microbiological assay were withdrawn at intervals and neutralized with 0.1% sodium thiosulfate before membrane filtration.

Colony counts were made after incubation for 72 h at 37°C, and the data were used to construct growth or survival curves. Plates showing no survivors at 72 h were held up to 7 days; these continued to show no growth and were recorded as no survivors.

RESULTS

Figure 1 shows growth patterns in CDW of 4 of the mycobacterial strains (TM strains) isolated from a hospital peritoneal dialysis unit, and four reference mycobacterial strains, over a period of 4 weeks. Cells of all 12 TM strains of

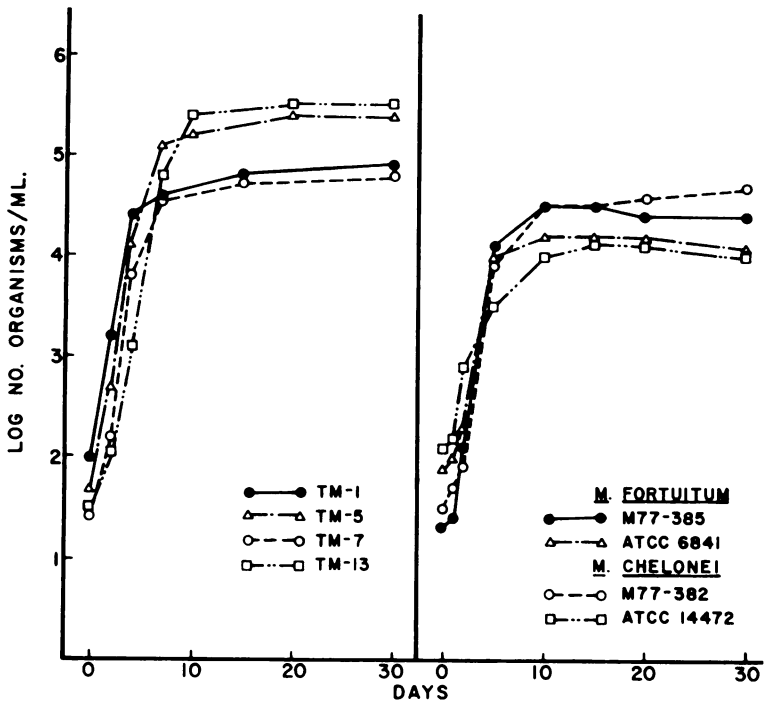

FIG. 1. *Comparative growth of M. chelonei and M. fortuitum strains in distilled water at 25°C.*

M. chelonei tested attained stationary-phase levels ranging from 5.0×10^4 to 5.0×10^6 organisms per ml within 10 days, with an average generation time of 12 h. Growth patterns of reference strains of *M. chelonei* and *M. fortuitum* were similar, although maximum population levels attained even after 30 days were slightly lower than those with TM strains. As we have reported previously with gram-negative water bacteria (6, 14, 15), acid-fast cells grown in CDW do not produce visible turbidity at levels of 10^5 to 10^6 cells per ml. Furthermore, cultures in CDW do not appear to enter phases of decline and death typically seen after growth in standard laboratory media, and assays conducted over a 1-year period showed that population levels remained fairly constant at 10^4 to 10^5 cells per ml.

Results of initial tests with HCHO showed that cells of a TM strain of *M. chelonei* cultured in CDW (1.6×10^3 cells per ml) were highly resistant, with viable counts reduced less than 2 logs after 24 h of exposure to 0.5, 1, or 2% HCHO. However, reference strains of *M. chelonei* (M77-385) and *M. fortuitum* (M77-382) showed no survivors after 2 h of exposure to 2% HCHO. Cells of the TM strain also were resistant to higher HCHO concentrations, with 10% survivors in 4% HCHO and 3% survivors in 5% HCHO after 4-h exposure times. In 10% HCHO solutions, the TM strain did not survive 2 h of exposure. Further studies were conducted with 8% HCHO, and Fig. 2 presents inactivation curves for five of the TM strains and two reference ATCC mycobacterial strains. TM strains were relatively resistant to 8% HCHO, with two strains showing survivors at 2 h and three strains showing survivors at 4-h exposure times. Reference strains of *M. chelonei* (ATCC 14472) and *M. fortuitum* (ATCC 6841) were rapidly inactivated, with $<10^1$ cells per ml surviving 5 min and no survivors detectable after 15 min of exposure. Cultures of *P. aeruginosa*, *P. cepacia*, *Klebsiella*, and *E. coli* ranging from 1.4×10^4 to 2.4×10^6 cells per ml in CDW showed no survivors in 2% HCHO at 10 min of exposure or in 8% HCHO at 2 min of exposure. Other gram-negative bacterial strains in a mixed flora in softened water (6.6×10^4 cells per ml) were slightly more resistant, with survivors detected up to 8 h in 2% HCHO and up to 15 min in 8% HCHO.

Survivor curves for five mycobacterial TM strains in 2% AG are presented in Fig. 3. Four of the five strains showed at least small numbers of survivors (<1 ml) at 40 min, and one strain showed low levels (10^1 cells per ml) remaining at 60 min of exposure. With ATCC reference strains of *M. chelonei* and *M. fortuitum*, as well as cells of *P. aeruginosa*, *P. cepacia*, *Klebsiella*, and *E. coli*, no survivors were detected after 2

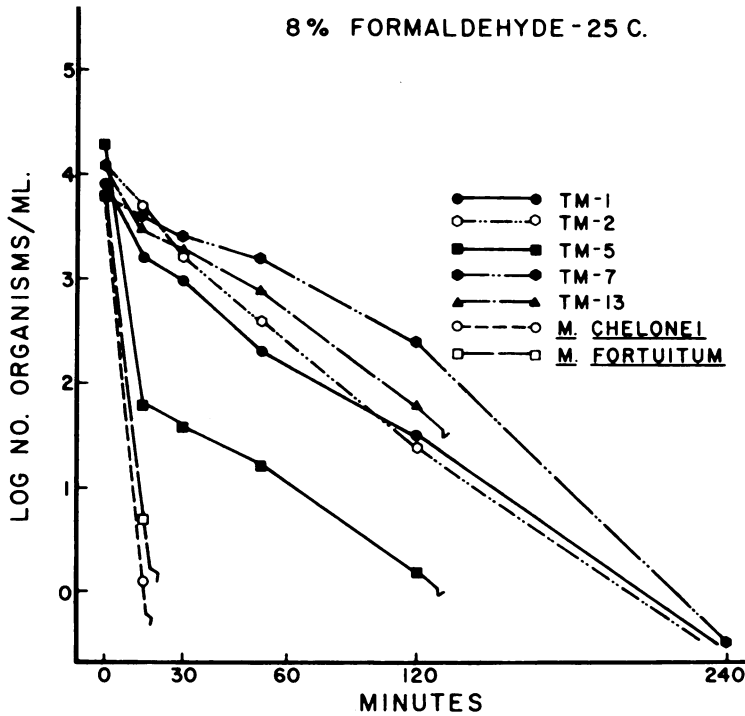

FIG. 2. *Comparative resistance of mycobacterial organisms cultured in distilled water to 8% formaldehyde at 25°C.*

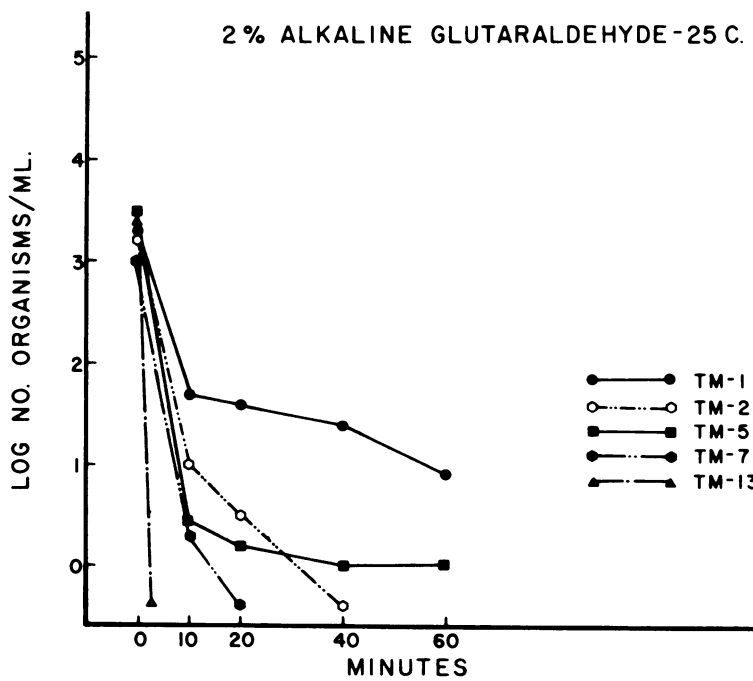

FIG. 3. *Comparative resistance of mycobacterial organisms cultured in distilled water to 2% AG at 25°C.*

min of exposure to 2% AG. In 0.2% AG (Fig. 4), TM strains were capable of prolonged survival, with viable cell counts ranging from 10^0 to 10^2 cells per ml after 96 h of exposure. Cells of *M. chelonei* also were resistant to the lower AG concentration, with survivors detected at 96 h, whereas with *M. fortuitum* no survivors were detected at 24 h.

In tests to determine comparative resistance to chlorine, sufficient sodium hypochlorite was added to flasks containing cells of *Klebsiella*, *E. coli*, *P. aeruginosa*, or *P. cepacia* in CDW (5.1×10^4 to 3.7×10^5 organisms per ml) to obtain mean levels of 0.15 and 0.35 μg of free chlorine per ml. At the lower chlorine levels, *Klebsiella* and *P. aeruginosa* showed some survivors (2.2×10^0 and 1.0×10^{-1}, respectively) at 5 min; *E. coli* and *P. cepacia* were rapidly inactivated, with no survivors at 2 min of contact time. All gram-negative bacterial strains tested failed to survive 1 min of exposure to 0.35 μg of free chlorine per ml. Results of comparative tests with mycobacterial strains, presented in Fig. 5, show that these acid-fast bacteria constitute a much more resistant flora, with all strains surviving 60 min of exposure to 0.3 μg/ml, and four of five strains surviving 30 min of exposure to 0.7 μg of free chlorine per ml.

DISCUSSION

The potential health hazards of microbial contamination in fluids associated with dialysis procedures in hemodialysis and peritoneal dialysis centers have previously been related (15, 16, 20, 25, 27, 29, 32) to a variety of causal factors including: (i) high levels of gram-negative bacteria or associated endotoxins in source waters used to prepare dialysis fluids; (ii) the efficacy of various types of water treatment systems (i.e., softening, deionization, reverse osmosis) in harboring or reducing microbial contaminants or in removing endotoxins; (iii) the effectiveness of disinfection procedures in eliminating or preventing colonization of filters, membranes, and modules in water treatment systems or piping in fluid distribution systems; and (iv) the problems of specific machine designs associated with failure of disinfection procedures.

Natural and treated waters also are increasingly being recognized as reservoirs and potential sources of human infections involving atypical mycobacteria. Kazda (22, 23) reported the ability of strains of *M. intracellulare* and *M. avium* to multiply in moorland water filtrates, in which maximum population levels of 10^4 to 10^5 cells per ml were attained in 21 days. Growth

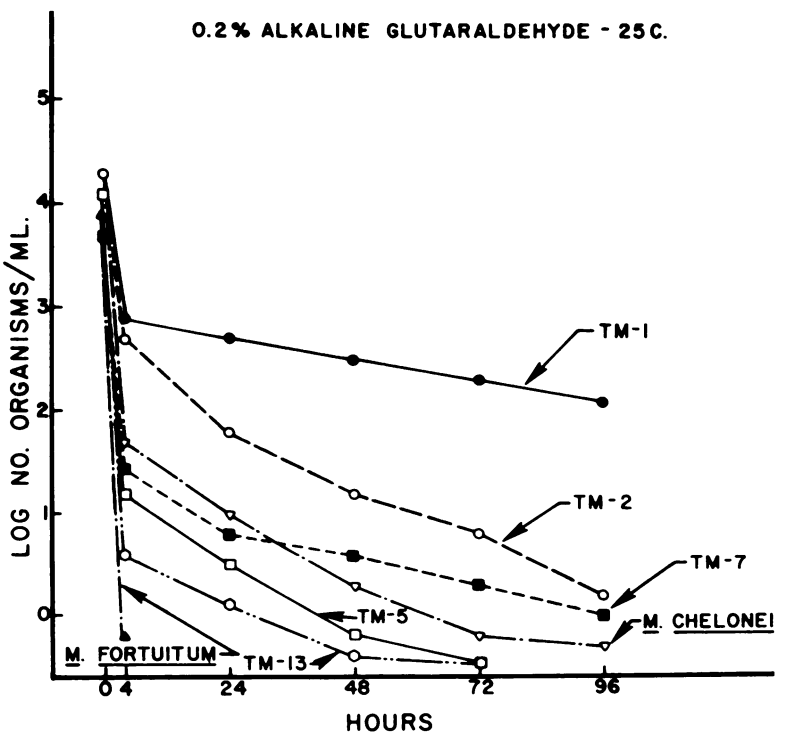

FIG. 4. *Comparative resistance of mycobacterial organisms cultured in distilled water to 0.2% AG at 25°C.*

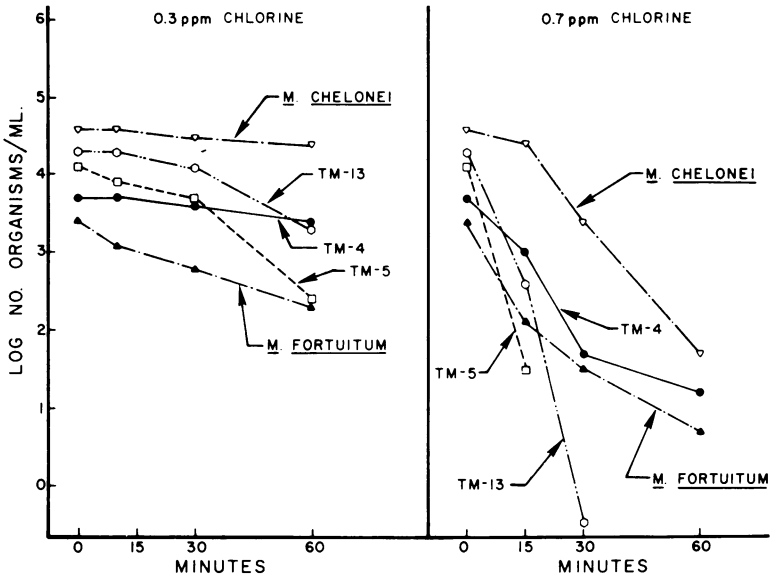

FIG. 5. *Comparative resistance of mycobacterial organisms cultured in distilled water to 0.3 and 0.7 μg of free chlorine per ml at pH 7.*

appeared to be pH dependent, with lower pH values (4.2 to 4.7) favoring multiplication and pH values > 6 being somewhat growth inhibitory for these acid-fast organisms. Chapman (8) noted, however, that most strains of atypical mycobacteria appear to be nonfastidious in growth requirements and are able to survive and multiply in simple media under a wide range of environmental conditions of pH and temperature. Goslee and Wolinsky (18) reported the isolation of 51 strains of *M. fortuitum* in samples collected from city water supplies, lakes, rivers and ponds, and also from reservoirs, swimming pools, and ice machines. Acid-fast organisms also have been isolated from the back-flush contents of water-softening units in private homes and hospitals (32). Borghans and Stanford (4) reported that strains of *M. fortuitum* and *M. chelonei* isolated from abscesses after injection of diphtheria-pertussis-tetanus-polio (DPTP) vaccine were able to survive up to 9 weeks at 4°C in sterile tap water and in DPTP vaccine containing 20 μg of a quaternary ammonium compound per ml. Other studies (19, 21) have implicated source waters and hemodialysis fluids in cases of septicemia and disseminated subcutaneous abscesses and osteomyelitis caused by organisms in the *M. fortuitum* group.

Studies reported here have shown that isolates of *M. chelonei* were able to multiply in distilled water, attaining population levels of 10^5 to 10^6 cells per ml. Over a period of 1 year, moreover, total viable counts of CDW stock cultures of these organisms declined only slightly. The prolonged maintenance of these stationary levels presumably reflects the ability of these bacterial organisms to utilize trace amounts of volatile or other nutrients which are inevitably introduced when bottled distilled water is added periodically to maintain constant fluid volumes in stock cultures.

The presence of low levels of free available chlorine (0.2 to 0.4 μg/ml) in municipal water supplies has been shown to reduce both the rate and extent of gram-negative bacterial colonization of reverse osmosis units, preventing the development of high levels of bacteria in reverse-osmosis product water (15, 16, 25). Acid-fast organisms, however, have been found in relatively high numbers in raw wastewater (1.5×10^4/100 ml), with chlorine-resistant strains of *M. fortuitum*, *M. phlei*, and *M. smegmatis* persisting (5.0×10^3/100 ml) in treated effluents (12, 13). These organisms were found to be 20 to 100 times more resistant than coliforms and other gram-negative bacteria to free chlorine residuals up to 1.0 μg/ml. Furthermore, cells of *M. fortuitum* survived 60 min of exposure to 2.0 μg of free chlorine per ml.

Results of disinfectant studies showed that CDW-grown cells of *M. chelonei* and *M. fortuitum* were markedly resistant to chlorine, with up to 60% survivors at 60 min of exposure to 0.3 μg of free chlorine per ml and up to 2% survivors at 60 min of exposure to 0.7 μg of free chlorine per ml. In tests to determine the comparative resistance of waterborne gram-negative bacteria, *Pseudomonas* and coliform bacteria were inac-

tivated by ≤ 5 min of exposure to 0.15 or 0.35 μg of free chlorine per ml. These results would tend to explain why atypical mycobacteria can be isolated from municipal and hospital water supplies that are considered to be adequately disinfected.

Routine disinfection of both reverse osmosis units and dialysis machines with 1.5 to 3.0% HCHO solutions has similarly been shown to prevent colonization of fluid pathways with gram-negative water bacteria (15, 16, 27, 28), and Katz (20) reported effective reduction of gram-negative bacterial contaminants in deionizers disinfected with 1.5% HCHO solutions. However, strains of *M. chelonei* (TM strains) associated with episodes of peritonitis among patients in a hospital peritoneal dialysis center were not eliminated from dialysis machines after overnight disinfection with 2% HCHO solutions. In laboratory studies these TM strains of *M. chelonei* demonstrated high resistance to HCHO. In 2% aqueous solutions survivors were detected up to 24 h, and in 8% HCHO only a 2-log reduction in viable counts was observed over a 2-h sampling period.

Reports of studies from other laboratories (9, 26) have shown 2% AG solutions to be highly effective for disinfecting various types of hospital equipment contaminated with tubercle as well as atypical mycobacterial organisms, although greater variation in inactivation rates was observed at AG concentrations of 1.0 or 0.5%. *M. chelonei* organisms have been reported as intrinsic contaminants of porcine prosthetic heart valve tissues treated and stored, respectively, in 1 and 0.2% buffered glutaraldehyde solutions (24, 33). As reported here, TM strains of *M. chelonei* showed survivors remaining after 60 min of exposure to 2% AG, although no survivors of ATCC strains of *M. chelonei* or *M. fortuitum* were detected in fluids assayed at 2 min of contact time. Whether the comparatively greater resistance of TM strains to aldehyde disinfectants reflects differences in their culture history as opposed to reference strains maintained in subculture over prolonged periods, or whether TM strains are, in fact, variant strains in the *M. fortuitum* complex could not be determined. With 0.2% AG, both TM and ATCC strains showed survivors at 96 h of exposure time. These results suggest that disinfectant solutions used in treating or packing of materials and/or equipment for patient use must be carefully evaluated in terms of their potential for harboring rather than eliminating microbiological contaminants.

The results of studies demonstrating the ability of acid-fast bacteria to persist for prolonged periods in fluids of low nutrient content or fluids containing disinfectant residues suggest that a variety of water sources can serve as reservoirs and transmission routes of infection. The studies reported here demonstrate that strains of *M. chelonei* and *M. fortuitum* are able not only to survive but also to multiply in distilled water, attaining levels of 10^4 to 10^6 cells per ml. These acid-fast organisms also appear to constitute a significantly more resistant flora with respect to certain disinfectants than do gram-negative water bacteria typically encountered in hospital water supplies. Inadequate disinfection of equipment and fluids in the hospital or dialysis environment may, therefore, result in the accumulation or amplification of mycobacterial contaminants, thereby constituting a health hazard to compromised patients.

LITERATURE CITED

1. **American Public Health Association.** 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, New York.
2. **Bailey, R. K., S. Wyles, M. Dingley, F. Hesse, and G. W. Kent.** 1970. The isolation of high catalase *Mycobacterium kansasii* from tap water. Am. Rev. Respir. Dis. **101**:430–431.
3. **Barksdale, L., and K-S. Kim.** 1977. *Mycobacterium.* Bacteriol. Rev. **41**:217–372.
4. **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.
5. **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.
6. **Carson, L. W., M. S. Favero, W. W. Bond, and N. J. Petersen.** 1973. Morphological, biochemical and growth characteristics of *Pseudomonas cepacia* from distilled water. Appl. Microbiol. **25**:467–483.
7. **Center for Disease Control.** 1977. Atypical mycobacteria wound infections—North Carolina, Colorado, p. 12–14. *In* National nosocomial infections study report, Annual Summary 1974. Center for Disease Control, Atlanta, Ga.
8. **Chapman, J. S.** 1971. The ecology of the atypical mycobacteria. Arch. Environ. Health **22**:41–46.
9. **Collins, F. M., and V. Montalbine.** 1976. Mycobactericidal activity of glutaraldehyde solutions. J. Clin. Microbiol. **4**:408–412.
10. **Croshaw, B.** 1971. The destruction of mycobacteria, p. 419–449. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press Inc., New York.
11. **Dizon, D., C. Mihailescu, and H. C. Bae.** 1976. Simple procedure for detection of *Mycobacterium gordonae* in water causing false-positive acid-fast smears. J. Clin. Microbiol. **3**:211.
12. **Engelbrecht, R. S., D. H. Foster, E. O. Greening, and S. H. Lee.** 1974. New microbial indicators of wastewater chlorination efficiency. U. S. Environmental Protection Agency, technology series no. EPA-670/2-73-082, Washington, D. C.
13. **Engelbrecht, R. S., B. F. Severin, M. T. Massarik, 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-600/2-77-052, Washington, D. C.

14. **Favero, M. S., L. A. Carson, W. W. Bond, and N. J. Petersen.** 1971. *Pseudomonas aeruginosa*: growth in distilled water from hospitals. Science **173**:836–838.
15. **Favero, M. S., N. J. Petersen, K. M. Boyer, L. A. Carson, and W. W. Bond.** 1974. Microbial contamination of renal dialysis systems and associated health risks. Trans Am. Soc. Artif. Intern. Organs **XX-A**: 175–183.
16. **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.
17. **Gangadharam, P. R., J. A. Lockhart, R. J. Awe, and D. E. Jenkins.** 1976. Mycobacterial contamination through tap water. Am. Rev. Respir. Dis. **113**:894.
18. **Goslee, S., and E. Wolinsky.** 1976. Water as a source of potentially pathogenic mycobacteria. Am. Rev. Respir. Dis. **113**:287–292.
19. **Graybill, J. R., J. Silva, Jr., D. W. Fraser, R. Lordon, and E. Rogers.** 1974. Disseminated mycobacteriosis due to *Mycobacterium abscessus* in two recipients of renal homografts. Am. Rev. Respir. Dis. **109**:4–10.
20. **Katz, D., H. Laney, J. A. Linquist, and E. C. Persike.** 1976. Formaldehyde disinfection to eliminate bacterial contamination of deionizers. Dialy. Transplant. **5**: 42–44.
21. **Katz, M. A., and A. R. Hull.** 1971. Probable *Mycobacterium fortuitum* septicaemia: complication of home dialysis. Lancet **i**:499.
22. **Kazda, J.** 1973. The importance of water for the spread of potentially pathogenic mycobacteria. I. Possibilities for the multiplication of mycobacteria. Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. 1 Orig. Reihe B **158**:161–169.
23. **Kazda, J.** 1973. The importance of water for the spread of potentially pathogenic mycobacteria. II. Growth of mycobacteria in water models Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B **158**: 170–176.
24. **Laskowski, L. F., J. J. Marr, J. F. Spernoga, N. J. Grank, H. B. Barner, G. Kaiser, and D. H. Tyras.** 1977. Fastidious mycobacteria grown from porcine prosthetic-heart-valve cultures. N. Engl. J. Med. **297**: 101–102.
25. **Lauer, J., A. Streifel, C. Kjellstrand, and R. DeRoos.** 1975. The bacteriological quality of hemodialysis solution as related to several environmental factors. Nephron **15**:87–97.
26. **Miner, N. A., J. W. McDowell, G. W. Willcockson, N. I. Bruckner, R. L. Stark, and E. J. Whitmore.** 1977. Antimicrobial and other properties of a new stabilized alkaline glutaraldehyde disinfectant/sterilizer. Am. J. Hosp. Pharm. **34**:376–382.
27. **Petersen, N. J., K. M. Boyer, L. A. Carson, and M. S. Favero.** 1978. Pyrogenic reactions from inadequate disinfection of a dialysis fluid distribution system. Dialy. Transplant. **7**:52–55.
28. **Petersen, N. J., L. A. Carson, and M. S. Favero.** 1977. Microbiological quality of water in an automatic peritoneal dialysis system. Dialy. Transplant. **6**:38–41.
29. **Runyon, E. H., L. G. Wayne, and G. P. Kubica.** 1974. Family II. *Mycobacteriaceae* Chester, p. 681–701. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. Williams & Wilkins Co., Baltimore.
30. **Segard, E. C., and G. E. Thompson.** 1952. Acid-fast bacilli as contaminants: presence in digestant and indicator in the examination for tubercle bacilli. Am. J. Clin. Pathol. **22**:294–295.
31. **Smith, C. R.** 1968. Mycobactericidal agents, p. 504–514. *In* C. A. Lawrence and S. S. Block (ed.), Disinfection, sterilization and preservation. Lea and Febiger, Philadelphia.
32. **Stamm, J. M., W. E. Engelhard, and J. E. Parsons.** 1969. Microbiological study of water-softener resins. Appl. Microbiol. **18**:376–386.
33. **U. S. Department of Health, Education and Welfare.** 1977. Isolation of mycobacteria species from porcine heart valve prosthesis. Morbid. Mortal. Weekly Rep. **26**:42–43.
34. **Vestal, A. L.** 1976. Procedures for the isolation and identification of mycobacteria. (revised ed.), Department of Health, Education and Welfare, publication no. CDC-76-8230. Center for Disease Control, Atlanta, Ga.