Factors Influencing Numbers of *Mycobacterium avium*, *Mycobacterium intracellulare* , and Other Mycobacteria in Drinking Water Distribution Systems

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Appl. Environ. Microbiol. **2001, 67(3):1225. DOI: 10.1128/AEM.67.3.1225-1231.2001.**

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2001, p. 1225–1231 Vol. 67, No. 3
0099-2240/01/$04.00+0 DOI: 10.1128/AEM.67.3.1225–1231.2001

Factors Influencing Numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and Other Mycobacteria in Drinking Water Distribution Systems

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Received 29 September 2000/Accepted 15 December 2000

Eight water distribution systems were sampled over an 18-month period (528 water and 55 biofilm samples) to measure the frequency of recovery and number of mycobacteria, particularly *Mycobacterium avium* and *Mycobacterium intracellulare*, in raw source waters before and after treatment and within the distribution system. The systems were chosen to assess the influence of source water, treatment, and assimilable organic carbon levels on mycobacterial numbers. Overall, mycobacterial recovery from the systems was low (15% of samples). Numbers of mycobacteria ranged from 10 to 700,000 CFU liter^{-1}. The number of *M. avium* in raw waters was correlated with turbidity. Water treatment substantially reduced the number of mycobacteria in raw waters by 2 to 4 log units. Mycobacterial numbers were substantially higher in the distribution system samples (average, 25,000-fold) than in those collected immediately downstream from the treatment facilities, indicating that mycobacteria grow in the distribution system. The increase in mycobacterial numbers was correlated with assimilable organic carbon and biodegradable organic carbon levels ($r^2 = 0.65$, $P = 0.03$). Although *M. intracellulare* was seldom recovered from water samples, it was frequently recovered (six of eight systems) in high numbers from biofilms (average, 600 CFU/cm^2). Evidently, the ecological niches of *M. avium* and *M. intracellulare* are distinct.

Members of the *Mycobacterium avium* complex (i.e., *M. avium* and *Mycobacterium intracellulare*) are environmental opportunistic human and animal pathogens (11, 21, 43). *M. avium* complex pulmonary infections are found in patients with predisposing lung conditions, such as silicosis and black lung (5, 43), and in patients with pulmonary alveolar proteinosis (42) and cystic fibrosis (23). Infections in elderly women without any of the known risk factors for *M. avium* complex infection have also been described (30). *M. avium*, but not *M. intracellulare*, infections are found in (and are limited to) the cervical lymph nodes of young children with erupting teeth (44). Immune deficiency resulting in AIDS (18) or due to interleukin-12 deficiency (1), malignancy (41), or immunosuppression associated with transplantation (33) is also a risk factor for *M. avium* complex infection. Infections in AIDS patients are disseminated (e.g., bacteremia [19]) and are almost entirely (i.e., 95%) due to *M. avium*, whereas both mycobacterial infections occur at equal frequencies in nonimmunodeficient patients with pulmonary disease (8, 17).

One source of *M. avium* infection in AIDS patients is water. DNA fingerprints of *M. avium* isolates from water to which AIDS patients were exposed were identical to those of the patients (38). Further, the high incidence of *M. avium* infections in AIDS patients in Finland correlated with high numbers of *M. avium* in drinking and environmental waters (31). Water also appears to be the source of *M. avium* infections in simian immunodeficiency virus-infected macaques, based on the identity of DNA fingerprints of *M. avium* recovered from the monkeys and their drinking water (25).

Members of the *M. avium* complex and other mycobacteria have been recovered from natural waters (12, 39) and drinking water systems (3, 6, 9, 15, 18) throughout the United States. The reported numbers of *M. avium* complex CFU per liter of sample have ranged from 0.8 to 100,000. *M. avium* complex organisms can grow in water (3, 14) and are highly resistant to ozone- and chlorine-based disinfectants (36). Further, *M. avium* numbers are higher in hospital hot water systems than in the source waters (10), and a single *M. avium* clone was shown to persist for as long as 41 months in a single water distribution system (38).

Although there have been reports of the presence of *M. avium* complex organisms in drinking water (3, 6, 9, 13, 15, 18, 29), there have been no systematic studies of individual systems over long periods of time and of the effects of water treatment practices. Thus, the basis for differences in numbers of *M. avium* complex organisms among systems is unknown. Because of the widespread prevalence of *M. avium* complex bacteria in drinking water, we sought to accurately describe their prevalence both in the source waters and within the distribution system and to identify factors associated with the presence of the *M. avium* complex. It was anticipated that the data could serve to guide the implementation of measures to control the numbers of *M. avium* complex organisms in drinking water. The specific objectives of the study included the measurement of the frequency of occurrence and the numbers of *M. avium* complex bacteria and other mycobacteria in

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TABLE 1. Characteristics of drinking water systems

Site	Source water	Disinfectant type (pre/post)[a]	AOC		Raw water NTU[b]
			Level	μg/liter (mean ± SD)	
1	Surface	Ozone/free Cl	High	234 ± 59	2.9
2	Surface	Free Cl/none	Moderate	113 ± 41	1.1
3	Surface	Free Cl/none	Low	61 ± 86	0.7
4	Ground	Free Cl/none	Low	28 ± 43	0.5
5	Surface	Free Cl/NH_2Cl	High	215 ± 110	57
6	Surface	Free Cl/NH_2Cl	Moderate	109 ± 80	28
7	Surface	Free Cl/NH_2Cl	Low	98 ± 53	7
8	Ground	Free Cl/NH_2Cl	Low	17 ± 9	0.5

[a] Pretreatment disinfectant/posttreatment or residual disinfectant in distribution system (NH_2Cl, monochloramine).
[b] NTU, nephelometric turbidity units (4).

water and biofilm samples collected from drinking water distribution systems throughout the United States. That data would be used to assess the efficacy of treatment in reducing *M. avium* complex numbers and to determine whether the growth of *M. avium* complex cells occurs in the distribution system.

MATERIALS AND METHODS

Description of the eight distribution systems. Three criteria were used to choose study distribution systems. Sites were selected on the basis of source of water (surface or ground), assimilable organic carbon (AOC) levels (high, moderate, and low), and type of disinfectant (chlorine, chloramine, or ozone). The eight sites and their characteristics are listed in Table 1.

Collection of water samples. Samples were collected monthly for each of the eight systems for a period of 18 months from October 1996 through March 1998. Water samples of 3 liters were collected at the following sites: 1, raw source water; 2, effluent from treatment plant; 3, middle of distribution system; and 4, dead ends of the distribution system. Only well, midpoint, and endpoint samples were collected from the two groundwater systems (sites 4 and 8). A total of 528 samples were collected. Sample containers were shipped containing 0.1 ml of 1% sodium thiosulfate to neutralize any free disinfectant immediately upon collection (4).

Collection of biofilm samples. Biofilm samples were received either as portions of pipe material or as water meters. Both were obtained as a consequence of routine maintenance (e.g., tapping new lines or replacing meters). The pipes or water meters were obtained at the study sites, wrapped in plastic wrap to prevent drying, and shipped by courier to the Virginia Polytechnic Institute and State University laboratory. A total of 55 biofilm samples were obtained from the eight study sites.

Recovery of mycobacteria from water. Water samples of 300 ml were centrifuged (5,000 × *g* for 20 min at 25°C), the pelleted cells were suspended in 1 ml of sterile distilled water, and 0.1-ml samples were spread on the surface of Middlebrook 7H10 agar medium (BBL Microbiology Systems, Cockeysville, Md.) containing 0.5% (vol/vol) glycerol and 10% oleic acid–albumin enrichment (M7H10). Each sample was spread on five plates, and the plates were incubated at 37°C and examined after 21 days of incubation. If the suspensions contained high numbers of nonmycobacterial colonies, cetylpyridinium chloride (CPC) was added to a final concentration of 0.005% (wt/vol) and the suspensions were incubated for 30 min at room temperature. The bacteria were pelleted by centrifugation (5,000 × *g* for 20 min at 25°C), the CPC-containing supernatant was discarded, and the cells were washed twice in homogenization buffer (2) and resuspended in 1 ml of sterile water before 0.1-ml volumes were spread. Again, each sample was spread on five plates, and the plates were examined after 21 days of incubation at 37°C.

Recovery of mycobacteria from biofilms. The material adhering to 4 cm^2 of the interior surface of either a pipe or a water meter was collected with a sterile Teflon scraper and suspended in 5 ml of homogenization buffer (2). The suspension was homogenized by three passages through a sterile tissue homogenizer, and the resulting suspension was plated as described for water samples. Because of the high numbers of bacteria in biofilm suspensions, CPC disinfection was quite commonly used.

Enumeration and identification of mycobacteria. Following 21 days of incubation at 37°C, the total number of colonies on each plate was counted; 18 colonies from a representative area of a plate (or plates) were picked and acid-fast stained. A representative area was chosen to avoid bias due to colony selection, and 18 colonies were picked to identify only isolates that represented greater than 5% of the total population (27). Any acid-fast colonies were streaked for isolation on M7H10 agar medium. Plates were incubated at 37°C, and colony formation and pigmentation were scored daily. Slowly growing (i.e., colony appearance after 7 days of incubation) and rapidly growing colonies were identified as representatives of the genus *Mycobacterium* or *M. avium* or *M. intracellulare* using a multiplex PCR (40). For *Mycobacterium* isolates that did not display the bands characteristic of either *M. avium* or *M. intracellulare*, PCR amplification of the *hsp-65* gene followed by *Bst*EII and *Hae*III restriction endonuclease digestion of the PCR product was used for identification (35, 37). Identification of *Mycobacterium* sp. isolates whose restriction patterns were not represented (35, 37) was carried out by cultural, biochemical, and enzymatic characterizations (16). Following identification, the total number of CFU of each species per liter of original sample was calculated.

Physiochemical characteristics of water samples. Data were obtained for the following parameters to describe the characteristics of each system: heterotrophic plate bacteria, coliforms, turbidity, residual disinfectant, pH, alkalinity, hardness, ammonia, nitrate, anions determined by ion chromatography (sulfate, chloride, and phosphate), heavy metals (zinc, lead, and copper), and total organic carbon levels (4). AOC (4) and biodegradable organic carbon (BDOC) (22) levels were measured for plant effluent samples.

RESULTS

***M. avium* complex and other mycobacteria in water samples.** A total of 528 water samples were collected over an 18-month period (October 1996 through March 1998) from the eight study sites. A total of 135 water samples yielded 708 individual acid-fast isolates, 307 of which were *Mycobacterium* spp. Among those isolates, 47 were identified as *M. avium* and 15 were identified as *M. intracellulare*. Eleven *Mycobacterium* sp. isolates recovered from water samples have not been identified. Fifteen percent of samples yielded slowly growing *Mycobacterium* spp., 2% yielded rapidly growing *Mycobacterium* spp., 3% yielded *M. avium*, and 1% yielded *M. intracellulare*. No samples were lost to contamination or overgrowth of other microorganisms.

The frequencies of recovery and the numbers of *M. avium* and *M. intracellulare* organisms in the drinking water samples were low (Tables 2 and 3). Because of the low numbers of recovered mycobacteria and the possible overgrowth of mycobacterial colonies by those of faster-growing bacteria, the values for the density of mycobacteria (Table 3) should be taken as estimates. Generally, *M. avium* was recovered from water samples that contained high microbial numbers. This result suggests that there was poor recovery or a low efficiency of plating of *M. avium. M. avium*, *M. intracellulare*, and other mycobacteria were seldom recovered from well water, in agreement with earlier data (26). *M. intracellulare* was seldom recovered from either raw or system water samples (Table 2).

In addition to *M. avium* and *M. intracellulare*, representatives of other *Mycobacterium* species were recovered from the water samples. Isolates of the slowing growing mycobacteria *Mycobacterium shimoidei* and *Mycobacterium szulgai* were isolated from raw water from system 7, *Mycobacterium haemophilum* was isolated from a distribution water sample from system 4, and *Mycobacterium marinum* was isolated from the raw water from system 6. *Mycobacterium terrae* was isolated from all eight systems, and *Mycobacterium gordonae* was isolated from all but system 3. Isolates of rapidly growing mycobacteria were also recovered. *Mycobacterium chelonae* was isolated from systems 1 and 7, *Mycobacterium flavescens* and *Mycobacterium fortuitum* were isolated from systems 5 and 7, *Mycobacterium abscessus*

TABLE 2. Frequency of recovery of mycobacteria from water

Site	Samples		No. (%) of samples yielding			
			Mycobacterium spp.		*M. avium*	*M. intracellulare*
	Type[a]	No.	Slowly growing	Rapidly growing		
1	Raw	17	6 (35)	1 (6)	1 (6)	1 (6)
	Plant	17	0	0	0	0
	Distr	34	0	0	0	0
2	Raw	18	3 (17)	0	0	0
	Plant	18	0	0	0	0
	Distr	36	1 (3)	0	0	1 (3)
3	Raw	18	2 (11)	1 (6)	1 (6)	0
	Plant	18	0	0	0	0
	Distr	36	2 (6)	0	0	0
4	Well	16	0	0	0	0
	Distr	34	10 (29)	0	2 (6)	1 (3)
5	Raw	17	9 (53)	3 (18)	5 (29)	1 (6)
	Plant	17	1 (6)	0	0	0
	Distr	34	4 (12)	0	1 (3)	0
6	Raw	18	7 (39)	0	2 (11)	0
	Plant	18	2 (11)	0	0	0
	Distr	36	6 (17)	0	0	3 (8)
7	Raw	18	10 (56)	4 (22)	4 (22)	0
	Plant	18	4 (22)	0	0	0
	Distr	36	11 (31)	2 (6)	0	0
8	Well	18	2 (11)	0	0	0
	Plant	36	1 (3)	0	0	0
Total	All	528	81 (15)	11 (2)	16 (3)	7 (1)

[a] Raw, raw source water; plant, plant effluent after treatment; distr, distribution system samples (midpoint and endpoint).

was isolated from system 7, and *Mycobacterium aurum* was isolated from system 3. Apart from the numbers of the slowly growing *M. terrae* and *M. gordonae*, the numbers of the other mycobacteria were low (i.e., <10 CFU liter^{-1}). There were differences between systems in the spectra of mycobacterial species recovered and numbers, suggesting that each drinking water system had a unique mycobacterial flora. For example, system 7 raw water had a wide diversity of mycobacterial species, including *M. shimoidei*, *M. szulgai*, *M. terrae*, *M. gordonae*, *M. fortuitum*, *M. abscessus*, *M. flavescens*, and *M. aurum*, whereas the well water samples of system 8 yielded only *M. terrae*.

The number of water samples yielding *Mycobacterium* or *M. avium* was higher in the two 6-month periods from October 1996 through March 1997 (12 of 48) and October 1997 through March 1998 (20 of 48) than in the single period from April 1997 through September 1997 (10 of 48). However, the differences were not statistically significant.

M. avium, *M. intracellulare*, and other mycobacteria were recovered from samples collected throughout the distribution systems (Table 2). Raw water samples more frequently yielded and had higher numbers of *M. avium* complex organisms than did samples collected immediately following disinfection (i.e., plant entries in Tables 2 and 3). In system 7, 10 of 18 raw water and 4 of 18 plant effluent samples yielded slowly growing *Mycobacterium* isolates (Table 2). However, the number of slowly growing *Mycobacterium* isolates was higher in the plant samples than in the raw water (Table 3). The reason for the higher numbers was not evident from a comparison of the characteristics of that system with those of the other surface water systems. The average reduction of slowly growing *Mycobacterium* sp. numbers (i.e., plant CFU/raw CFU) was almost 2 log units (1.98), with a range of 1.96 to 4 log units, for the five surface water systems (Table 3). This result demonstrated the efficacy of treatment processes in reducing the number of *M. avium* cells. However, *M. avium* and *M. intracellulare* cells were recovered from distribution system samples, although they were not isolated from the raw waters (i.e., sites 2, 3, and 5), suggesting that the organisms were resident in the distribution systems, independent of the source waters.

One objective of the study was to test the hypothesis that mycobacteria can grow in distribution systems and that AOC or BDOC levels influence growth. Accordingly, a value for growth in the distribution system was calculated for slowly growing *Mycobacterium* spp. (i.e., average CFU liter^{-1} for midpoint and endpoint divided by CFU liter^{-1} for plant effluent). The calculation was not made for *M. avium*, *M. intracellulare*, or other mycobacteria because there was an insufficient number of values. In the seven systems where mycobacteria were recovered from the distribution systems (system 1 excluded), slowly growing *Mycobacterium* sp. colony counts increased in six and decreased in one (system 8). Three systems (i.e., 2, 6, and 7) had moderate levels of growth in the distribution sys-

TABLE 3. Number of mycobacteria in water samples

Site	Sample		CFU of the following liter of sample^{-1b}:			
			Mycobacterium spp.		*M. avium*	*M. intracellulare*
	Type[a]	No.	Slowly growing	Rapidly growing		
1	Raw	17	760	720	100	40
	Plant	17	<10	<10	<10	<10
	Distr	34	<10	<10	<10	<10
2	Raw	18	20	<10	<10	<10
	Plant	18	<10	<10	<10	<10
	Distr	36	20	<10	<10	20
3	Raw	18	2,200	2,000	40	<10
	Plant	18	<10	<10	<10	<10
	Distr	36	3,200	<10	<10	<10
4	Well	16	<10	<10	<10	<10
	Distr	34	72,000	<10	170	880
5	Raw	17	55,000	5,500	14,000	110
	Plant	17	40	<10	<10	<10
	Distr	34	700,000	<10	20	<10
6	Raw	18	10,000	<10	3,000	<10
	Plant	18	830	<10	<10	<10
	Distr	36	4,100	<10	<10	160
7	Raw	18	14,000	20	1,800	<10
	Plant	18	220,000	<10	<10	<10
	Distr	36	240,000	50	<10	<10
8	Well	18	21,000	<10	<10	<10
	Distr	36	5,700	<10	<10	<10

[a] Raw, raw source water; plant, plant effluent after treatment; distr, distribution system samples (midpoint and endpoint).
[b] Limit of detection, 10 CFU liter^{-1}.

TABLE 4. Frequency of recovery of mycobacteria from biofilm samples

Site	No. of samples tested	No. (%) of samples yielding			
		Mycobacterium spp.		*M. avium*	*M. intracellulare*
		Slowly growing	Rapidly growing		
1	6	6 (100)	0	0	2 (33)
2	9	3 (22)	1 (11)	0	0
3	6	2 (33)	0	0	1 (17)
4	7	4 (57)	0	0	0
5	6	6 (100)	0	1 (17)	4 (67)
6	6	1 (17)	0	0	1 (17)
7	9	9 (100)	1 (11)	2 (22)	4 (44)
8	6	3 (50)	0	0	1 (17)
Total	55	34 (62)	2 (4)	3 (5)	13 (24)

tems (average, 2.7-fold increase). Three systems (i.e., 3, 4, and 5) had substantial increases (average, 61,000-fold increase).

Associations between waterborne mycobacteria and system characteristics. Comparisons between mycobacterial numbers in water samples and physiochemical characteristics of the systems identified some statistically significant associations. High raw water turbidity (i.e., >2 nephelometric turbidity units) was significantly associated with *M. avium* ($r^2 = 0.93$, $P = 0.0001$) and slowly growing ($r^2 = 0.73$, $P = 0.0073$) and rapidly growing ($r^2 = 0.62$, $P < 0.02$) *Mycobacterium* sp. colony counts per milliliter of raw water. Only the frequency of recovery of *M. avium* from water samples (Table 2) was significantly associated with high turbidity ($r^2 = 0.63$, $P < 0.02$). That association can be demonstrated by an example. Heavy rains in January 1997 led to flooding and an increase in raw water turbidities at system 1. A raw water sample collected at that time was the only raw water sample from that system to yield *M. avium* (130 CFU liter^{-1}). There was no association between *M. intracellulare* numbers in raw water samples and turbidity. There were significant associations between colony counts per milliliter of raw water for rapidly growing *Mycobacterium* spp. and *M. avium* ($r^2 = 0.79$, $P = 0.0034$) or slowly growing *Mycobacterium* spp. ($r^2 = 0.65$, $P < 0.02$). These data suggest that enumeration of rapidly growing mycobacteria can be used as a surrogate for the numbers of *M. avium* complex cells and other slowly growing *Mycobacterium* cells in drinking water. There were no correlations between mycobacterial numbers and raw water pH or colony counts of heterotrophic bacteria and fecal or total coliforms.

There were no statistically significant associations between colony counts for any of the mycobacterial groups and system characteristics, including residual disinfectant concentration, AOC, BDOC, NO_3, PO_4, alkalinity, and hardness (alone or in combination). However, there was a positive association between AOC ($r^2 = 0.65$, $P = 0.029$) and BDOC ($r^2 = 0.64$, $P = 0.031$) concentrations and the calculated growth values for slowly growing *Mycobacterium* spp. in the distribution systems. Further, AOC and BDOC concentrations were highly correlated ($r^2 = 0.81$, $P = 0.002$). Thus, the growth of slowly growing *Mycobacterium* spp. appears to be limited by the AOC levels in distribution system waters.

***M. avium* complex and other mycobacteria in biofilms.** A total of 55 biofilm samples were collected over an 18-month period (October 1996 through March 1998) from the eight study sites. The majority of the biofilm samples (76%) were collected from water meters. The average age of the water meters was 21.6 years (range, 5 to 70 years). Because water meters are placed at residences or places of business, they are at the ends of the distribution systems. Pipe sections were taken from main lines of the distribution systems and thus represented the midpoint of the systems. Biofilm samples from water meters seldom required CPC treatment, whereas those from pipe sections were quite thick and were always treated with CPC.

Forty biofilm samples (69%) (Table 4) yielded 450 individual acid-fast isolates, 267 of which were *Mycobacterium* spp. The only biofilm samples that yielded *Mycobacterium* sp. isolates were recovered from water meters. The pipe sections had thicker biofilms, required disinfection, and had higher numbers of CFU even after disinfection. Thus, it was possible that any mycobacterial colonies were overgrown and were not isolated when 18 colonies were picked. Thirteen of the 40 biofilm samples that yielded acid-fast isolates contained *M. intracellulare*, and at least one biofilm sample from every site except sites 2 and 4 contained *M. intracellulare* (Table 4). Among the 267 biofilm *Mycobacterium* sp. isolates, 131 were identified as *M. intracellulare* and 4 were identified as *M. avium*. The average number of *M. intracellulare* organisms in systems yielding that mycobacterium was 600 CFU cm^{-2} (range, 1 to 2,850) (Table 5). As was noted for water samples, the mycobacterial biofilm densities (Table 5) should be taken as estimates only. *M. avium* was recovered from biofilms from only two of the eight distribution systems (systems 5 and 7 in Table 4), and the numbers were low (<0.5 CFU cm^{-2}) (Table 5). Thirty *Mycobacterium* sp. isolates recovered from biofilm samples have not been identified. No samples were lost to contamination, although the overgrowth of other microorganisms may have prevented the detection of infrequent mycobacterial colonies.

The material supporting the biofilms did not appear to make a difference in the frequency of recovery of mycobacteria. *Mycobacterium* spp. were recovered from biofilms growing on brass or bronze (63%), galvanized (100%), or plastic (64%) surfaces, and *M. intracellulare* was isolated from 25% of brass or bronze, 0% of galvanized, and 43% of plastic surfaces. The

TABLE 5. Number of mycobacteria in biofilm samples

Site	No. of samples tested	CFU of the following cm^{-2}[a]:			
		Mycobacterium spp.		*M. avium*	*M. intracellulare*
		Slowly growing	Rapidly growing		
1	6	4,300	<0.01	<0.01	2,900
2	9	1.4	4.8	<0.01	<0.01
3	6	650	<0.01	<0.01	430
4	7	85	<0.01	<0.01	<0.01
5	6	71	<0.01	0.2	20
6	6	2	<0.01	<0.01	1.3
7	9	570	0.6	0.3	17
8	6	880	<0.01	<0.01	274
Avg[b]		820	2.7	0.3	600

[a] Limit of detection, <0.01 CFU cm^{-2}.
[b] Average CFU per square centimeter of biofilm samples yielding mycobacteria.

number of samples in each category was too small for the differences to be statistically significant. The surface composition might influence the numbers of *Mycobacterium* spp. and *M. intracellulare* in biofilms. The average numbers of *Mycobacterium* spp. were 2,100 CFU cm^{-2} on bronze or brass, 130 on galvanized, and 78 on plastic surfaces. The numbers of *M. intracellulare* CFU per square centimeter on brass or bronze surfaces were 4,400; the corresponding value for plastic surfaces was 70. However, the different surfaces were necessarily in different systems, each with a unique mycobacterial flora. Proof that surface composition influences mycobacterial numbers will require comparison of different surfaces in the same system.

There was no apparent effect of residual disinfectant type or concentration on the frequency of recovery of *Mycobacterium* spp. or *M. intracellulare* from biofilm samples (Table 3). Biofilm samples from systems using monochloramine yielded both *Mycobacterium* and *M. intracellulare* isolates.

Although *M. intracellulare* was not recovered from samples from two of the three systems using free chlorine as the only disinfectant (i.e., systems 2 and 4), the number of *M. intracellulare* CFU per square centimeter for the other, system 3, was quite high (Table 5); this result suggests that free chlorine was not capable of reducing biofilm numbers. Because the pipe sections and water meters were obtained only as a consequence of normal operations of the collaborating water systems, they were not collected on a regular basis. That fact, coupled with the fact that only 55 samples were obtained from the eight systems, prevented any analysis of the effect of season on the recovery of mycobacteria from the meters.

In addition to members of the *M. avium* complex, representatives of other slowly growing *Mycobacterium* species were recovered. Biofilm samples yielded *M. haemophilum* from systems 6 and 7, *M. shimoidei* from system 5, and *M. marinum* from system 1. *M. terrae* was isolated from biofilm samples collected from systems 1, 3, 4, 5, and 7, and *M. gordonae* was isolated from systems 1, 4, 5, 6, 7, and 8. Rapidly growing mycobacteria were rare in biofilm samples. Only *M. aurum* (site 2) was recovered from the biofilm samples. Although *M. flavescens* was shown to readily form biofilms (30) and was isolated from raw water samples collected from systems 5 and 7, it was not recovered from distribution water or biofilm samples. Possibly, its numbers in raw water were too low to permit survival following treatment.

Associations between biofilm mycobacteria and system characteristics. There were no statistically significant associations between biofilm colony counts for any of the mycobacterial groups and system characteristics, including residual disinfectant concentration, AOC, BDOC, NO_3, PO_4, alkalinity, and hardness (alone or in combination). Although biofilm samples collected from systems with higher AOC and BDOC concentrations did yield higher numbers of biofilm *M. intracellulare* and slowly growing *Mycobacterium* sp. organisms (Table 5), the associations were weak and not significant.

DISCUSSION

Although the data confirm that members of the genus *Mycobacterium* are present in drinking waters, the numbers and frequencies of recovery of *M. avium* and *M. intracellulare* are low in drinking waters. In this study, at least one water sample from every system yielded mycobacteria, and either *M. avium* or *M. intracellulare* was isolated from every system, except system 8, a well water system (Table 2). The frequencies of recovery of *M. avium* and *M. intracellulare* from drinking water samples reported here were lower than (6) or similar to (13, 15) values reported previously. Although other species of mycobacteria were recovered, their numbers, with the exception of *M. terrae* and *M. gordonae*, were low and isolation was infrequent. Interestingly, *Mycobacterium scrofulaceum*, which used to be frequently isolated from water samples (12), was not isolated in this study. Because isolation is rather infrequent and numbers of mycobacteria are low, single samples from separate systems are unlikely to yield mycobacteria. The data demonstrate that one factor influencing mycobacterial recovery is the system. Some systems are characterized by an absence of mycobacteria in raw water (e.g., well water sources), some are characterized by the presence of mycobacteria in raw water but their absence in the distribution system (e.g., system 1), and some are characterized by substantial numbers in raw water and the distribution system.

There was no strong seasonal effect on mycobacterial recovery, perhaps because these organisms are permanent residents in the systems. Samples collected in the fall and winter more frequently yielded mycobacteria than did those collected in the spring and summer. In contrast, spring potable water samples collected in the Czech Republic had a higher frequency of mycobacteria than did samples collected in the fall (24). However, this result occurred only when the medium was incubated at 25°C. Recovery frequencies for spring and fall samples were similar when incubation was carried out at 37°C (24). Thus, incubation temperature influences recovery of mycobacteria.

Although the results document the presence of *M. avium* and other mycobacteria in drinking water systems, the efficacy of current treatment methods for reducing their numbers is also demonstrated. In five of six systems using surface source water, current treatment methods decreased mycobacterial numbers by almost 2 log units (Table 3). The reason for the failure of the system 7 treatment plant to reduce mycobacterial numbers has not been identified. Because of the resistance of *M. avium* (36) and other mycobacteria (3, 28) to chlorine, chloramine, and ozone, mechanisms other than disinfection (e.g., filtration) must have been responsible for the reduction in mycobacterial numbers in the raw source waters. The demonstration that *M. avium* and *Mycobacterium* sp. numbers in raw water samples correlated with turbidity suggests that reductions in turbidity ought to be associated with decreases in mycobacterial numbers entering treatment plants. The fact that the increased number of slowly growing *Mycobacterium* spp. in the distribution system was correlated with AOC and BDOC levels offers a second approach to reducing mycobacterial numbers in drinking water. A reduction in AOC and BDOC levels would be expected to result in less growth of mycobacteria in the distribution system.

This is the first study designed to measure the frequencies of *M. avium*, *M. intracellulare*, and other mycobacteria in biofilms in a wide range of drinking water distribution systems. Biofilms in drinking water distribution systems appear to be rich sources of mycobacteria. The values for CFU per square centimeter are 1 order of magnitude lower than those for silicon tube biofilms formed in a warm water distribution system (32) and

are of the same order of magnitude as those for biofilms recovered from drinking water systems in Finland (20). Biofilms are significant sources of mycobacteria in drinking water systems. Although bronze and brass surfaces had higher numbers of *M. intracellulare* and other mycobacteria in biofilms, all surfaces examined had biofilms with mycobacteria. The length of pipe in the collaborating drinking water systems ranged from 75 to 7,100 miles. If the biofilms sampled are representative of the entire systems, the numbers of mycobacteria, in particular, *M. intracellulare*, are enormous.

The results also provide insight into the ecology of the different species. In particular, the positive association between *M. avium* CFU and raw source water turbidity suggests that *M. avium* cells were bound to colloidal or suspended particles. Therefore, one approach to reducing the numbers of *M. avium* organisms in drinking water would be to reduce the turbidity of the raw water of the system. However, that procedure might not be sufficient to completely eliminate *M. avium* from a distribution system. The presence of *M. avium* was not always associated with recovery from raw source water. For example, *M. avium* was not recovered from raw water samples from site 3 but was recovered from distribution system samples (Table 2). Persistence in a drinking water system may be due, in part, to the presence of *M. avium* in biofilms. Thus, in confirmation of other data (10, 38), *M. avium* should be considered a normal inhabitant of drinking water systems.

M. intracellulare appears to occupy a different environmental niche than *M. avium* in drinking water systems and, by extension, in natural waters. Although *M. intracellulare* was seldom recovered from water samples, including raw water and system samples (Table 2), it was recovered frequently from biofilm samples (Table 4). Further, there were high numbers of *M. intracellulare* organisms in biofilms (average, 600 CFU cm^{-2}) (Table 5). Therefore, one normal habitat of *M. intracellulare* is the biofilm of a distribution system, and the source of *M. intracellulare* in suspension would be the biofilm.

The recovery of two *M. haemophilum* isolates from biofilm samples suggests that this species, like *M. intracellulare*, resides normally in biofilms. The recovery of these isolates from biofilm and from water was surprising in light of their requirement for chelated iron (7, 34). The isolates formed larger colonies on M7H10 agar containing 60 μM hemin than on that agar lacking hemin. They also shared the published restriction endonuclease fragment patterns of the amplified *hsp-65* gene (37) and those of a clinical isolate of *M. haemophilum*. Recovery on the primary isolation medium was likely due to the presence of other bacteria that could have provided chelated iron. Further, it is not unusual for mycobacterial colonies to be quite small. The recovery of two of the three *M. haemophilum* isolates from biofilms suggests that appropriate samples for the detection of *M. haemophilum* are biofilms rather than water.

These results demonstrate that accurate detection of mycobacteria in a drinking water distribution system requires repeated sampling of both water and biofilms, because the distributions of individual mycobacterial species between suspended and attached fractions are not the same. For example, the data in this report suggest that it would be inappropriate to determine whether *M. intracellulare* is present in a distribution system by testing water samples alone. Fortunately, water meters can be used to sample this portion of the mycobacterial flora of drinking water systems. In addition, water meters are relatively easy (compared to pipe sections) to obtain for sampling.

ACKNOWLEDGMENTS

This work was supported by a grant from the American Water Works Association Research Foundation (AWWARF).

These investigations could not have been carried out without the cooperation of the eight study site water systems and the guidance of the AWWARF Project Advisory Committee and Emerson Lomaquahu of the AWWARF. The *M. haemophilum* isolate was generously provided by Timothy E. Kiehn of the Sloan-Kettering Cancer Center. We acknowledge the expert technical assistance of Myra D. Williams.

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