

Isolation of *Mycobacterium avium* Complex from Water in the United States, Finland, Zaire, and Kenya

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Disseminated infection with organisms of the *Mycobacterium avium* complex (MAC) is a common complication of AIDS in the United States and other developing countries, but it is rare or absent in sub-Saharan Africa. To assess the comparative likelihood of exposure to MAC in these geographic areas, we used a standard protocol to culture 91 water samples from environmental sites and piped water supply systems in the United States, Finland, Zaire, and Kenya. MAC was isolated from all geographic areas and from 22 of 91 (24%) samples. Isolation rates were 13 of 47 (28%) for environmental samples and 9 of 44 (20%) for water supply samples. Overall isolation rates were 18 of 52 (35%) samples in the United States and Finland, whereas they were 4 of 39 (10%) samples in Zaire and Kenya ($P = 0.015$). MAC isolation rates from water supply systems were 8 of 25 (32%) samples in the United States and Finland and 1 of 19 (5%) samples in Zaire and Kenya ($P = 0.056$). MAC was isolated from hospital water in the United States and Finland but not in hospital water in Zaire and Kenya. Serovar determinations showed that six of eight isolates from the United States were serovar 4 or 8. One MAC isolate from Zaire was identified as an "X" mycobacterium. These data suggest that exposure to MAC in water is likely in diverse areas of the world, but that the likelihood of human exposure to the organism in water may be slightly less in sub-Saharan Africa than in developed countries in the Northern Hemisphere.

Organisms of the *Mycobacterium avium* complex (MAC) are environmental mycobacteria which cause disseminated infection in as many as 20 to 40% of patients with late-stage AIDS in the United States and other developed countries (11, 14, 18, 19). MAC can be isolated from numerous sources in the environment, including water, aerosols, soil, mud, and plants (7). Although the specific environmental source of most MAC infections in patients with AIDS is not known, water or water aerosols are a leading possibility (11). Exposure to water is common enough to explain a disease which affects as many as 40% of people with AIDS. In addition, nosocomial common source outbreaks of other nontuberculous mycobacteria have been traced to contaminated water (1-3, 16).

Although studies from various parts of the world have shown the widespread distribution of environmental MAC isolates, disseminated MAC infection has not been found in clinical and autopsy studies of patients with AIDS in Africa (17, 24). This observation could be explained by variations in the environmental distribution of MAC, differences in behaviors which result in exposure to MAC, or differences in host resistance to MAC. We examined these issues in an international epidemiologic study of disseminated MAC in-

fection in patients with AIDS at five geographically diverse sites chosen to represent areas where the prevalence of disseminated MAC in patients with AIDS was expected to be high (United States [two sites], Finland) or low (Kenya, Zaire). In this report we describe the results for cultures of water samples obtained from these same five sites to assess the relative likelihood of human exposure to MAC in each of these areas.

(The study described here was presented in part at the 93rd General Meeting of the American Society for Microbiology, Atlanta, Ga., 16 to 20 May 1993 [6a].)

MATERIALS AND METHODS

Collection of water samples. Water samples were collected from numerous environmental sites (including rivers, streams, lakes, ponds, harbors, marshes, and standing water) as well as from piped water supply systems (including piped municipal and private drinking water supplies) from March 1990 through February 1992. Samples were collected in sterile 250-ml wide-mouth plastic bottles at ambient temperature during one or more different months (New Hampshire, February and March; Boston, February, August, and October; Finland, February, May, and July; Kenya, January and April; Zaire, December). Bottles were either dipped into the water (for most environmental samples) or filled from a

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tap. In most cases the bottles were filled completely; the minimum volume collected was 100 ml per sample. Bottles were sealed with Parafilm (American National Can, Greenwich, Conn.), capped, and shipped to Virginia for processing. All samples were processed within 5 weeks of collection.

Processing of water samples. Twenty-five-milliliter water samples were centrifuged at $5,000 \times g$ for 30 min at 22°C in sterile, 50-ml screw-cap centrifuge tubes. The supernatant was decanted and its pH was measured. The pellet was suspended in 1 ml of sterile distilled water by vortexing, and 0.1 ml (in triplicate) of the liquid was spread to dryness onto Tsukamura minimal-Tween 80-cycloheximide agar medium plates (8) containing 0.073 M KH_2PO_4 , 0.002 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 M $(\text{NH}_4)_2\text{SO}_4$, 1% (vol/vol) Tween 80 (Sigma Chemical Co., St. Louis, Mo.), and 1.5% agar (BBL Microbiology Systems, Cockeysville, Md.). The pH of the medium was adjusted to 5.5 with KOH, and the medium was sterilized by autoclaving. To prevent fungal growth, cycloheximide (Sigma Chemical Co.) at 500 mg/liter was added to cooled sterile medium before medium was poured into the plates. After the liquid was spread, the plates were sealed with Parafilm (American National Can) and were incubated at 37°C in air. Colony formation was observed daily for up to 60 days.

Identification of organisms. Colonies of different morphologies were stained by the Ziehl-Neelsen method, and acid-fast colonies of the same morphology were counted. Representatives of each colony type were streaked on Lowenstein-Jensen slants or Middlebrook 7H10 agar plates, and the slants and plates were incubated at 37°C in air. Growth, as judged by the appearance of colonies, was followed daily for up to 60 days. Rapidly growing mycobacteria (i.e., growth within 7 days) were discarded. Slowly growing mycobacteria (i.e., growth after more than 7 days) were kept for identification. Pigmentation was noted, and isolates were grown in 10 ml of Middlebrook 7H9 broth for serotyping and identification by DNA probe analysis and were streaked onto Middlebrook 7H10 agar medium plates to ensure pure cultures. Putative MAC isolates were identified by using DNA probes, and organisms which hybridized with the MAC probes were then tested with probes to distinguish *M. avium* from *Mycobacterium intracellulare* and "X" mycobacterium (SNAP DNA Probe; Digene Diagnostics Inc., Silver Spring, Md., or Gen-Probe, San Diego, Calif.). Selected MAC isolates were serotyped by the State Health Laboratory, Queensland Department of Health, Brisbane, Queensland, Australia (5).

RESULTS

MAC isolates were recovered from water samples collected at each of the five geographic locations and were recovered as frequently from piped water supply systems as from environmental sites (Table 1). Isolation rates were higher from samples collected in developed countries than from those collected in developing countries: 18 of 52 (35%) samples from the United States and Finland compared with 4 of 39 (10%) samples from Zaire and Kenya ($P = 0.015$). Concentrations of MAC in water samples positive for MAC ranged from 0.2 to 1,000 CFU/ml; median concentrations were 0.4 to 40 CFU/ml at the five sites and did not differ among the geographic sites. Two environmental sources yielded 1,000 CFU of MAC per ml: the Charles River in Boston and lake water from a bucket in a Finnish sauna. MAC was isolated during 6 of the 8 months when samples

TABLE 1. Recovery of MAC from water by geographic site and source of sample

Site	No. of MAC-positive samples/no. of samples (%)		
	Environmental ^a	Water supply ^b	Total
New Hampshire	2/6 (33)	1/6 (17)	3/12 (25)
Boston	5/13 (38)	2/8 (25)	7/21 (33)
Finland	3/8 (37)	5/11 (45)	8/19 (42)
Zaire	1/3 (33)	1/5 (20)	2/8 (25)
Kenya ^c	2/17 (12)	0/14 (0)	2/31 (6)
Total	13/47 (28)	9/44 (20)	22/91 (24)

^a Environmental samples were collected from rivers, streams, lakes, ponds, harbors, marshes, and standing water.

^b Water supply samples were collected from wells, hot and cold municipal water supplies, showers, and standpipes.

^c Includes five water samples collected from northern Tanzania.

were collected (exceptions were May in Finland and February in New Hampshire).

Among samples collected from water supply systems, MAC isolation rates were 8 of 25 (32%) samples from developed countries (New Hampshire, Boston, and Finland) and 1 of 19 (5%) samples from developing countries (Kenya and Zaire) ($P = 0.056$; Fisher's exact test). MAC was not isolated from water supply systems in Kenya. MAC could be recovered from hospital water supplies in the United States and Finland but not from hospital water supplies in Kenya and Zaire (Table 2). Among water supply systems, MAC was isolated from both hot water (4 of 14 [29%] samples) and cold water (5 of 30 [17%] samples) ($P = 0.61$). Five water supply samples (all culture negative) were collected from wells; the remaining 39 samples were collected from municipal chlorinated water systems (chlorine levels were not measured). Pipes for municipal water supplies were made from a variety of materials, including cement, iron, and plastic. Pipes in hospital water systems were also constructed from a variety of materials; galvanized pipes were used only in the two Finnish hospitals (for cold water).

Among samples collected from the environment, MAC isolation rates were not significantly different in developed (10 of 27 [37%] samples) and developing (3 of 20 [15%] samples) countries ($P = 0.18$). MAC was isolated more frequently from rivers and streams (7 of 12 [58%] samples) than from lakes and ponds (2 of 16 [12%] samples) ($P = 0.017$; Fisher's exact test). Most water samples had pH values from 6.0 to 7.9; MAC isolation rates were not related to pH within this narrow range.

MAC was isolated from water samples that were processed as long as 5 weeks after collection, including one set

TABLE 2. Recovery of MAC from water collected from patient care facilities (clinics and hospitals)

Site	No. of MAC-positive samples/no. of samples (%)		
	Hot water	Cold water	Total
New Hampshire	1/2 (50)	0/2 (0)	1/4 (25)
Boston	1/3 (33)	0/4 (0)	1/7 (14)
Finland	2/5 (40)	3/5 (60)	5/10 (50)
Zaire	0/1 (0)	0/2 (0)	0/3 (0)
Kenya	0/2 (0)	0/4 (0)	0/6 (0)
Total	4/13 (31)	3/17 (18)	7/30 (23)

of water samples collected in Finland in which six of eight samples were positive for MAC. MAC isolation rates did not differ by interval from the time of collection to processing, as follows: ≤ 1 week, 7 of 25 (28%) samples; 2 weeks, 5 of 32 (16%) samples; 3 weeks, 0 of 4 (0%) samples; 4 weeks, 2 of 8 (25%) samples; 5 weeks, 8 of 22 (36%) samples.

The 22 positive samples yielded 29 MAC isolates with distinct colonial morphologies. Species probes were performed on 24 isolates; 23 were *M. avium* and one was an "X" mycobacterium. The serovars of eight *M. avium* isolates from four samples collected in New Hampshire and Boston were determined, with the following results: serovar 4, two isolates; serovar 8, four isolates; multiple serovars, one isolate; and nontypeable, one isolate. One MAC isolate from Zaire was identified as an "X" mycobacterium (4).

DISCUSSION

Previous environmental surveys have documented the presence of MAC in water and other environmental samples from both Africa and the United States (7, 10, 17, 20), but this is the first study to compare by similar methods rates of isolation of MAC from diverse geographic sites in both developed and developing countries. Overall MAC isolation rates were higher in developed countries than in developing countries, principally because of higher isolation rates from piped water supply systems. The use of water for drinking and bathing (piped water supply systems in the present study) is more likely than the use of water present in rivers, lakes, and other environmental sources to result in exposure of adults to MAC. Thus, the higher rates of isolation of MAC from water supply systems in the United States and Finland compared with those from water supply systems in Kenya and Zaire are especially significant; many patients with late-stage AIDS in developed countries have been hospitalized and exposed to hospital water. Other studies have documented the presence of MAC in chlorinated hospital water, especially hot water, with high concentrations of organisms in shower heads (6). We did not test sufficient numbers of samples to determine whether MAC is more common in hospital than in residential water supplies. However, some hospitals use galvanized pipes made with zinc alloys, and natural waters with zinc concentrations of >0.75 mg/liter are more likely to contain MAC (12). Furthermore, continuous recirculation of hot water in hospitals may allow mycobacteria to persist and multiply (6, 15).

Some human MAC infections may be caused by recreational exposure to natural water sources or inhalation of aerosols in the vicinity of natural bodies of water. In addition, surface water may serve as the source of water for municipal water supplies. In the present study, MAC was found in numerous natural water sources and was more likely to be isolated from streams and rivers than from standing bodies of water. There are several possible explanations for this observation. Rivers and streams collect runoff from a wider variety of sources and may contain more organic material or elements (e.g., zinc) conducive to the growth of MAC. Results of studies in Finland in which nontuberculous mycobacteria were recovered from 100% of brook water samples indicate that low pH and high organic content favor the isolation of mycobacteria (13).

The lower recovery rates of MAC from one set of samples from Zaire (processed at 3 weeks) and two sets of samples from Kenya (processed at 2 and 5 weeks, respectively) were not explained by delays in processing. MAC isolates are extremely hardy organisms, and the number of CFU per

milliliter in natural water sources has been demonstrated to remain stable at 9.4 to 15.5°C and to increase by ≥ 1 log unit at $\geq 17.8^\circ\text{C}$ when samples are held for 30 days before processing (9).

Serovars of *M. avium* associated with infection in patients with AIDS were identified in *M. avium* isolates from water. In parallel clinical studies in the United States, the most common serovars infecting patients with AIDS have been serovars 4 and 8 (11 of 23 [48%] patients) (21). These two serovars accounted for six of eight (75%) of the environmental *M. avium* isolates typed in the present study. We are unable to find previous reports of the isolation of "X" mycobacteria from Africa. In a previous report from the United States, "X" mycobacteria represented only 3% of clinical MAC isolates and were found only in respiratory specimens from recent immigrants to the United States (4).

The present findings add to other studies that we have conducted on the epidemiology of disseminated MAC infection in patients with AIDS. Results of skin test studies with an *M. avium* sensitin conducted in the United States, Finland, Trinidad, and Kenya have suggested that healthy subjects at all sites have low but comparable levels of prior infection with MAC and that levels of prior infection are too low to explain the high rates of disseminated MAC infection in patients with AIDS in developed countries solely on the basis of reactivation (23). Overall reactivity to mycobacterial antigens is higher among healthy subjects from Kenya than among healthy subjects from the United States and Finland. Studies in Kenya among AIDS patients with CD4 cell counts of <200 mm^3 have confirmed a low rate of disseminated MAC infection, and studies in Finland and the United States have shown rates of disseminated MAC infection similar to those presented in other published reports from developed countries (22). The finding of MAC isolates in environmental water samples from all five sites examined in the present study fits with skin test data showing similar rates of prior infection among healthy subjects at four of the sites. Disseminated MAC infection in patients with AIDS is probably acquired late in human immunodeficiency virus disease as a new infection. Patients in Kenya may be protected from this infection by a combination of broad mycobacterial immunity, reduced levels of exposure to environmental water as they become ill, and the low likelihood of exposure to MAC-infected drinking water. Patients in developed countries, on the other hand, lack broad mycobacterial immunity, survive to a point when their CD4 cell counts may be low, and have continued exposure to MAC-infected piped water both at home and during hospital admissions.

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