PERSISTENT COLONISATION OF POTABLE WATER AS A SOURCE OF MYCOBACTERIUM AVIUM INFECTION IN AIDS

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Section: Public health Summary

The source of Mycobacterium avium infection in AIDS has not been identified and it is not known whether most patients with AIDS acquire the organism from recent infection or by reactivation of previous infection. As part of a prospective epidemiological study, we isolated multiple colonies of M avium from patients with AIDS and from potable water to which they had been exposed. All isolates were analysed with pulsed field gel electrophoresis (PFGE).

As judged by PFGE, 29 (81%) of 36 patients were infected with one or more unique clinical strains of M avium. 7 patients (19%) were infected with three groups of common strains. Group 1 included 3 patients who lived in separate rural areas and had no common exposures apart from treatment at hospital A. The same strain was isolated repeatedly during 41 months from a recirculating hot water system at hospital A; residential water cultures were negative. Group 2 included 2 patients with no common exposures apart from treatment at hospital B; the same strain was isolated repeatedly over a period of 24 months from a recirculating hot water system at hospital B. Patients in groups 1 and 2 had numerous possible exposures to hospital hot water. Group 3 included 2 patients treated at the same methadone treatment facility.

In an institution the hot water system may be persistently colonised with a particular strain of M avium. HIV-infected patients exposed to these water sources can develop disseminated M avium infection.

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Introduction

Disseminated infection with organisms of the Mycobacteriuint avium complex (MAC) develops in as many as 40% of patients with advanced AIDS in the USA.[1, 2] MAC are environmental mycobacteria found in soil, water, aerosols, and animals,[3] but the specific source of MAC causing human infection has not been identified. Because infection with MAC is common in late HIV infection and is distributed equally among patients in all HIV transmission categories,[4] it is likely that these organisms are acquired from an environmental source to which most people are exposed. Serotypes of M avium that cause human infection (eg,
serovar 4) correlate with serovars found in environmental sources;[4-7] since M avium strains expressing the same serovar show considerable genetic diversity,[8] serovars cannot be used to conclusively link strains of MAC isolated from individual patients to a specific environmental source.

Investigations of epidemics involving other non-tuberculous mycobacteria have provided evidence that water can be a source of infection or specimen contamination; in most studies the association with water was provided by epidemiological analysis rather than by a unique molecular marker.[9-12] In one study,[13] pulsed field gel electrophoresis (PFGE) was used to identify an epidemic strain of Mycobacterium fortuitum; shower water was implicated as the likely source of colonisation.

In 1991 we started an international epidemiological study of disseminated MAC in AIDS to find the environmental source of infection. Clinical and environmental MAC isolates have been collected from five geographic sites (New Hampshire, Boston, Finland, Trinidad, and Kenya). PFGE of blood isolates from 13 patients with invasive disease showed that each patient was infected with one or more unique strains of M avium.[8] Here we report an analysis of an expanded set of clinical isolates plus environmental isolates of M avium from the two US sites.

**Methods Environmental cultures**

Sterile plastic bottles were used to collect water samples (50-500ml) from environmental sources (eg, lakes, rivers) and water supply systems (eg, home municipal water, home well water, hospital water) in New Hampshire and Boston. Samples were processed either by centrifugation or by filtration.

25 ml aliquots were centrifuged at 5000 g for 30 min; the resulting pellet was re-suspended in 1 ml sterile distilled water, and 0.1 ml triplicate aliquots of the liquid were spread to dryness onto Tsukamura minimal, Tween-80, cycloheximide (TTC) agar[14] with 500 [mu]g cycloheximide/ml and incubated at 37 degrees C in air for 6 weeks.[7] Representatives of distinct acid-fast colony types were counted and streaked onto Lowenstein-Jensen slants or Middlebrook 7H10 agar plates (Beeton-Dickinson, MD) and incubated at 37 degrees C in air. Putative slow-growing MAC isolates were identified with DNA probes according to manufacturers' instructions (SNAP DNA Probe, Digend Diagnostics Inc., MD or Gen-Probe, CA).

Before filtration, each sample was decontaminated with cetylpyridium chloride (CPC) at a final concentration of 0.04% (volume/volume) for 18 h at room temperature.[15] 250 ml volumes were filtered through a sterile 0-45 [mu]m filter (Nalge Co, Rochester, NY). The filter was washed with 500 ml sterile distilled water and placed onto modified 7H10 agar plates (pH 5.5) which were sealed and incubated at 44 degrees C in sealed plastic bags for 6 weeks.[16] Up to 10 representative acid-fast colonies per sample were inoculated into 7H9 broth and then subcultured to routine Middlebrook 7H10 agar. Slow-growing organisms were characterised with DNA probes (Gen-Probe).

**Patient cultures**

Patients with HIV infection and peripheral blood lymphocyte CD4 counts of less than 200/[mu]l were enrolled in a study of the epidemiology of disseminated MAC infection in New Hampshire and Boston. A clinical history was obtained and an environmental exposure questionnaire was administered to all patients. Stool, sputum, and blood cultures for mycobacteria were obtained and processed at Dartmouth-Hitchcock Medical Center as described previously.[8] For each specimen yielding mycobacteria, three individual colonies were picked, subcultured, saved, and analysed as independent isolates, Species-specific DNA probes were used to identify isolates (Gen-Probe). All patients were seen again at 6 months for repeat stool, sputum, and blood cultures. When possible, MAC isolates from routine clinical cultures taken before or after completion of the two study visits were also obtained. In addition to isolates from the MAC epidemiological study, isolates from HIV-infected patients treated at Boston City Hospital were obtained from the Massachusetts State Laboratory, Mycobacteriology Division.
PFGE analysis of isolates

Whole cellular DNA was extracted from each strain as previously described. The isolates were analysed with PFGE to resolve AseI restriction profiles in a 1% agarose gel (SeaKem GTG, FMC Bioproducts, Rockland, ME) in a CHEF DR II apparatus (BioRad, Richmond, CA) at 200V, 15 degrees C, with switch times of 1-40 s and linear ramping. The DNA was visualised over UV light after staining with ethidium bromide (0.5 [mu]g/ml). The restriction profiles of the isolates were compared; when profiles differed by 3 or more bands isolates were regarded as separate strains, and when profiles were indistinguishable or differed by 1 or 2 bands consistent with a single genetic event (eg, insertion, deletion, or the gain or loss of a restriction site), isolates were regarded as the same strain. We have previously observed such variations among separate colonies of the same strain of M avium from an individual patient.

Results PFGE analysis of clinical and water isolates

Disseminated M avium infection has been recorded in 47 (20%) of 234 New Hampshire and Boston patients entered into the epidemiological study to date. M avium isolates from 28 patients were available for examination by PFGE--9 from New Hampshire and 19 from Boston. 25 patients had multiple colonies available for analysis; 5 (20%) have polyclonal infection, 2 of whom have been reported previously. Single-colony isolates from 8 additional patients seen at Boston City Hospital were also analysed by PFGE. Comparison of clinical isolates from the 36 patients revealed that the same strain of M avium was isolated from 3 patients at hospital A (group 1), that a second strain was isolated from 2 patients at hospital B (group 2), and that a third unique strain was isolated from 2 patients living in Boston (group 3) (table 1). Each of the remaining 29 patients (81%) was infected with one or more unique strains of M avium.

MAC were isolated from 10 (30%) of 33 water samples collected from environmental and municipal water sources in Boston and New Hampshire between March, 1990, and February, 1992; recovery rates, colony forming units/ml sample, and other characteristics of these isolates are described elsewhere. A second set of water samples was collected from hospital A and hospital B and the homes of patients treated at hospital A between December, 1992, and July, 1993. Three isolates cultured from hot water collected 41 months apart at hospital A were the same as isolates from the 3 group I patients; two isolates cultured from water samples collected during 24 months at hospital B were the same as isolates from the 2 group 2 patients; and two isolates, one from the Charles River (Boston) and one from a Boston patient (group 4) were the same. Results of the hospital water cultures from groups 1 and 2 are summarised in table 2.

Epidemiological investigations

Group 1 Each of the 3 patients had symptomatic disseminated M avium infection. All 3 patients had been admitted or treated on numerous occasions as inpatients or outpatients in hospital A (table 3). None of them had been in the hospital on the same day. While in the hospital, all patients had had showers and had consumed drinking water or ice. Hospital A has a recirculating hot water system maintained at an average temperature of 49 degrees C without a holding tank. The 3 patients had undergone bronchoscopy and 2 had also had multiple gastrointestinal endoscopies (none on the same day). Endoscopes were routinely disinfected in an automated glutaraldehyde unit which used a hospital hot-water connection. Final aspiration of bronchoscope channels with isopropyl alcohol was begun in May, 1991. All patients had received aerosolised pentamidine treatments in the building, but never on the same day. They all lived in towns or rural locations more than 40 km apart and had separate water supplies (two municipal, one drilled well). Hot and cold water samples obtained from the homes of the 3 patients in July, 1993, were negative for MAC. These patients had never met outside the hospital and there were no other common water exposures.

Group 2 The 2 patients in this group had symptomatic disseminated M avium infection and had been treated on numerous occasions as inpatients or outpatients in hospital B (table 3). For 1 week in February, 1991, they were admitted to hospital at the same time, but there was no known direct contact between them, and the patient who
was already known to have M. avium infection (patient 1212) did not have pneumonia or diarrhoea during that time. Possible inpatient water exposures included shower and tap water; patient 1212 had not been admitted before the onset of disseminated M. avium infection but had been treated numerous times in the outpatient department served by the same water system. The patient was known for his habit of entering occupied outpatient examination rooms unannounced to drink water by putting his mouth around the nozzle of the tap (faucet). Hospital B is served by a recirculating hot-water system maintained at a minimum temperature of 49 degrees C with two 19 000 L holding tanks. 1 patient had bronchoscopy; bronchoscopes were disinfected manually by a wash with tap water, a 25 min glutaraldehyde soak, a rinse with sterile water, and a final channel aspiration with isopropyl alcohol. The patients had not received aerosolised pentamidine. They had lived in numerous residences in different parts of the same urban area and had no known common exposures outside the hospital.

Group 3 The first patient in this group was a 47-year-old man with a history of injecting drug use who developed fever; M. avium was isolated from a liver biopsy in January, 1992, and he died in November, 1992. The second patient was a 41-year-old man with a history of injecting drug use who developed a fever in May, 1991, and had M. avium isolated from blood; he died in July, 1991. They had never received aerosolised pentamidine. These men were not known to have shared drugs or injection paraphernalia. Both had their primary residences in the South End of Boston, but were intermittently homeless and may have used a homeless shelter in this same neighbourhood. Both men received their medical care at Boston City Hospital and attended the same methadone treatment programme located in a trailer near the hospital. The clinic dispenses each daily dose to clients by dissolving the methadone tablets in Boston municipal water heated in a coffee urn. Specimens obtained from the coffee urn and the tap water in June, 1993, did not yield MAC.

Group 4 The patient was a 51-year-old former junk-yard worker with no history of intravenous drug use, blood transfusion, or homosexuality; his partner died of AIDS in February, 1991 (she had no history of mycobacterial disease and had a negative blood culture for MAC in February, 1991). In April, 1991, he had positive cultures for M. avium from blood, sputum, and bone marrow. The patient died in July, 1991, and further information about possible contact with the Charles River could not be obtained.

Discussion

In this study we have extended our observations on the clinical and molecular epidemiology of M. avium strains infecting AIDS patients and have identified four groups of strains, each isolated from two or more epidemiologically related sources. For three of these strains the epidemiological data implicate water as the source of infection, including two strains associated with nosocomial acquisition.

To our knowledge this report is the first documentation that a water system may be persistently colonised with a particular strain of M. avium. The two hospitals are served by recirculating hot water systems, as was the hospital in a report of hot water colonisation with M. terrae.[12] Recirculating hot water systems, which are used in large institutions (including hospitals, hotels, and apartment buildings) may allow thermophilic organisms with moderate resistance to chlorine such as non-tuberculous mycobacteria[19, 20] and legionellae[21] to persist and multiply once they are introduced from municipal water systems. For example, in a previous study from Boston, MAC were isolated from 42% of municipal water samples[22] and were present at high concentrations in hospital hot water, especially from shower heads.[23] The temperature of the hot water system may also influence the likelihood of colonisation since mycobacteria can proliferate at temperatures up to 51 degrees C,[24] and water temperatures must be held at 70 degrees C for 5-60 min to produce a 99% kill rate of MAC.[23] Over the past two decades many institutions have lowered the temperature of hot-water systems from 71 degrees C to 49-60 degrees C to conserve energy and reduce scald injuries (Eugene Sullivan, Robert W Sullivan, Inc, Consulting Engineers, Boston, MA). During this same interval, disease due to MAC among patients in northeastern USA with and without AIDS has increased 5-10-fold.[26, 27]
The specific route of infection for these cases is not known. Infection with MAC is thought to occur from colonisation of the gastrointestinal tract (eg, drinking water), although respiratory colonisation has also been documented. Patients with AIDS may be especially susceptible to M avium infection while in hospital when intercurrent conditions may transiently decrease their resistance to MAC colonisation (eg, pneumonia, antibiotic therapy). Hot water was the source of the positive water cultures in our study, and showers have been suggested as a source of infection for MAC and other non-tuberculous mycobacteria. We do not exclude drinking water as a possible source, since hot and cold water may be delivered through a common tap. Other non-tuberculous mycobacteria have been transmitted by endoscopes contaminated with hospital water and all patients in group 1 were exposed to endoscopes (relevant endoscope cultures could not be done since processing methods had been changed at hospital A). All the group 1 patients had received aerosolised pentamidine in hospital A, but only sterile water is used in mixing the aerosol. Person-to-person transmission is unlikely since the patients in group 1 were not admitted to hospital at the same time, and the index patient in group 2 did not have a clinical condition that might have encouraged respiratory or faecal transmission during the brief time in hospital with the other group 2 patient. Respiratory spread during outpatient aerosolised pentamidine therapy could not be excluded in group 1, but all patients were always treated in separate rooms.

The patients reported in groups 1 and 2 represent only 14% (5 of 36) of all patients for whom M avium isolates were available for testing with PFGE during the study at the two participating institutions. Most patients were infected with unique and sometimes multiple strains of M avium distinct from the strains identified in the hospital water supplies, suggesting acquisition from diverse environmental sources. The patient in group 4, for example, was infected with a strain of M avium isolated from the Charles River, and the two drug injecting patients in group 3 may have been infected from a common environmental source in their community. Specific confirmation of other environmental sources may be more difficult since most ambulatory adults have numerous possible water exposures. We have not found MAC in single family residential water supplies (data not shown), possibly because they are not usually served by the recirculating hot water systems which may be important in amplifying or perpetuating colonisation of potable water. A similar observation has been made with water cultures for legionellae from single family homes, although here and in the previous Boston mycobacterial studies it was suggested that more frequent colonisation of institutional water supplies was due to large hot water storage tanks facilitating the growth and persistence of the organisms.

Previous studies have been unable to determine with certainty whether M avium is newly acquired in AIDS or represents reactivation of previous infection. We have recently reported M avium skin test studies which suggest that previous infection with M avium is not sufficiently common in healthy populations to explain the high incidence of disseminated M avium in AIDS solely on the basis of reactivation. The identification of water as the source of infection for 5 patients confirms that infection was acquired recently and was not the result of reactivation of latent infection. None of the group 1 patients had ever lived in the town where hospital A was located and therefore had no previous exposure to water from the same municipal water supply. These findings have important implications for the prevention of disseminated M avium infection in AIDS, since they suggest that additional sources of infection may be identifiable.

The recommended approach to the prevention of M avium in AIDS is to use rifabutin chemoprophylaxis in patients with CD4 counts less than 100/μL. Rifabutin is 50% effective in preventing disseminated MAC for patients with CD4 counts less than 200/μL. However, rifabutin prophylaxis has several important limitations: incomplete efficacy in preventing MAC bacteraemia; the absence of a statistically significant survival benefit among patients given prophylaxis; concerns about the potential impact of inadvertent administration of rifabutin to patients with active tuberculosis or disseminated MAC on mycobacterial drug resistance patterns; side-effects, including uveitis; the potential for a wide variety of drug interactions with rifabutin; and the cost of the drug.

Our findings suggest another potential approach to the prevention of M avium infection in AIDS—namely, specific identification and avoidance of environmental sources of M avium infection for patients with CD4 counts less than 100/μL. For example, patients might be advised to avoid institutional showers and...
exposure to non-sterile potable water; colonised institutional water systems might be sterilised, as recommended in the prevention of nosocomial legionella infection. If effective, these risk-reduction strategies might supplement the partial protection against M avium afforded by chemoprophylaxis.

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Table 1: Clinical and environmental M avium Isolates representing the same attain as judged by pulsed field gel electrophoresis

Legend for Chart:
A - Group
B - Site
C - Isolate
D - Isolate class
E - Isolate source
F - Date of isolation

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<tbody>
<tr>
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<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td>1</td>
<td>Hospital A</td>
<td>5502</td>
<td>Environmental</td>
<td>Hospital water</td>
<td>March, 1990[a]</td>
</tr>
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<td></td>
<td>5002</td>
<td>Clinical</td>
<td>Blood</td>
<td>May, 1991[b]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5026</td>
<td>Clinical</td>
<td>Stool</td>
<td>May, 1991[b]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5028</td>
<td>Clinical</td>
<td>Blond</td>
<td>January, 1992[b]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5537</td>
<td>Environmental</td>
<td>Hospital water</td>
<td>July, 1993[a]</td>
</tr>
<tr>
<td>2</td>
<td>Hospital B</td>
<td>1212</td>
<td>Clinical</td>
<td>Marrow</td>
<td>October, 1990[c]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1508</td>
<td>Environmental</td>
<td>Hospital water</td>
<td>August, 1991[a]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1060</td>
<td>Clinical</td>
<td>Stool</td>
<td>April, 1992[c]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1225-HA</td>
<td>Environmental</td>
<td>Hospital water</td>
<td>October, 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1225-HBs</td>
<td>Environmental</td>
<td>Hospital water</td>
<td>July, 1993</td>
</tr>
<tr>
<td>3</td>
<td>Boston</td>
<td>1031</td>
<td>Clinical</td>
<td>Blood</td>
<td>April, 1991[d]</td>
</tr>
<tr>
<td>4</td>
<td>Boston</td>
<td>1027</td>
<td>Clinical</td>
<td>Liver</td>
<td>January, 1992[d]</td>
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<td></td>
<td>1503</td>
<td>Environmental</td>
<td>Charles River</td>
<td>February, 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1213</td>
<td>Clinical</td>
<td>Blood</td>
<td>April, 1991[e]</td>
</tr>
</tbody>
</table>

Each group of isolates represents a distinct strain as judged by PFGE.

a See table 2 for additional environmental cultures, b Treated at hospital A; no other common exposures. c Treated at hospital B; no other common exposures. d Treated at same methadone clinic. e No known direct exposure to Charles River water.

Table 2: Persistent colonisation of hospital water supplies with the same strain of M avium

<table>
<thead>
<tr>
<th>Result (CFU/mL sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Hospital A
March, 1990  5501  Cold tap  Building 1  NG
5502  Hot shower  Building 1  5.2
July, 1993  5536  Cold tap  Building 2  NG
5537  Hot tap  Building 2  0.4
5538  Hot Shower  Building 2  1.2

Hospital B
August, 1991  1507  Cold tap  Building 1  NG
1508[*]  Hot tap  Building 1  4.2
1509[*]  Hot/cold (mix) tap Building 3  4.2
October, 1992  1225-HA  Hot/cold (mix) tap Building 2, Floor 5  0.2
1225-HB  Hot/cold (mix) tap Building 2, Floor 5  NG
1225-HC  Hot/cold (mix) bath Building 2, Floor 2  0.2
1225-HD  Cold tap Building 2, Floor 2  NG
1225-HE  Hot/cold (mix) bath Building 2, Floor 2  0.8
1225-HF  Hot/cold (mix) tap Building 2, Floor 2  NG
July, 1993  1225-HBs  Hot tap  Building 2, Floor 2  0.4
1225-HCs[*]  Hot tap  Building 2, Floor 2  3.3

All isolates from hospital A represent same strain designated group 1 in table 1: with the exception noted below, all isolates from hospital B represent same strain designated group 2 in table 1.

* Each of these three samples also contained a second strain of M avium as resolved by PFGE; these strains have not been detected in patient specimens analysed to date.

NG = no growth, CFU = colony-forming unit.

Table 3: Exposure histories of two groups of patients infected with M avium strains cultured repeatedly from hospital water.

Information is presented in the following order: Patient; age (yr)/sex; HIV transmission category; site (date first positive culture); CD4 count[a]; times in hospital before positive culture; interval from last hospital stay to positive culture (mo); type (no) endoscopies before positive culture; no of aerosol pentamidine treatments (outpatient, inpatient)

Group 1

- 5002; 36/M; Bisexual; Blood (May 3, 1991); 4; 7; 15; EGD (2), B (1.); 3, 0
- 5026; 37/M; Homosexual; Stool[b] (May 14, 1991); 17; 6; 11; B (1); 0, 6
- 5028; 62/F; Transfusion; Blood (Jan 9, 1992); 4; 6; 2; EGD (1); 5, 1

; ; ; ; ; ; B (2), ERCP (1)

Group 2

- 1212; 35/M; injecting drug use; Marrow (Oct 25, 1990); 4; 0 (outpatient visits only); NA; 0; 0
- 1060; 33/F; Heterosexual; Stool (Nov 4, 1992)[c]; 2; 12; 1; B (1); 0
a Most recent CD4 count before positive M avium culture. b Polyclonal infection; M avium isolate from blood had a different PFGE pattern. c At this time patient positive in stool and sputum; bone-marrow culture positive for same strain on Sept 21, 1992, confirming disseminated disease.

EGD = oesophagogastroduodenoscopy, B = bronchoscopy, ERCP = endoscopic retrograde cholangiopancreatography, NA = not applicable.

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