The Recovery of *Mycobacterium avium-intracellulare* Complex (MAC) from the Residential Bathrooms of Patients with Pulmonary MAC

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The distribution of *Mycobacterium avium-intracellulare* complex (MAC) in residences was examined. MAC was only recovered from bathrooms but not from other sites of residences. The appearance ratio in the bathrooms of patients with pulmonary MAC was significantly higher than that in healthy volunteers' bathrooms ($P = .01$). For 2 patients, the genotypes of environmental isolates were identical to their respective clinical isolates.

The *Mycobacterium avium-intracellulare* complex (MAC) is an opportunistic pathogen. However, MAC occasionally causes a progressive lung disease, leading to death, even in patients without a history of lung diseases or immunodeficiency [1]. MAC is widely distributed in rivers, soil, birds, farm animals, public pools, hot tubs, and hot water supplies [2]. In the absence of proof of person-to-person spread of MAC, it is now generally accepted that environmental sources are the reservoir for most human infections caused by MAC [1, 2]. The number of cases of pulmonary MAC disease has been increasing in many countries, especially in advanced countries rather than in developing countries [2]. O'Brien et al. [3] hypothesized that the increased prevalence of MAC lung disease relates to the change in our hygiene habits from bathing to showering. However, the distributions of MAC in individual residences have not been examined. Therefore, we investigated the distribution of MAC in residences to assess the hypothesis of O'Brien and colleagues. Furthermore, we compared the recovery ratio between patients' and healthy volunteers' houses. We also compared the isolates recovered from patients' houses with those from their respective sputum samples using restriction fragment–length polymorphism and PFGE.

Participants and methods. We collected 7 samples from each individual's residence, including water supply–related samples (i.e., three 200-mL water samples, with 1 each from the kitchen tap, showerhead, and used bathtub water), scale on the surface of the showerheads, and slime in 3 drains (i.e., the kitchen basin, washbasin, and bathroom). In addition, we collected the dust from air conditioners as an eighth environmental sample. Participants were outpatients experiencing pulmonary MAC disease ($n = 49$) and healthy volunteers without lung disease ($n = 43$). Patients and control subjects shared the same water supply system, which was disinfected with chlorine. The bathroom was separated from the lavatory in each residence. All patients and 40 volunteers lived with their families and were not the only persons who used the bathrooms in each residence. Patients received a diagnosis of pulmonary MAC disease according to the diagnostic criteria of the American Thoracic Society 1997 [4]. Of 49 patients, 8 were smokers, and none were alcohol abusers; 19 had predisposing lung disease (16 with previous pulmonary tuberculosis and 3 with chronic obstructive pulmonary disease). Radiographic findings were nodular bronchiectasis lesions in 23 patients and fibrocavitary lesions in 8 patients. Forty-seven of the 49 patients had previously undergone multidrug chemotherapy, including clarithromycin therapy, but cultures of sputum samples from 30 patients were consecutively positive for MAC. Informed consent was obtained from all participants before the collection of samples. This study was approved by the Toneyama National Hospital (Osaka, Japan) institutional review board and complies with international guidelines for studies involving human subjects.

Water samples were centrifuged at 11,800 *g* for 30 min at 4°C, and pellets from the tap water or the shower water were then suspended in 0.5 mL of phosphate buffer (pH, 6.8), 200 μL of which was inoculated onto a Middlebrook 7H11 agar plate containing 0.2% glycerol, 10% oleic acid-albumin-dextrose-catalase enrichment medium (Becton Dickinson), 30 U/mL polymyxin B, 0.3% amphotericin B, 1.2% nalidixic acid, 0.3% trimethoprim, and 0.36% azlocillin (7H11 PANTA plate). The pellet from the used bathtub water was treated with 3 mL of 2% sodium hydroxide solution for 10 min. After adding 6

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Table 1. Recovery of *Mycobacterium avium-intracellulare* complex (MAC) from different residential areas.

Residence	Bathroom				Kitchen		Washbasin	Living room	All locations
	Shower-head	Shower water	Bathtub water	Drain	Tap water	Drain	Drain	Dust of air conditioner	
Patients' residences	2/37	3/46	3/48	2/49	0/48	0/49	0/49	0/45	10/371[a]
Healthy volunteers' residences	0/39	0/43	1/38	0/43	0/43	0/43	0/43	0/41	1/333

NOTE. Data are no. of MAC-containing samples/no. of test samples. Eleven of 343 total samples from bathrooms contained MAC ($P < .001$; statistically significant difference in isolate source [bathroom vs. other places]). None of 361 total samples from the kitchen, washbasin, and living room contained MAC.

[a] $P = .01$; statistically significant difference in participant groups between the patients' group and healthy volunteers' group.

mL of phosphate buffer to this alkali-treated sample, it was centrifuged at 2000 *g* for 15 min and then resuspended in 0.5 mL of phosphate buffer. The collected samples on the swabs were suspended in 1 mL of tryptic soy broth and then incubated at 25°C for 3 h, followed by alkali treatment, and the pellets were then suspended in 1 mL of phosphate buffer solution. One hundred to 200 μL of these suspensions were inoculated onto 7H11 PANTA plates and incubated at 37°C for 3 weeks. Growing colonies were examined microscopically by Ziehl-Neelsen staining. The isolated acid-fast bacterial species were

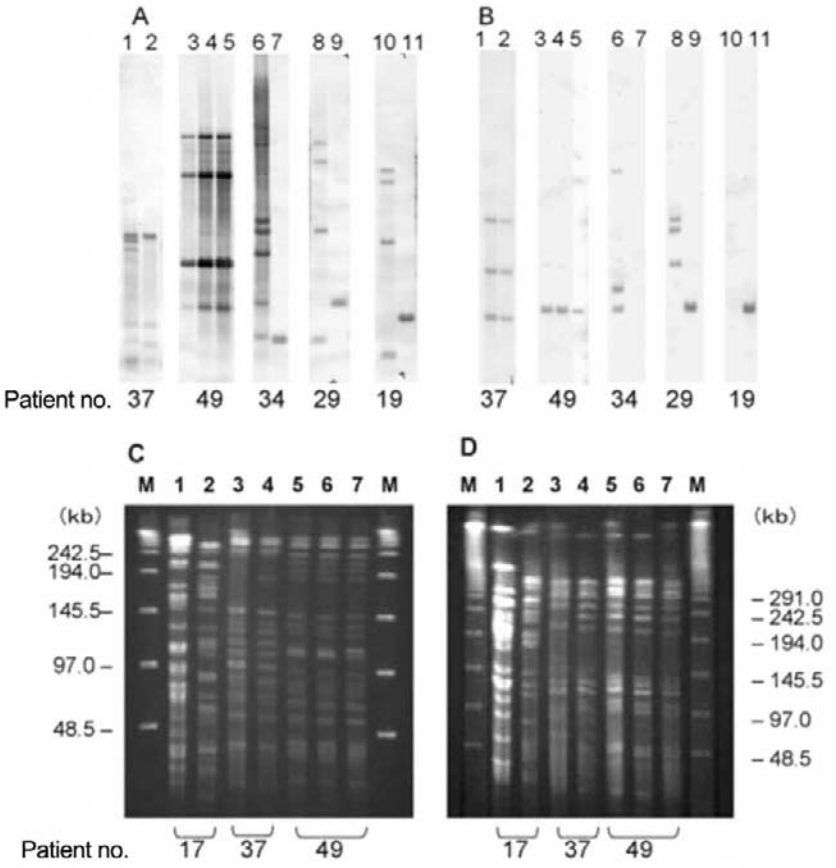

Figure 1. Molecular typing of environmental and clinical *Mycobacterium avium-intracellulare* complex (MAC) isolates. *A* and *B,* Restriction fragment–length polymorphism analyses were performed by Southern blotting of *Pvu*II-digested genomic DNA probed with IS*1245 (A)* and IS*1311 (B). Lanes 1* and *2,* Isolates from bathtub water and a sputum sample from patient 37. *Lanes 3–5,* Isolates from bathtub water, bathroom drain, and a sputum sample from patient 49. *Lanes 6* and *7,* Isolates from shower water and a sputum sample from patient 34. *Lanes 8* and *9,* Isolates from bathtub water and a sputum sample from patient 29. *Lanes 10* and *11,* Isolates from shower water and a sputum sample from patient 19. *C* and *D,* PFGE patterns of clinical and environmental MAC isolates from patients 17, 37, and 49. Large restriction fragment patterns of genomic DNA digested with *Xba*I *(C)* and *Dra*I *(D). Lanes 1* and *2, M. intracellulare* isolates from the showerhead and a sputum sample from patient 17. *Lanes 3* and *4, M. avium* isolates from bathtub water and a sputum sample from patient 37. *Lanes 5–7, M. avium* isolates from bathtub water, bathroom drain, and a sputum sample from patient 49. M, marker of λ DNA standards.

Table 2. Summary of phenotypic and genotype profiles of *Mycobacterium avium-intracellulare* complex (MAC) strains isolated from patients' bathrooms and their respective clinical isolates.

Patient no. (MAC type), sample	Species	Serovar	RFLP[a]		PFGE[a]	
			IS*1245*	IS*1311*	*Xba*I	*Dra*I
37 (F)						
Bathtub water	*M. avium*	Apolar	Related	Identical	Identical	Related
Sputum	*M. avium*	Apolar	…	…	…	…
49 (F)						
Bathtub water	*M. avium*	8	Identical	Identical	Identical	Identical
Drain	*M. avium*	8	…	…	…	…
Sputum	*M. avium*	8	…	…	…	…
17 (N)						
Showerhead	*M. intracellulare*	16	NT	NT	Unrelated	Unrelated
Sputum	*M. intracellulare*	16	…	…	…	…
34 (F)						
Shower water	*M. avium*	4	Unrelated	Unrelated	NT	NT
Sputum	*M. avium*	Apolar	…	…	…	…
29 (N)						
Bathtub water	*M. avium*	Apolar	Unrelated	Unrelated	NT	NT
Sputum	*M. avium*	2	…	…	…	…
19 (N)						
Shower water	*M. avium*	8	Unrelated	Unrelated	NT	NT
Sputum	*M. avium*	Apolar	…	…	…	…
5 (N)						
Shower water	*M. avium*	Apolar	NT	NT	NT	NT
Sputum	*M. intracellulare*	7	…	…	…	…
20 (N)						
Showerhead	*M. intracellulare*	16	NT	NT	NT	NT
Sputum	*M. avium*	NG	…	…	…	…
21 (N)						
Drain	*M. intracellulare*	14	NT	NT	NT	NT
Sputum	*M. avium*	NG	…	…	…	…

NOTE. Apolar, apolar glycopeptidolipid; F, fibrocavitary type; N, nodular bronchiectasis type; NG, lack of glycopeptidolipid; NT, not tested; RFLP, restriction fragment–length polymorphism.

[a] Data describe the comparison of patterns between the samples for each patient. RFLP and PFGE genotypic patterns were defined as follows: identical, when 1 case was not distinguishable from another; related, when the genotypic pattern differed only for 1–3 bands; and unrelated, when the genotypic pattern was completely different.

identified by sequence analysis of the 16S–23S rRNA internal spacer region [5].

Genotypic analyses were performed using restriction fragment–length polymorphism [6] and PFGE [7], as described previously, with minor modifications in the method of PFGE of the lysis buffer composition containing lysozyme (5 mg/mL), 1 M of NaCl, 10 mmol/L Tris/HCl, 0.1 M of EDTA (pH, 8.0), 0.5% polyoxyethylene (20) cetyl ether, 0.5% deoxycholate, and 0.5% *N*-lauroyl sarkosyl.

MAC has been classified into 28 serovars on the basis of the antigenic glycopeptidolipids (GPLs) that are present on the cell surface. The serovars of MAC isolated from residential samples and sputum samples were identified by liquid chromatography/mass spectrometry [8]. In some cases, we found lack of GPL or apolar GPL instead of serospecific GPL. Apolar GPL did not have serospecific oligosaccharides in the GPL. The distribution of the isolated MAC was analyzed with the χ^2 test. P values $<.05$ were considered to be statistically significant.

Results and discussion. Samples were obtained from 49 patients' and 43 healthy volunteers' residences. Eleven samples were found to contain MAC isolates: 3 from shower water, 2 from showerheads, 4 from bathtub water, and 2 from bathroom drains (table 1). No isolates were recovered from any other places. Ten of the MAC isolates were from 9 (18.4%) of the 49 patients' residences, whereas only 1 isolate (2.3%) was found in 43 healthy volunteers' residences (in bathtub water). Thus, MAC isolates were recovered from patients' residences at a significantly higher frequency than from residences of control subjects ($P = .01$). Of the 11 MAC isolates from the 10 residences, 8 were *M. avium*, and 3 were *M. intracellulare*. This

ratio of *M. avium* to *M. intracellulare* among the environmental isolates reflects the characteristics of patients with pulmonary MAC in Osaka, Japan [9].

Ten MAC isolates from patients' bathrooms were analyzed and compared with isolates from their respective sputum samples by genotyping and serotyping. For 2 patients (patients 37 and 49), MAC isolates from bathtub water and sputum samples had identical molecular profiles by restriction fragment–length polymorphism and PFGE analysis (figure 1 and table 2) and possessed the same serovar. Both patients had undergone complete chemotherapy but had retained low-level MAC positivity intermittently, as determined by culture of sputum samples. Both patients had fibrocavitary lesions. For 4 patients (patients 17, 34, 29, and 19), MAC isolates from bathrooms and sputum samples were the same species but were different in terms of restriction fragment–length polymorphism or PFGE profiles (figure 1 and table 2). For the 3 remaining patients (patients 5, 20, and 21), MAC isolates from bathrooms were different species from the respective clinical isolates (table 2). Of these latter 7 patients, 4 had nodular bronchiectasis, and 2 had prior pulmonary tuberculosis. MAC isolates from patients' bathrooms were identified as serovars 4, 8, 14, or 16; these had been most commonly detected in the sputum specimens from patients with MAC who were reported in our previous study [10].

The possibility has been suggested that MAC can colonize urban water systems, where development of biofilms of nontuberculous mycobacteria has been observed [11]. However, we could obtain no MAC isolates from kitchen tap water, although the same system was supplying water to both the kitchen tap and the shower in the bathroom. Furthermore, the family residences examined in this study did not use a recirculating hot water system, which has been reported to be a source of disseminated MAC infection in large institutions, such as hospitals [12]. Therefore, our results suggest that MAC organisms predominantly colonize the bathroom. The temperature and humidity in the bathroom might provide favorable conditions for the growth of MAC. It is known that MAC organisms are resistant to chlorine [13] and to a wide range of temperatures [14] and pHs [15]. Auchuleta et al. [16] reported that desiccated *M. avium* lose viability at a constant rate (half-life, 2.3 days).

The frequency of obtaining MAC isolates from patients' homes was lower than expected (9 [18.4%] of 49 patients). This might have occurred because we did not collect samples from inside the showerhead or inside the bathtub inlet. The traditional style of Japanese bath has a water inlet below the water level. Biofilms may develop in these places, and biofilm cultures might, therefore, have yielded MAC organisms. Further efforts to detect biofilm formation and to recover MAC from these places may increase the detection rate in patients' homes.

On the basis of genotypic analysis, it was revealed that MAC isolates from 2 patients' bathrooms were identical to those recovered from their respective sputum specimens. Our results support the hypothesis by O'Brien et al. [3] that the increased prevalence of MAC lung disease relates to the change in our hygiene habits from bathing to showering. Nonetheless, our results suggest that residential bathrooms could be a reservoir for MAC organisms; our results could not sufficiently prove the bathroom to be one of the infection sources of MAC disease. The possibility remains that organisms transfer from the patient to their bathroom, instead of the organism in the bathroom being the source of the patient's infection. To assess the source of infection, precise genetic analysis may be required between environmental and clinical isolates.

In summary, the present study revealed that MAC is recovered more frequently from patients' bathrooms than from control subjects' bathrooms but not in other domestic locations. MAC preferentially localized the bathroom (i.e., shower water, showerheads, and bathtub water).

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