



Effective Infection Control Measures by *Mycobacterium avium* Complex Infection from Potted Plants in Medical Facilities

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Abstract

Background: *Mycobacterium Avium* Complex (MAC) is a group of related opportunistic pathogens. Previous studies have suggested that residential soils would be a likely source of pulmonary MAC infection. Hence, we analyzed Variable Number of Tandem Repeats (VNTR) patterns of MAC isolates from potted plants surrounded by fences and patients infected with MAC to evaluate the efficacy of fences on infection control.

Methods: Clinical isolates were recovered from the sputum of pulmonary MAC infection patients who had been examined at the Aichi Medical University Hospital. Soil sample was recovered from potted plants surrounded by fences unable to touch by patients and stuffs in patient lobbies. Bacterial species were identified by *hsp65* gene. Comparing genotypes of MAC samples were used by Variable Number of Tandem Repeats (VNTR) analysis.

Results: Both clinical samples and soil sample were identified as *M. avium* subsp. *hominissuis* determined as *hsp65* sequence. Both samples were identified as DQ284765 deposited in Gen Bank. VNTR patterns of clinical sample and soil sample were different.

Conclusion: It was considered that the contact with potted plants is reduced by enclosing fences. Soil infection with MAC might be prevented by enclosing fences the potted plants, further clinical study would be needed.

Keywords: *Mycobacterium avium*; Infection control; Variable number of tandem repeats

Introduction

Mycobacterium avium Complex (MAC) is a group of related opportunistic pathogens, consisting of *Mycobacterium avium*, *Mycobacterium intracellulare* and several less commonly encountered species. The proportion of pulmonary MAC disease is high in elderly people, and it is not uncommon to merge lung cancer [1,2]. MAC isolates have been isolated from various animal hosts and environmental sources, including soil, water and dust. In 1990, *M. avium* was divided into further four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *Silvaticum*, *M. avium* subsp. *hominissuis* due to the development of various biochemical and molecular tools [3,4]. In medical facilities, we often see fresh flowers, dried flowers and potted plants. Flowers are important as spiritual care for patients. But, Fujita et al. [5] reported that high soil exposure in farming or gardening was likely to increase the risk of transmission of MAC from environmental soils so that residential soils are a likely source of pulmonary MAC infection [5,6]. In fact, CDC guidelines recommend to remove flowers and plants in the area where immuno compromised patients exist. Therefore, it seems that reducing contact with potted plants is conducive to prevent MAC infection from soil. However, the efficacy remains unclear. In our study, as an initial study, we analyzed Variable Number of Tandem Repeats (VNTR) patterns of MAC isolates from an inpatient and potted plants surrounded by fences to prove the possibility of infection control.

Material and Methods

Bacterial strains

The standard MAC strain *M. avium* subsp. *avium* (ATCC700898) was used to identify the

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Figure 1: Potted plants isolated soil sample.

pathogens from clinical sample and environmental sample. Clinical isolates were recovered from inpatients of Aichi Medical University Hospital. Sputum of pulmonary MAC infection patients was taken and identification of *M. avium* strains was performed using the COBAS Taq Man MAI test (Roche Diagnostics, Basel, Switzerland). Clinical isolate was sub cultured on Middle brook 7H11 agar plates (BD). Soil sample was recovered from potted plants at the lobby in the Aichi Medical University Hospital. The area around potted plants was not affected by air conditioning and surrounded by fences unable to touch by patients and stuffs (Figure 1). Soil samples were processed as described by Parashar et al [7]. After cultivation using the BACTEC MGIT 960 system, positive cultures were subjected to PCR analysis for identification of *M. avium* or *M. intracellulare*, as reported previously [8]. PCR-positive cultures were sub cultured on Middle brook 7H11 agar plates to obtain single colonies.

Sequencing of the *hsp65*

Amplification of the *hsp65* gene was performed in a 50 µl final reaction volume consisting of 5 ng to 50 ng of DNA template, 2.5 mM MgCl₂, 1_PCR buffer (Invitrogen, Carlsbad, CA), 5 µl of 50% acetamide, 0.2 mM deoxynucleoside triphosphates, 0.5 µM of each primer, and 1 U of Taq DNA polymerase (Invitrogen). Primers used for amplification of the *hsp65* fragment were MACHsp65F (5'-CGGTTTCGACAAGGGTTACAT-3') and MACHsp65R (5'-ACGGACTCAGAAGTCCATGC-3') [9]. PCR was performed using Applied Biosystems Gene Amp PCR system 2700 and the following conditions: 95°C for 10 min; 35 cycles with intervals of 94°C (60 s), 58°C (120 s) and 72°C (1 min); and 72°C for 10 min followed by holding of the reaction mixture at 4°C. Sequence comparisons of the 3' portion of the *hsp65* gene were performed in-house using only the strains tested in this study, since availability of data spanning this region in public databases is limited. Comparative analyses spanning the 441-bp region were performed by BLAST analysis in NCBI [10]. Phylogeny reconstruction of all sequence alignments was performed in MEGA 3.1 using the neighbor-joining method [11].

VNTR analysis

Primer sets for 15 *M. avium* subsp. hominissuis VNTR loci (MATR-VNTR; excluding MATR-VNTR-10) were used in the VNTR analysis, as described previously [12]. For amplifying *M.*

Table 2: VNTR allelic distribution in *M. avium* strains.

Isolate	VNTR locus															
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	
Clinical sample	2	1	1	2	2	2	1	2	2	2	3	0	2	2	3	
Soil sample	2	0	1	2	2	2	2	2	1	2	3	2	2	2	3	

avium subsp. hominissuis VNTR loci, the PCR program consisted of an initial denaturing step at 95°C for 10 min, followed by 38 cycles of denaturing at 98°C for 10 s, annealing at 68°C for 30 s, extension at 72°C for 60s, and then a final extension step at 72°C for 7 min. The PCR products were electrophoresed with the TrackIt 50-bp DNA ladder (Invitrogen, San Diego, CA) in a 2% agarose gel (BMBio). Template DNA from *M. avium* ATCC700898 was used as a control in each experiment. After electrophoresis, the gel was photographed with Gel-Doc (Bio-Rad), and the number of base pairs in the target VNTR loci was estimated using Quantity One (Bio-Rad) analysis software. The numbers of repetitions of various VNTR loci of each strain were determined and regarded as an allele profile.

Results

Strain identification

Both clinical sample and soil sample were identified as *M. avium* subsp. hominissuis determined as *hsp65* sequence (Table 1). Both samples were identified as DQ284765 deposited in Gen Bank.

VNTR analysis

As shown in Table 2, clinical sample and soil sample showed allele profiles representing 2 different patterns. VNTR patterns of clinical sample and soil sample was not matched.

Discussion

Numerous studies have revealed a continuous increase in the worldwide incidence and the prevalence of Non-Tuberculous Mycobacteria (NTM) diseases, especially pulmonary MAC diseases. Mycobacterial communities are also likely to occur in these infection sources in households. These infection sources include areas with frequent human contact, such as soil and bathrooms, indicating that individuals may carry NTM organisms that concomitantly attach to their household belongings. Nishiuchi et al. [13] suggested that the formation of aerosols containing NTM arising from shower water, soil, and pool water can be infection sources. And, clinical isolates were genetically identical to environmental ones from household tap water, bathrooms, potting soil, and garden soil were detected [13]. In the environmental sources, NTM organisms can form bio films, survive within amoebae and exist in a free-living state. Hence, some reports have suggested that aerosolized water contaminants, including NTM can cause febrile respiratory illness and hypersensitivity pneumonitis [14]. Of note, medical treatment of pulmonary MAC disease does not always provide curative effects and is frequently hampered by recurrence. This suggests the presence of a reservoir for MAC in the environment surrounding patients. As described above, MAC isolates are widely distributed both in the natural and living environment, and these environmental organisms are thought to be

Table 1: Hsp65 sequence of the *M. avium* strains.

Representative strain or feature (hsp65 code no.)	Nucleotide at indicated base pair position														
	645	861	1092	1128	1136	1218	1269	1272	1296	1350	1435	1468	1488	1536	
Clinical sample	C	G	G	C	C	G	G	C	C	G	A	G	G	G	
Soil sample	C	G	G	C	C	G	G	C	C	G	A	G	G	G	

a source of infections [15,16]. There are some reports that water in living environment may be the source of MAC infection [17,18]. In previous study, the appearance ratio in the bathrooms of patients with pulmonary MAC was significantly higher than that in healthy volunteers' bathrooms and predominantly colonized in the bathtub inlets [17]. Nearly half of the 15 bathrooms that harbored MAC strains (47%, 7/15) had strains with a genetically close relationship to their respective clinical isolates [18]. Thus, water distribution systems are a transmission route from natural water reservoirs to household tap water. Similarly, there are some reports that soil may be the source of MAC infection [5,19,20]. MAC is isolated from environmental soil samples at a high rate of 20% to 60% [21,22]. As previous study revealed, patients with pulmonary MAC disease have significantly more soil exposure than non-infected control patients and occupational exposure to agricultural soil was associated with MAC infection [19,20]. Thus, residential soils are also a likely source of pulmonary MAC infection [5,23,24]. Additionally, MAC is the most abundant mycobacterium in potting soils [23]. MAC was isolated from 24% of the indoor samples, while only 3% of the outdoor samples yielded MAC isolates. A similar result was obtained when samples were collected from areas with frequent human contact, including soil from flower pots and compared with samples involving less probable human contact [25]. Based on their study, we enclosed the potted plants by fences to reduce the contact by patients. Consequently, VNTR patterns of clinical sample and soil sample were different. Hence, we thought that MAC patient was not infected from potted plants surrounded by fences. And the result suggested that soil infection from potted plants can be prevented by enclosing fences. However, several considerations should be noted when interpreting our results. The first, there were few number of analyzed case. We should conduct further studies to prove our hypothesis. The seconds, the possibility remains that organisms transfer from the patient to the potted plants, instead of the organism in the potted plants 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. A prospective cohort study would be required to clarify that the fences prevent MAC infection. However, there were few reports investigated soil infection prevention. Fujita reported that high soil exposure in farming or gardening is likely to increase the risk of transmission of MAC from environmental soils so that residential soils are a likely source of pulmonary MAC infection [5]. Therefore, our results suggested that the contact with potted plants is reduced by enclosing fences so that soil infection can be prevented in patient lobbies. In conclusion, the enclosing the potted plants in hospital by fences can be a one of the means to prevent soil infection with MAC.

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