Case Report

Recurrence of pulmonary *Mycobacterium avium* complex disease due to endogenous reactivation

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Introduction: An official American Thoracic Society and Infectious Disease Society of America statement has shown that patients with pulmonary *Mycobacterium avium* complex (MAC) disease who complete 10–12 months of negative cultures on therapy but then have either single or multiple positive MAC cultures are more likely to have reinfection with a new MAC strain.

Case presentation: A 63-year-old woman was diagnosed with pulmonary disease caused by clarithromycin (CAM)-susceptible MAC. Before initiating chemotherapy using a four-drug regimen containing CAM, an investigation of the patient's residential bathroom was conducted and one of the *M. avium* isolates recovered from the bathtub inlet was found to be genetically identical to sputum-derived isolates by variable number tandem repeats analysis using *M. avium* tandem repeat loci (MATR-VNTR). A second investigation of the bathroom during chemotherapy showed no *M. avium* isolates, and five consecutive sputum cultures were negative for 12 months until chemotherapy was discontinued. A recurrence occurred 3 months after the end of chemotherapy (at age 65 years). A third investigation of the bathroom was performed and MATR-VNTR analysis revealed that the VNTR profile of the *M. avium* isolates recovered from the sputum at recurrence was identical to that of the isolates recovered from the sputum at initial diagnosis and the bathroom at the first investigation.

Conclusion: The recurrence occurred due to endogenous reactivation of the initial *M. avium* isolate despite drug treatment for 12 months after sputum culture conversion. Further genetic analyses of MAC isolates recovered from patients and environments should be encouraged.

Keywords: endogenous reactivation; pulmonary *Mycobacterium avium* complex disease; reinfection; residential bathroom; VNTR analysis.

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Introduction

Elucidation of the sources and routes of *Mycobacterium avium* complex (MAC) infections remains a challenge. Residential bathrooms in patients' homes are currently

believed to be a source and route of MAC infection. Nishiuchi *et al.* (2007) showed that MAC isolates recovered from the sputa of patients with pulmonary MAC disease were genetically identical to those recovered from their residential bathrooms, suggesting the potential for MAC infection in bathrooms. Environmental soil exposure is also considered to be a source and route of MAC infection. Maekawa *et al.* (2011) reported in a case-control study that patients with pulmonary MAC disease

Abbreviations: ATS/IDSA, American Thoracic Society and Infectious Disease Society of America; CAM, clarithromycin; MAC, *Mycobacterium avium* complex; MATR, *M. avium* tandem repeat; VNTR, variable number tandem repeats

had significantly more soil exposure than non-infected control patients, suggesting that environmental soil exposure is a probable risk factor for the development of pulmonary MAC disease.

An official statement from the American Thoracic Society and Infectious Disease Society of America (ATS/IDSA) says that patients who complete 10–12 months of negative cultures on therapy but then have either single or multiple positive MAC cultures are more likely to have reinfection with a new MAC strain (Griffith *et al.*, 2007), and it is conceivable that repeated reinfection by MAC strains from patients' residential bathrooms or various environmental sources might be a cause of recurrence. However, the mode of recurrence and the optimum duration of treatment have not been sufficiently clarified.

This report presents a case of recurrence due to endogenous reactivation of an initial MAC strain despite treatment with a clarithromycin (CAM)-containing regimen for pulmonary disease caused by CAM-susceptible MAC for 12 months after sputum culture conversion, which was revealed by variable number tandem repeats (VNTR) analysis.

Case report

The patient, a 65-year-old woman with a history of hypertension, hyperlipidaemia and multiple lacunar infarctions, had an unremarkable family medical history. She had not smoked, consumed alcohol or performed any yard or gardening activities, and had worked until the age of 60 years selling tickets at a cycle-race track. At about the age of 52 years, the patient's residential bathroom was remodelled, and a fully automatic hot water system was installed. At 63 years (October 2010), the patient presented with a persistent low-grade fever. Chest computed tomography revealed infiltrations around bronchiectatic lesions in the right middle lobe and the patient was diagnosed with the nodular/bronchiectatic form of pulmonary MAC disease compatible with ATS/IDSA diagnostic criteria (Griffith *et al.*, 2007). The CAM MIC of the isolate was $0.25 \mu\text{g ml}^{-1}$. An initial investigation of the bathroom was conducted before initiating chemotherapy, and a total of five samples were taken for culture. The resultant isolates were identified at subspecies level and genotyped by VNTR analysis using *M. avium* tandem repeat loci (MATR-VNTR) as described previously (Inagaki *et al.*, 2009). One of the *M. avium* subsp. *hominissuis* isolates recovered from the bathtub inlet was genetically identical to the sputum-derived isolate by MATR-VNTR analysis, indicating that the patient could have developed residential infection, as reported previously (Taga *et al.*, 2012). A four-drug regimen consisting of rifampicin (450 mg day^{-1}), ethambutol (750 mg day^{-1}), CAM (800 mg day^{-1}) and sitafloxacin (100 mg day^{-1}) was initiated in November 2010. The pipe leading from the water heater outside the home to the bathtub was replaced

in April 2011, but the inside of the bathroom was not remodelled. Although the patient's husband cleaned the bathroom after the discovery of MAC isolates, the patient continued to use the bathroom daily. The patient did not resume any yard or gardening activities. At the second investigation of the bathroom in June 2011 (during chemotherapy after sputum culture conversion), a total of five samples were collected using a seed swab from the bathtub inlet, bathtub tap, bathtub drain, showerhead surface and shower faucet. All samples were negative for *M. avium* culture. The patient had a good clinical course with nearly complete resolution of pulmonary infiltrations, and five consecutive negative sputum cultures over 12 months (January 2011 to January 2012); therefore, the chemotherapy was discontinued at the end of January 2012.

Three months after discontinuing therapy (April 2012), three consecutive sputum cultures revealed *M. avium* isolates and chest computed tomography showed new infiltrations around bronchiectatic lesions in the right middle and lower lobes: the pulmonary MAC disease had relapsed. At recurrence, no abnormal findings on physical and blood examinations were found. The CAM MIC values of these MAC isolates were all $0.25 \mu\text{g ml}^{-1}$, identical to the initial MAC isolate recovered from the patient's sputum. A third investigation of the bathroom in May 2012 (after recurrence) also showed no *M. avium* isolates (a total of five samples were also collected from the same sites as the second investigation).

M. avium isolates recovered from the bathroom at the first investigation, from the sputum at initial diagnosis and at recurrence were subjected to subspecies identification and MATR-VNTR analysis. Twelve *M. avium* isolates recovered from the bathtub inlet (E1–E5, E6-1, E6-2, E7-1, E7-2, E8-1, E8-2 and E9) (Taga *et al.*, 2012), two isolates recovered from sputum at initial diagnosis (L1 and L2) (Taga *et al.*, 2012) and 10 isolates recovered at recurrence (X1–X10) were identified as *M. avium* subsp. *hominissuis*. MATR-VNTR analysis revealed that the VNTR profile of the isolates recovered from the sputum at recurrence (X1–X10) was identical to that of the isolates recovered from the sputum at initial diagnosis (L1 and L2) and one of the isolates recovered from the bathroom at the first investigation (E9) (Fig. 1). Therefore, the recurrence of pulmonary MAC disease occurred due to endogenous reactivation of the initial *M. avium* isolate that had remained in the bronchiectatic lesions despite drug treatment for 12 months after sputum culture conversion.

Discussion

Several studies have used VNTR analysis to elucidate the sources and routes of MAC infection (Taga *et al.*, 2012; Fujita *et al.*, 2013). However, few studies have investigated the recurrence of pulmonary MAC disease. Wallace *et al.* (1998, 2002) used PFGE to determine that recurrences occurred more commonly from reinfection by new MAC

Fig. 1. Twenty-four loci MATR-VNTR analysis dendrogram. E refers to *M. avium* isolates recovered at the first investigation of the bathroom (detailed MATR-VNTR analysis showed that E6, E7 and E8 were mixtures of two different clones, E6-1 and E6-2, E7-1 and E7-2, E8-1 and E8-2, respectively); L refers to *M. avium* isolates recovered from the patient's sputum at initial diagnosis; X refers to *M. avium* isolates recovered from the patient's sputum at recurrence. The Manhattan distance is indicated at the bottom.

isolates than endogenous reactivation of the initial MAC isolate if patients had completed 10–12 months of negative cultures during therapy. Ichikawa *et al.* (2010) developed a VNTR analysis method for *Mycobacterium intracellulare* infection and showed that isolate genotypes were stable for up to 4 years. They also reported a patient with an *M. intracellulare* infection that recurred 1 year after treatment; VNTR analysis showed that isolates collected from the patient before and after recurrence were genetically identical. Therefore, molecular epidemiological analyses, including VNTR, are useful to determine whether pulmonary MAC disease recurrence is due to reinfection or to endogenous reactivation (Ogawa, 2013), evidence that not only provides insights into possible sources and routes of MAC infection but also has implications for treatment of pulmonary MAC disease.

The present case with pulmonary MAC disease caused by CAM-susceptible MAC was treated with a four-drug regimen containing CAM. The sputum cultures had been negative for 12 months until chemotherapy was discontinued; however, recurrence occurred 3 months after the end of the chemotherapy and was shown by VNTR analysis to be due to endogenous reactivation of the same genotype. The official ATS/IDSA statement is that patients with pulmonary MAC disease should be treated until cultures are negative for 1 year (Griffith *et al.*, 2007), a guideline that is based on reports that recurrence at

10–12 months after sputum culture conversion are usually due to reinfection (new MAC isolates) rather than disease relapse (Wallace *et al.*, 2002). However, as the present study revealed, treatment for 12 months after sputum culture conversion might be insufficient. The second and third environmental investigations of the residential bathroom of this patient were negative for *M. avium* isolates recovered from the bathtub inlet. These results might be due to replacement of the pipe leading to the bathtub or a change in the role of bathroom cleaning from the patient to the patient's husband, and probably led to a reduced risk of reinfection from the bathroom environment. The present study has certain limitations in that we could not rule out the possibility of MAC isolate transfer from the patient to the bathroom, or MAC infection from an environment other than the bathroom, such as soil, although the patient did not perform any yard or gardening activities. Further genetic analyses of MAC isolates recovered from patients and environments should be encouraged to clarify the sources and routes of MAC infection and the mode of recurrence, and to investigate the optimum duration of treatment for pulmonary MAC disease.

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