ORIGINAL RESEARCH LETTER

Prevention of aerosol isolation of nontuberculous mycobacterium from the patient's bathroom

To the Editor:

Recent clinical studies have revealed that reappearance of the same nontuberculous mycobacterium (NTM) infection is common after successful standard treatment [1, 2]. Using pulsed-field gel electrophoresis analysis, Wallace *et al.* [1] found that ~75% of *Mycobacterium avium-intracellulare* complex (MAC) isolates identified after successful treatment are the result of reinfection. According to a recent study conducted by Koh *et al.* [2] using repetitive sequence-based PCR analysis, all re-identified *M. abscessus* subsp. *abscessus* isolates had a unique genotype. Therefore, patients with NTM are exposed to large amounts of microbes in their daily lives, particularly in cases of reinfection.

We performed environmental investigations for pulmonary NTM disease cases using an air sampling method. Interestingly, we identified a case with recurrent pulmonary *M. abscessus* subsp. *massiliense* infection who was exposed an identical genotype rather than a different genotype during and after treatment. This report is also the first to show that aero-environmental interventions may be able to prevent reinfection.

All patients were outpatients at Fukujuji Hospital who were diagnosed with pulmonary NTM disease, according to the guidelines of the American Thoracic Society/Infectious Disease Society of America [3]. Chart reviews were used to collect participants' characteristics, including age, sex, infecting mycobacterial species, radiological disease type, and clinical course. Air sampling was performed in the sitting room, the garden (if the patients possessed one) and the bathroom using a SAS SUPER ISO (International PBI S.p.A., Milan, Italy). Air was collected at a rate of 100 $L \cdot min^{-1}$ for 10 min at each site, resulting in a total of 1000 L. In the bathroom, air sampling was performed three times under different conditions: 1) a dry condition, in which the bathroom was not used for over 12 h prior to sampling; 2) a humid condition, in which hot water was running into the bathtub; and 3) a humid condition created 10–15 min after the bathtub was filled with hot water. The temperature of the water supply was lower than 46°C. Additional air sampling was performed if a patient possessed a garden and had previously been exposed to soil, regardless of frequency.

A Middlebrook7H11 (Becton Dickinson, Maryland, USA)-based agar plate was prepared according to the method described by Thomson *et al.* [4], with some modification. Whole-genome sequencing (WGS) was performed to identify the genotype of each isolate [5–10]. All single-nucleotide variant (SNV) sites were extracted by VarScan v2.3.4 [9] using the default parameters, followed by the exclusion of SNV sites in repeat and prophage regions to determine SNV sites in core-genome regions. This study was approved by the Institutional Review Board of the Fukujuji Hospital, Kiyose, Tokyo (No. 17003), and all patients provided written informed consent.

Five patients with pulmonary NTM were investigated. The patients' demographics and clinical courses are detailed in table 1. All patients were female and had nodular-bronchiectatic (NB)-type NTM disease [3]. MAC and *M. abscessus* complexes were isolated from sputum samples in two and three patients, respectively. All patients had been treated for more than at least 2 months at the time of sampling. Case 5

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TABLE 1 Summary of the results from swab samples and air-sampling tests for each patient

Number	Clinical species	Age years, sex	Disease type	Swab samples				Air-sampling test					
				Hot water inlet	Cover of the hot water inlet	Shower head	Kitchen sink	Bathroom: dry[#]	Bathroom: hot water running[¶]	Bathroom: bathtub full of water[+]	Sitting room	Potting soil	Garden
1	*M. massiliense*	57, female	NB	AFB 73 colonies	AFB 3+	AFB 1 colony	AFB 2+	AFB 2 colonies	AFB 3+	N/A	N/A	Contamination with mould	AFB 25 colonies
2	MAC	69, female	NB	AFB 33 colonies	Negative	Negative	Negative	Negative	AFB 132 colonies	AFB 86 colonies	N/A	N/A	AFB 2 colonies
3	MAC	69, female	NB	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	N/A	N/A
4	*M. abscessus*	66, female	NB	AFB 33 colonies	Negative	Negative	AFB 2+	AFB 1 colony	AFB 2+	AFB 16 colonies	N/A	N/A	AFB 2 colonies
5	*M. massiliense*	71, female	NB	Negative	N/A	Negative	AFB 3+	AFB 1 colony	*M. massiliense* 65 colonies	Negative	Negative	AFB 9 colonies	AFB 1 colony
5 (Second investigation)				Negative	Negative	AFB 2 colonies	N/A	Negative	*M. massiliense* 19 colonies	Negative	N/A	Negative	N/A

M. massiliense: Mycobacterium abscessus subsp. *massiliense*; NB: nodular-bronchiectatic; AFB: acid-fast bacilli; N/A: not available; MAC: *Mycobacterium* avium complex; *M. abscessus: Mycobacterium abscessus* subsp. *abscessus*. [#]: a dry condition, in which the bathroom was not used for over 12 h prior to sampling; [¶]: a humid condition, in which hot water was running into the bathtub; [+]: a humid condition created 10–15 min after the bathtub was filled with hot water.

was under a second course treatment that had lasted for 5 months. The first treatment ended 10 months ago and recurrence was diagnosed 7 months ago. Negative conversions of culture were obtained in patients 1, 3 and 5, whereas the other two patients showed consecutive culture-positive results for more than 24 months.

Regarding the results of air sampling in the bathroom, several NTM colonies were isolated from the sampled air, even 12 h after the last use (*i.e.* under relatively dry conditions) (table 1). However, the number of NTM colonies in the air increased markedly when hot water was running (generating water vapour), with the exception of the sample from the bathroom of patient 3. Furthermore, the number of colonies decreased under less humid conditions in the bathrooms of all four patients after the bathtub was filled with hot water. In patient 5, whose was infected with *M. massiliense*, the same species was detected in the air while hot water was running (65 colonies), whereas no NTM was detected in the air after supplying hot water or from the swab samples. WGS analysis using two and three isolates from the patient's specimens and the environment, respectively, identified only three SNVs among the five, which were thought to be identical [11].

The first examination of patient 5 was conducted during treatment of her second *M. massiliense* disease, which was successfully completed 1-year after culture reversion (8 months after the examination). The patient stopped using the bathtub after the first investigation. The next environmental investigation was conducted 1 year after the first investigation; *M. massiliense* with an identical genotype was again isolated after running hot water into the bathtub (table 1). After the patient stopped bathtub use, no recurrence of *M. massiliense* infection was observed, even with persistent bronchiectatic changes. The sputum samples were negative 12 times and no radiological progression was observed.

In case 5, the two separate collections of swab samples from the hot water inlet did not yield *M. massiliense*, but the pathogen was isolated from the air with running hot water. We therefore concluded that NTM might exist deep inside of the piping system and that simple cleaning of the inlet may be insufficient to remove the pathogen. Interestingly, even with the same system, *M. massiliense* was not detected (only seven colonies of other NTM were identified) from the air when the shower was running. Thus, the patient stopped using the bathtub after the first examination, but still uses the shower. Based on this result, the infection control measure, *i.e.* no use of the bathtub and suppression of the aerosol in this case, may be able to prevent reinfection with NTM.

As a certain NTM in the environment can exist as various genotypes, reinfection is generally thought to be caused by different genotype [1, 2, 12]. Discrimination of genotypes in first and second NTM infections has been performed using several genetic methods. Recently, WGS analysis has shown the highest discrimination power. Studies performing WGS analyses of *M. tuberculosis* isolates showed that most relapses are caused by reactivation of the same pathogen/genotype and reinfection with a different genotype is rare. This finding may be due to the rare chance of exposure to *M. tuberculosis* even in a setting with high TB prevalence, and the acquired immunity against TB would lower the possibility of reinfection with another genotype [11]. In addition, NTM patients have a high risk of reinfection because of the ubiquitous presence of NTM. Based on the air sampling and WGS results from the present study, NTM patients may be frequently exposed to aerosolised pathogens and infected repeatedly by the identical genotype from their environment, particularly in a small space like the bathroom. Therefore, identification of the infection route and identical genotype by WGS will be of great importance to demonstrate the occurrence of relapse. We believe that further analyses to clarify this infection control measure, including comprehensive environmental analysis of NTM genotype, is warranted.

In conclusion, this study identified, for the first time, the same genotype of *M. massiliense* from a patient and the aero-environment. Our findings also indicate that appropriate infection control measures should be based on environmental investigation. The preventive measure taken in this study indirectly demonstrates that recurrent NTM infections can be caused by reinfection and not by relapse. Overall, the proportion of recurrent cases due to reinfection may be higher than expected.

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