Research paper

Genetic relatedness of *Mycobacterium avium* subsp. *hominissuis* isolates from bathrooms of healthy volunteers, rivers, and soils in Japan with human clinical isolates from different geographical areas

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ABSTRACT

Japan reportedly has high incidence rate of nontuberculous mycobacterial lung disease (14.7 cases per 100,000 person in 2014). In Japan, the most common etiology is *Mycobacterium avium* subsp. *hominissuis* (MAH). MAH is a typical inhabitant of the environment, especially bathrooms, which are considered as a potential source of infection. To corroborate this hypothesis, we determined the detection rate of MAH in bathrooms of healthy volunteers by an ordinary culture method and we analyzed the genetic relatedness of these isolates with those from patients and other sources. We collected swabs of bathtub inlets, showerheads, bathroom drains, and shower water from 180 residences throughout Japan. The overall MAH detection rate was 16.1%, but the rate varied among regions: it was high in Kanto (9/34, 26.5%) and Kinki (9/33, 27.3%), but low in Kyushu (0/11, 0%), Tohoku (1/23, 4.3%), and Hokkaido (2/23, 8.7%). MAH was detected primarily in bathtub inlet samples (25 out of 170 residences). Variable numbers of tandem repeats (VNTR) analysis was used to examine the genetic relatedness of 57 MAH isolates from bathrooms of the healthy volunteers with human clinical isolates. A minimum spanning tree generated on the basis of the VNTR data indicated that isolates from the bathrooms of the healthy volunteers had a high degree of genetic relatedness with those from Japanese patients, bathrooms of patients, and river water, but not with those from Russian patients and Japanese pigs. These results showed that bathtub inlets in Japan provide an environmental niche for MAH and suggest that bathrooms are one of the important infection sources of MAH in Japan. Understanding country-specific lifestyle habits, such as bathing in Japan, as well as the genetic diversity of MAH, will help in elucidating the sources of this pathogen.

1. Introduction

Mycobacterium avium subsp. *hominissuis* (MAH) is one of the most clinically relevant non-tuberculous mycobacteria (Tortoli, 2014), which can infect humans and other animals, notably pigs (Leao et al., 2014).

The increasing trend in the incidence of MAH infections worldwide has led to a growing awareness of the pathogen and its recognition as a global public health concern (Hoefsloot et al., 2013; Nishiuchi et al., 2017). The incidence rate of nontuberculous mycobacterial pulmonary disease in Japan is reported 14.7 cases per 100,000 person in 2014

(Namkoong et al., 2016) and MAH is the most common etiology of this disease in Japan. Existing therapies for MAH infection are lengthy (typically more than one year) and have low efficacy in a large fraction of the patient population (Griffith et al., 2007). Therefore, prevention of MAH infection is critical. Human-to-human transmission is considered to be negligible, and the primary source of MAH infection appears to be the environment (Ichijo et al., 2014).

Recent investigations of reservoirs of MAH suggest that household water is one of the possible sources of infection based on the similarity in genotypes between isolates from the residential environment of patients (shower water, showerhead biofilm, bathroom, and hot bathtub) and clinical isolates (Falkinham III et al., 2008; Nishiuchi et al., 2007). Nishiuchi et al. (Nishiuchi et al., 2007; Nishiuchi et al., 2009) reported a predominant colonization of *M. avium* in bathtub inlets of bathrooms of patients in Japan based on culture isolation and pulsed field gel electrophoresis-based genotyping. Later, Iwamoto et al. (2012) reported a high degree of genetic relatedness between isolates from pulmonary MAH patients and isolates from their bathrooms based on variable numbers of tandem repeats (VNTR) analysis of 19 loci. These reports implied that bathrooms potentially are a major source for MAH in Japan. However, a major limitation of these studies was that the possibility of transmission of the pathogen from the patients to their bathroom was not ruled out.

To address this limitation, it is necessary to study isolates from the bathrooms of healthy individuals which lessen the effect of possible contamination of bathrooms from an infected patient. Here, we performed nationwide sampling of the swabs of bathtub inlets, bathroom drains, shower heads, and shower water to obtain MAH isolates as well as to determine the detection rate of MAH in there. The obtained MAH isolates were used for the analysis of 19-loci VNTR and compared the data with those retrieved from previous reports (Iwamoto et al., 2014; Iwamoto et al., 2012; Starkova et al., 2013; Yano et al., 2017).

The main objective of this study was to clarify the genetic relatedness between MAH isolates from the bathrooms in residences of healthy volunteers and those from various other sources, including bathrooms of patients, human clinical isolates from different countries (Japan, Korea, and Russia), and pigs in Japan. Moreover, we included isolates from river surface water and soils as well, to broaden our understanding of the genetic diversity of MAH strains in Japan.

2. Materials and methods

2.1. MAH isolates obtained in this study

Seventeen MAH isolates from river surface water, five isolates from soil, and 56 isolates from the bathrooms of healthy volunteers were obtained in this study. Numbers of MAH isolates and sampling points are listed in Table 1.

2.1.1. MAH isolates from bathrooms of healthy volunteers

The typical Japanese bathroom was depicted in Fig. 1. The bathtub inlet, which is connected to a boiler, is installed inside the bathtub, below the water level. Bathing in a bathtub and washing body at outside a bathtub is a common lifestyle practice in Japan. Therefore, there is a bathroom drain and shower is installed at the wall not above the bathtub.

In a nationwide sampling at six regions in Japan (Fig. S1) between Sep 2017 and Jan 2019, we received 149 shower water samples and 146 bathtub inlet swabs from 149 healthy volunteer households with the consent from the volunteers (study 1). Two liters of shower water was used to isolate MAH. The water was filtered through a polycarbonate membrane (47 mm in diameter) with 0.2-μm pore size (ADVANTEC CO., LTD, Tokyo, Japan). The membrane was treated with 2% NaOH for 10 min, neutralized with phosphate-buffered saline (PBS), and incubated on a 7H11 PANTA plate (Nishiuchi et al., 2007) at 37 °C for 3–4 weeks for bacterial culture. Biofilm samples from bathtub inlets

Table 1
Sample information and MAH isolates from natural and living environment in this study.

Sample background	Origin of samples	Sample type	Number of sampling points	Number of sampling points with MAH	% of samples with MAH	Number of genotypes of MAH isolates	Sampling period	Case[a]
Natural environment	River	Surface water	65	11	16.9	11	Aug, 2015–Aug, 2018	Study 1
	Soil	Surface water	3	2	66.7	6	Feb, 2011	Study 2
		Flower pots with plants, Soil around trees, Gardens, Other places with plants, Place without plants	28	2	7.1	5	Apr, 2014–Sep, 2014	Study 2
Living environment of healthy volunteer	Bathroom	Shower water	149	8	5.4	8	Sep, 2017–Jan, 2019	Study 1
		Swab of bathtub inlet	146	17	11.6	29	Sep, 2017–Jan, 2019	Study 1
		Swab of bathtub inlet	24	8	33.3	14	Dec, 2010–Jun, 2011	Study 2
		Swab of showerhead inside	14	0	0	0	Dec, 2010–Jun, 2011	Study 2
		Swab of showerhead surface	23	0	0	0	Dec, 2010–Jun, 2011	Study 2
		Swab of drain outlet	25	1	4	5	Dec, 2010–Jun, 2011	Study 2

[a] Study 1 was performed at Kobe Institute of Health; study 2 was performed at Osaka University. Regarding the samples from bathroom, study 1 was nationwide sampling in Japan and study 2 was local sampling at Osaka in western Japan.

Fig. 1. Typical Japanese bathroom.

were collected using sterilized swabs (4 N6 FLOQSwabs; Copan, Brescia, Italy). Each swab sample was suspended in 500 μl of PBS and centrifuged at 15,000 ×g for 10 min. The pellets were resuspended in 0.1 ml of PBS. To 75 μl of the 0.1-ml PBS suspension, an equal volume of 4% NaOH was added, left to stand for 10 min, and neutralized with 1 ml of PBS. The neutralized solution was centrifuged at 15,000 ×g for 10 min, and the pellet was resuspended in 100 μl of PBS. Fifty microliters of the suspension was inoculated onto a 7H11 PANTA plate that was incubated at 37 °C for 3–4 weeks. Growing colonies were picked up, recultured, and DNA was extracted using a simple boiling method,

according to a previous report (Iwamoto et al., 2012). The DNA was used for species identification and VNTR analysis (Iwamoto et al., 2012). MAH identification was carried out by *hsp65* sequence analysis as previously described by Turenne et al. (2006).

In a local sampling at Osaka in western Japan between Dec 2010 and Jun 2011, we collected 86 swab samples from 24 bathtub inlets, 25 bathtub drains, and 37 showerheads (14 from inside and 23 from the surface) from 31 healthy volunteer households, as described previously (Ichijo et al., 2014), with consent of the volunteers (study 2). MAH was isolated as described above, but with larger swabs (Large Alpha Swab TX714A; Texwipe, Kernersville, NC). Accordingly, each sample was suspended in 10 ml of PBS. To 2 ml of the 10-ml PBS suspension, an equal volume of 2% NaOH was added, left to stand for 15 min, and neutralized with 50 ml of PBS. The neutralized solution was centrifuged at 14,000 ×g for 13 min, and the pellet was resuspended in 1 ml of PBS. One hundred microliters of the suspension was inoculated onto a 7H11 PANTA plate and incubated at 37 °C for 3–4 weeks. MAH isolation and identification were conducted as described above.

2.1.2. MAH isolates from rivers

Between Aug 2015 and Aug 2018, 65 river water samples were collected from two rivers (Yodogawa river and Inagawa river) in the Kinki region of Japan (study 1). The river water (500 ml) was pre-filtered through a 0.8-μm pore-size membrane and was used to isolate MAH by the method described for nationwide sampling of shower water. Separately, three water samples were collected from the Kanzakigawa, Yodogawa, and Inagawa rivers on Feb, 7, 8, and 24, 2011, respectively (study 2). The river water (5 l) was prefiltered through a 10-μm pore-size membrane and then through a 3-μm pore-size membrane. Then, the water was filtered through a polycarbonate membrane with 0.4-μm pore size. The filter was soaked in 10 ml of distilled water and the tube was vigorously vortexed to release the bacterial cells from the filter. An equal volume of 2% NaOH was added to the suspension, which was then used for MAH isolation as described for swab samples from Osaka.
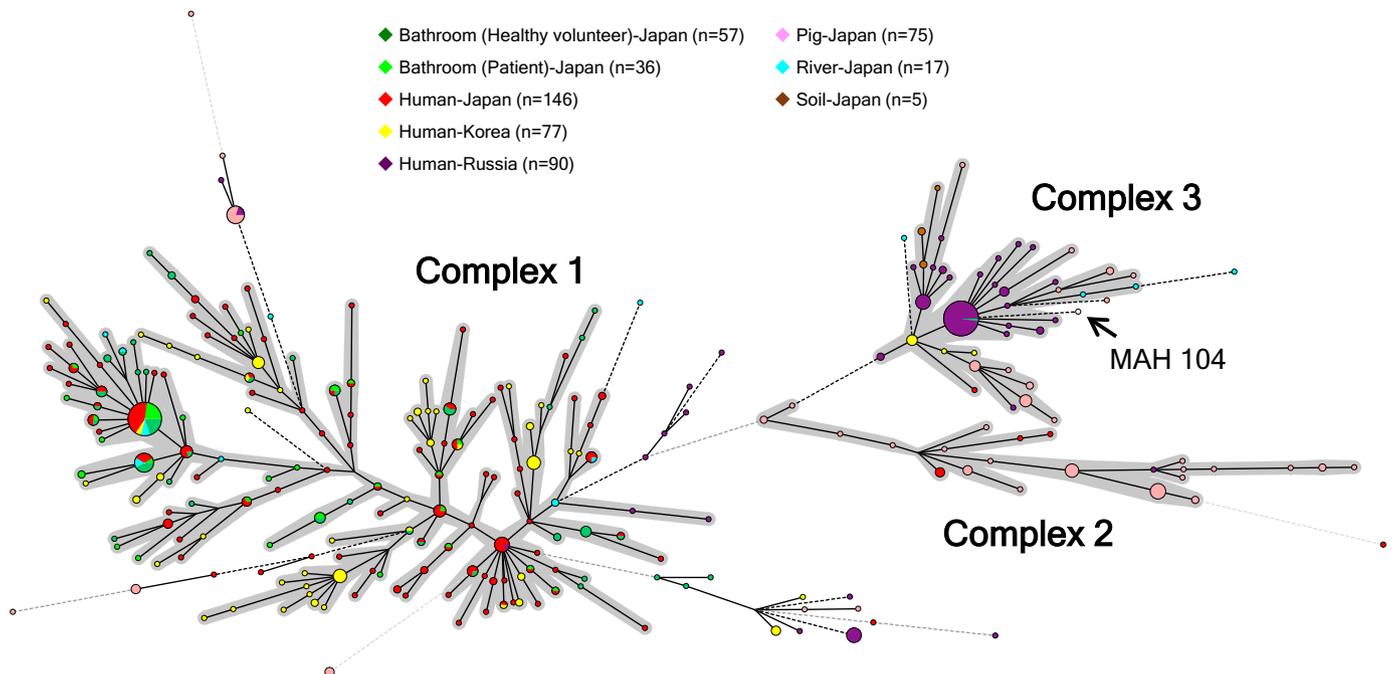

Fig. 2. Minimum spanning tree (MST) based on 19-loci VNTR profiles of 503 MAH strains isolated from various sources (human patients, pigs, biofilm of bathroom, shower water, river water, and soil), and geographic regions (Japan, Korea, and Russia). Circles indicate different VNTR genotypes. Their sizes are proportional to the number of isolates sharing an identical pattern. MAH 104 is indicated by an arrow. Linkages within triple-locus variations are indicated by lines, the lengths of which correspond to the number of variations. Four- or more-locus variations are indicated by dots. Clonal complexes shaded in gray were created following the rule described in the Materials and Methods section.

Table 2
Distribution of clonal complexes of the MAH strains with different origins.

Origin of samples	Clonal complex No.				Total
	1	2	3	Others	
Human_Japan_P (%)	135 (92.5)	5 (3.4)	1 (0.7)	5 (3.4)	146 (100)
Bathroom_Japan_H (%)	53 (93)	0 (0)	1 (1.8)	3 (5.3)	57 (100)
Bathroom_Japan_P (%)	36 (100)	0 (0)	0 (0)	0 (0)	36 (100)
Human_Korea_P (%)	66 (85.7)	0 (0)	6 (7.8)	5 (6.5)	77 (100)
Human_Russia_P (%)	3 (3.3)	1 (1.1)	68 (75.6)	18 (20)	90 (100)
River_Japn (%)	11 (64.7)	0 (0)	2 (11.8)	4 (23.5)	17 (100)
Pig_Japan (%)	0 (0)	36 (48)	19 (25.3)	20 (26.7)	75 (100)
Soil_Japan (%)	0 (0)	0 (0)	5 (100)	0 (0)	5 (100)
Total (%)	304 (60.4)	42 (8.3)	102 (20.3)	55 (10.9)	503 (100)

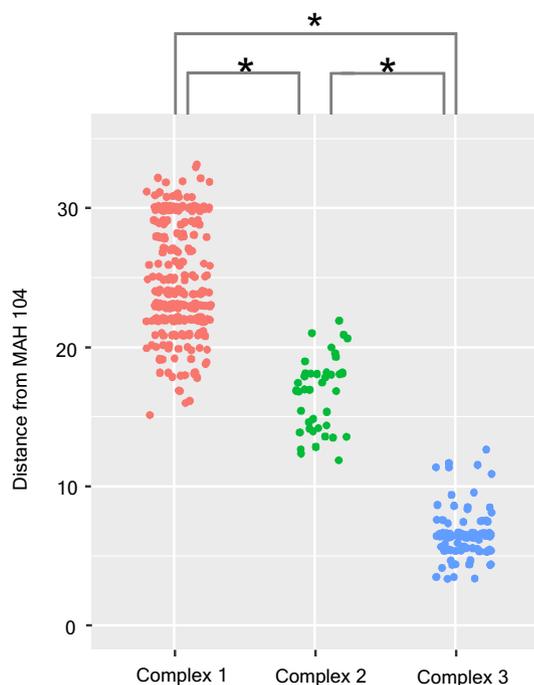P: Patient, H: Healthy volunteer.

Fig. 3. Genetic distance relationships of clonal complexes 1, 2, and 3. Genetic distance was calculated as the Manhattan distance from MAH 104. Pairwise comparison was conducted by the Mann–Whitney *U* test followed by Bonferrroni correction. *$p < .001$ was considered significant.

2.1.3. MAH isolates from soil

In total, 28 soil samples were collected from flower pots with plants (n = 4), soil around trees (n = 10), gardens (n = 3), other places with plants (n = 8), and places without plants (n = 3) at Osaka University campus in Osaka between Apr 2014 and Sep 2014. The soil samples were collected at a depth of 3 cm. MAH were isolated according to the method of Parashar et al. (2004). Briefly, 5 g of soil sample was mixed with 20 ml of sterile water for 1 min and centrifuged at $600 \times g$ for 5 min at 4 °C. To the precipitate, 10 ml of each of 6% sodium dodecyl sulfate solution and 8% NaOH solution was added, and the mixture was vortexed and then left to stand at room temperature for 30 min. After centrifugation at $8000 \times g$ for 15 min at 4 °C, 20 ml of 2% cetrimide solution was added to the precipitate, and the mixture was left to stand at room temperature for 15 min and then centrifuged at $8000 \times g$ for 15 min at 4 °C. The precipitate was washed twice with 20 ml of sterile water followed by centrifugation at $8000 \times g$ for 15 min at 4 °C. The precipitate was suspended in 0.5 ml of sterile water, and 100 μl of this suspension was applied onto a 7H11 PANTA plate and cultured at 37 °C for 3 weeks. MAH were identified as described for the nationwide shower water samples.

2.2. VNTR analysis

VNTR analysis on the basis of 19 loci (Iwamoto et al., 2012), including 15 loci reported by Inagaki et al. (2009) and 7 loci reported by Thibault et al. (2007), was conducted using the DNA samples that had been used for MAH identification. The fluorescently labeled primers used for multiplex PCR are shown in Table S1. Multiplex PCR was conducted using Ex Taq Hot start Version with GC buffer I (Takara Bio Inc., Shiga, Japan) using the following conditions: 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, and finally, 72 °C for 3 min. When amplification failed, the PCR polymerase was changed to Kod-FX (Toyobo, Osaka, Japan), and the PCRs were conducted using the following conditions: 94 °C for 2 min, 30 cycles of 98 °C for 10 s, 58 °C for 30 s, and 68 °C for 1 min, and finally, 68 °C for 3 min. The multiplex PCR products were diluted 20-fold with purified water and analyzed on a 3500 Genetic Analyzer (Thermo Fisher Scientific, USA), as described previously (Iwamoto et al., 2012).

2.3. Minimum spanning tree (MST)

Nineteen-loci VNTR data for MAH isolates from other sources were retrieved from previous reports: Japanese patients (n = 146), bathrooms of patients (n = 36), the bathroom of a healthy volunteer (n = 1; bathtub water), and Japanese pigs (n = 75) (Iwamoto et al., 2012); Korean patients (n = 77) (Iwamoto et al., 2014); and Russian patients (n = 90) (Starkova et al., 2013; Yano et al., 2017). VNTR data profiles of all isolates used in this study are listed in Table S2. An MST was generated based on the 19-loci VNTR data including 78 samples from this study (17 river isolates, five soil isolates, and 56 isolates from the bathrooms of healthy subjects) and the 425 previously reported isolates, using Bionumerics v.5.10 (Applied Maths, Sint-Martens-Latem, Belgium) for clustering analysis. We used the following reconstruction rules for the MST: a categorical coefficient was selected, the priority rule was set such that the type with the highest number of single-locus variants would be linked first, and creation of hypothetical types was permitted. The creation of clonal complexes was defined by setting the maximum number of changes in VNTR to fewer than three loci with more than ten genotypes.

2.4. Genetic distance

Genetic distances of the 503 MAH isolates were determined using *M. avium* 104 as a reference to estimate the Manhattan distances, according to previous reports (Ichikawa et al., 2015; Kikuchi et al., 2009). Distances among isolates were evaluated using the Mann–Whitney *U* test. Statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (Kanda, 2013), a graphical user interface for R 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria). Values of $p < .001$ were considered significant based on a previous report (Ichikawa et al., 2015).
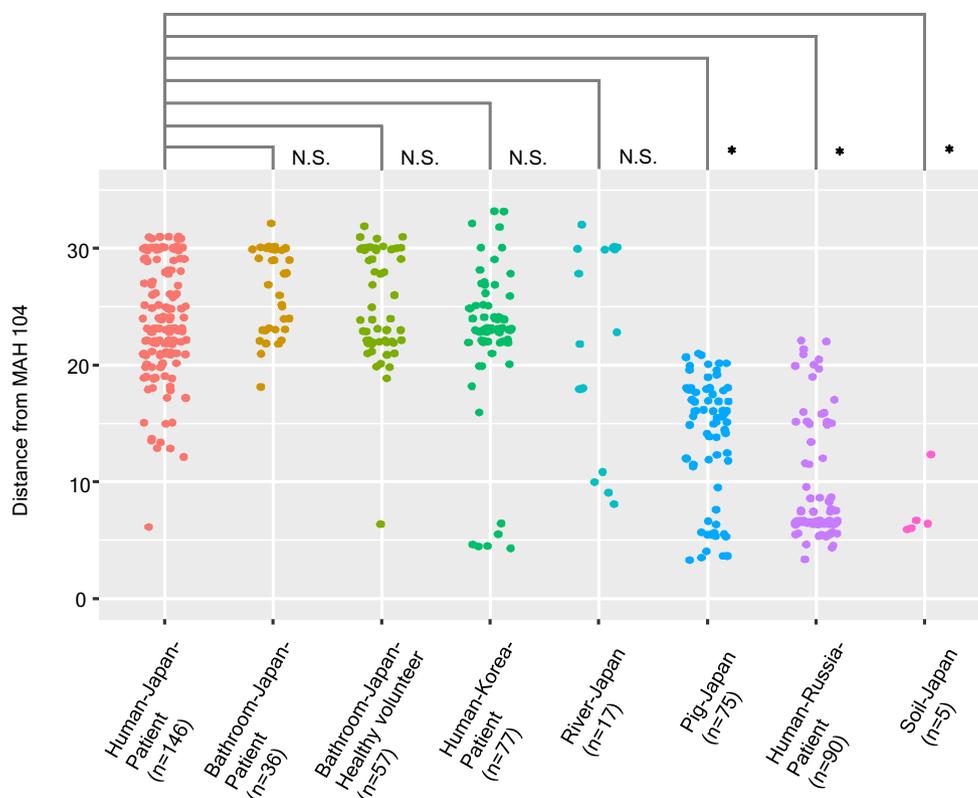
Fig. 4. Genetic distance relationship of MAH strains isolated from different hosts. Genetic distance was calculated as the Manhattan distance from MAH 104. Pairwise comparison was conducted by the Mann–Whitney *U* test. *$p < .001$ was considered significant. N.S., not significant.

3. Results

3.1. MAH isolation from environmental samples

We recovered MAH from 21 out of 149 bathrooms sampled in the nationwide sampling (14.1%); 17 isolates from 146 bathtub inlet swabs (11.6%) and eight isolates from 149 shower water samples (5.4%) (Table 1). In the local sampling at Osaka, we isolated MAH from eight out of 31 bathrooms (25.8%); eight isolates from 24 bathtub inlet swabs (33.3%); and one from 25 drain outlet swabs (4%). MAH was not recovered from the 37 showerhead samples. Some samples showed polyclonal colonization as indicated by VNTR analysis. In total, 56 genotypes of MAH were obtained from the 29 bathrooms. The overall detection rate of MAH was 16.1% (29 out of 180 bathrooms). However, the detection rate varied among regions; it was high in Kanto (9/34, 26.5%) and Kinki (9/33, 27.3%), but low in Kyushu (0/11, 0%), Tohoku (1/23, 4.3%), and Hokkaido (2/23, 8.7%) (Fig. S1).

Eleven MAH isolates were obtained from 65 river surface water samples collected in study 1, and six isolates were obtained from three surface water samples collected in study 2 (Table 1, Table S2). Five MAH isolates were obtained from two flower pot soil samples, whereas no MAH was recovered from the other 26 soil samples.

3.2. VNTR genotypes of 503 MAH isolates from different origins

VNTR analysis based on 19 loci discriminated 262 genotypes among the 503 MAH isolates, with a Hunter–Gaston discriminatory index value of 0.986 (Hunter and Gaston, 1988). We used an MST to compare the VNTR genotyping profiles of the 503 isolates to analyze clonal complexes (Fig. 2). In total, 448 isolates were classified into three clonal complexes (Table 2). Most of the isolates from the Japanese and Korean patients, and from bathrooms of both patients and healthy subjects belonged to complex 1. Additionally, out of 17 river isolates, 11 belonged to complex 1. One-half (48%) and one-quarter of the pig isolates (25.3%) belonged to complexes 2 and 3, respectively, whereas the remaining isolates were found scattered in the MST. Of the 90 isolates from Russian patients, 68 (75.6%) belonged to complex 3. All five soil isolates belonged to complex 3.

3.3. Comparison of the genetic distance of MAH isolates from different origins

Genetic distances among the 503 MAH isolates were estimated based on Manhattan distances, using *M. avium* 104 as a reference. This approach discriminated the three clonal complexes identified by MST, with statistically significant differences (Fig. 3). The genetic distance from *M. avium* 104 for isolates from Japanese patients was significantly different from those for isolates from Russian patients, Japanese pigs, and soil, whereas no statistically significant differences were found between isolates from Japanese patients and those from bathrooms of patients and healthy volunteers, rivers, and Korean patients (Fig. 4). Further, there were no significant differences between isolates from bathrooms of patients and healthy volunteers ($p = .173$). These findings were in good agreement with the MST analysis results, which showed that most of the isolates from Japanese patients, bathrooms of patients and healthy volunteers, river water, and Korean patients belonged to clonal complex 1 (Fig. 2, Table 2). The same result was obtained when we compared the river isolates with the other isolates, i.e., significant differences were found only with Russian patients, Japanese pigs, and soil samples (Fig. S2). Pig isolates were significantly distinct from the isolates of all of the other sources, except soil, of which there were only five samples (Fig. S3). In contrast with previous reports from Japan (Adachi et al., 2016; Uchiya et al., 2018), no significant differences were found between isolates from Russian HIV-positive and -negative patients (Figs. S4, S5). Most Russian isolates belonged to clonal complex 3, regardless of the HIV status of the patient.

4. Discussion

We determined the genetic relatedness between 57 MAH isolates from residential bathrooms of healthy individuals (43 from bathtub inlets, eight from shower water, five from drains, and one from bathtub water) and isolates from various other sources, including bathrooms of patients, human clinical isolates from different countries (Japan, Korea, and Russia), pigs, river surface water, and soil. Our results obtained by VNTR based on 19 loci revealed that the isolates from the bathrooms of healthy volunteers were closely related with the isolates from Japanese patients and bathrooms of patients. The rate of MAH detection in swab samples of bathtub inlets from residences of healthy volunteers was relatively high (25 out of 170 samples, 14.7%), whereas the detection rate in shower water was lower (8/149, 5.4%). Our data, along with previous reports (Ichijo et al., 2014; Nishiuchi et al., 2007; Nishiuchi et al., 2009), confirmed that bathtub inlets in Japan provide an environmental niche for MAH and therefore, bathrooms are one of the important sources of MAH in Japan. In a traditional Japanese bathtub, the bathtub inlet, which is connected to a boiler, is installed inside the bathtub, below the water level. Therefore, colonized MAH at bathtub inlet have a chance to be released into bathtub water. Bathing in a bathtub and washing body using bathtub water outside a bathtub is a common lifestyle practice in Japan. Japanese people like bathing in hot water to relax. Although none of the special practice is taken for producing aerosol, prolonged bathing for relaxing would increase a risk to be exposed to vapor from bathtub hot water. Moreover, used bathtub water is sometimes reserved in the bathtub until the next day for washing clothes or to provide for a disaster such as an earthquake (Nishiuchi et al., 2009). These country-specific lifestyle habits would create a unique colonization and exposure risk for MAH.

Comparison of the isolates from Japanese patients with those from Russian patients revealed that the prevalent clinical genotypes in these two countries are different. Recently, Uchiya et al. (Uchiya et al., 2018) and Adachi et al. (Adachi et al., 2016) reported that MAH isolates from HIV-positive patients in Japan form a unique genetic group that is rarely detected in HIV-negative patients. Our MST analysis including five strains representative of this unique group (DH-2, IH-068, NH-127, TH29, and TH89) (Fig. S6) revealed that they belonged to clonal complex 3, which consisted predominantly of Russian isolates, regardless of HIV infection, and a fraction of the pig isolates from Japan. Further, some of the river isolates and all five soil isolates belonged to this complex. These results strongly suggest that MAH occurring in Japan is genetically diverse and is not limited to clonal complex 1, to which most of the clinical isolates in Japan belong.

Given that the incidence rate of nontuberculous mycobacterial pulmonary disease in Japan is assumed to be the highest globally (Namkoong et al., 2016) and MAH is the most common etiology of this disease in Japan, the uniqueness of the Japanese MAH isolates, i.e., isolates in clonal complex 1, may imply they are more virulent than other isolates, such as those in complex 3, which includes Japanese HIV patients, Russian patients, and pigs in Japan. A recent study by Yano et al. (2017) based on whole genome-based population structure analysis revealed two predominant lineages among Japanese patients, MahEastAsia 1 and MahEastAsia 2. These lineages could be equivalent to our clonal complex 1. The alleles unique to these two lineages were identified in the loci responsible for trehalose biosynthesis (*treS* and *mak*) and in one mammalian cell entry operon. Further studies on these lineages are expected to clarify whether they are more virulent than others.

There are limitations to our study. The sampling was performed by each individual healthy volunteers and sent to us via courier. Therefore, the condition for the samples were not uniform. Moreover, we used different culture-based methods to determine the detection rate of MAH at two institutes, i.e., Kobe Institute of Health and Osaka University. Therefore, the detection rate of MAH in this study should be biased. A standardized sampling method including transportation system and using culture-independent method might have yielded a different picture for the detection rate. Moreover, the sampling sites and sample numbers were limited, especially for river water and soils. More samples from river water as well as soils are needed to understand the genetic diversity in various MAH habitats in Japan. Home dust also need to be investigated. Meanwhile, Fujita et al. (2013) reported that residential soils are a likely source of MAH. In Germany, MAH reportedly is predominant in soil and home dust (Lahiri et al., 2014).

In conclusion, the present study revealed that MAH isolates from bathtub inlets and shower water in residences of healthy individuals in Japan are genetically closely related with isolates from Japanese patients. This finding reinforces the hypothesis that bathrooms are one of the major sources of MAH infection in Japan. Moreover, our data from river water and soils indicate that MAH genotypes that are rarely isolated in Japanese clinical sites, but are commonly isolated from Russian patients and pigs in Japan, are prevalent in the natural environment. Further studies are needed to further elucidate environmental niches in both the inhabited environment and the pristine natural environment, which could contribute to reducing the frequency of exposure to MAH from environmental sources and thus, aid in the prevention of MAH infections.

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103923.

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