

RESEARCH ARTICLE

Association of ISMav6 with the Pattern of Antibiotic Resistance in Korean *Mycobacterium avium* Clinical Isolates but No Relevance between Their Genotypes and Clinical Features

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Abstract

The aim of this study was to genetically characterize clinical isolates from patients diagnosed with *Mycobacterium avium* lung disease and to investigate the clinical significance. Multi-locus sequencing analysis (MLSA) and pattern of insertion sequence analysis of *M. avium* isolates from 92 Korean patients revealed that all isolates were *M. avium* subspecies *hominissuis*. In *hsp65* sequevar analysis, codes 2, 15, and 16 were most frequently found (88/92) with similar proportions among cases additionally two isolates belonging to code N2 and an unreported code were identified, respectively. In insertion element analysis, all isolates were IS1311 positive and IS900 negative. Four of the *M. avium* subsp. *hominissuis* isolates did not harbor IS1245 and 1 of the *M. avium* isolates intriguingly harbored DT1, which is thought to be a *M. intracellulare*-specific element. *M. avium* subsp. *hominissuis* harboring ISMav6 is prevalent in Korea. No significant association between clinical manifestation and treatment response has been found in patients with the *hsp65* code type and ISMav6, indicating that no specific strain/genotype among *M. avium* subsp. *hominissuis* organisms was a major source of *M. avium* lung disease. Interestingly, the presence of ISMav6 was correlated with greater resistance to moxifloxacin. Conclusively, the genotype of Korean *M. avium* subsp. *hominissuis* isolates is not a disease determinant responsible for lung disease and specific virulent factors of *M. avium* subsp. *hominissuis* need to be investigated further.

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Abbreviations: MAC, *Mycobacterium avium* complex; MLSA, multi-locus sequencing analysis; IS, insertion sequences; RFLP, restriction fragment length polymorphism; PRA, PCR restriction fragment length polymorphism analysis; NTM, non-tuberculous mycobacteria; CLM, clarithromycin; RIF, rifampin; EMB, ethambutol; MXF, moxifloxacin; MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; VNTR, variable number of tandem repeats.

Introduction

A rise in the incidence of pulmonary disease caused by nontuberculous mycobacteria (NTM) has been reported worldwide [1, 2]. *Mycobacterium avium* complex (MAC) is the most frequent etiology of NTM lung disease [3]. MAC initially included two species, *M. avium* and *M. intracellulare*. *M. avium* is the most clinically significant species for humans and animals within the MAC and is divided into four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum* [4, 5].

Although subspecies of *M. avium* in different geographic regions or populations may have different levels of virulence due to co-evolutionary processes, consequently leading to varying epidemiological dominance, most cases of *M. avium* human disease are due to *M. avium* subsp. *hominissuis*. Recently, lymphadenitis patients in France were found to be infected by only *M. avium* subsp. *hominissuis* among *M. avium* subspecies [6]. More recently, a subspecies identification analysis of *M. avium* clinical strains in the USA showed *M. avium* subsp. *hominissuis* to be the dominant *M. avium* subspecies (92.6%), followed by *M. avium* subsp. *avium* (7.4%) [7]. All German *M. avium* strains isolated from children and adults were identified as *M. avium* subsp. *hominissuis* [8].

Many studies have emphasized the importance of taxonomy in distinguishing species and subspecies of MAC because non-sequencing methods or 16S rRNA sequencing frequently fails to distinguish closely related species [9, 10]. Multi-locus sequencing analysis (MLSA) has been suggested as the new standard method for identifying *Mycobacterium* species that are not well discriminated by 16S rRNA gene sequences alone [11–14].

The presence and distribution of various insertion sequences (IS) among *M. avium* subspecies have provided an unprecedented opportunity to define the genomic differences between *M. avium* subspecies as well as to develop molecular typing methods with sufficient discriminatory power to differentiate *M. avium* subspecies and isolates [15].

At our institution, the *rpoB*-PCR restriction fragment length polymorphism (RFLP) analysis [PRA] method was used for species identification and diagnosis of MAC lung disease until 2009 [16–18]. To gain better insight into *M. avium* lung disease in Korea, we used sequencing-based methods for subspecies identification and genotyping and compared clinical characteristics and treatment outcomes according to genotype. Furthermore, we investigated patterns of antibiotic resistance according to mycobacterial genotype as well as the presence or absence of IS*Mav6*.

Materials and Methods

Study subjects

Clinical isolates from 92 patients with newly diagnosed *M. avium* lung disease from Jan. 2008 to Dec. 2009 at Samsung Medical Center (Seoul, Korea) were collected and stored. The data in the present study are part of an ongoing prospective observational cohort study investigating NTM lung disease (ClinicalTrials.gov Identifier: NCT00970801). The study protocol for isolates collection and genotyping analysis was approved by the institutional review board of the Samsung Medical Center (IRB approval 2008-09-016), and written informed consent was obtained from all participants. All patients met the diagnostic criteria for NTM lung disease [3]. All patients were immunocompetent and none of the patients tested positive for human immunodeficiency virus. All isolates were collected before initiating antibiotic treatment for NTM lung disease. Additionally, *M. avium* species initially identified by PRA based on the *rpoB* gene at the time of diagnosis as previously described were used for subsequent analysis.

Drug susceptibility test

Drug susceptibility testing was performed at the Korean Institute of Tuberculosis. The minimum inhibitory concentrations (MICs) of clarithromycin (CLR) and moxifloxacin (MXF) were determined using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) [19]. Isolates with CLR MIC of ≥ 32 $\mu\text{g/ml}$ and MXF MIC of ≥ 4 $\mu\text{g/ml}$ were considered to be resistant, according to the guidelines of the CLSI [19].

Molecular characterization of *M. avium* clinical isolates by MLSA

M. avium strains were propagated in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI, USA) supplemented with 10% (vol/vol) oleic acid-albumin-dextrose-catalase (OADC; BD Diagnostics, Sparks, MD, USA). Mycobacterial DNA was extracted using a DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). MLSA including *hsp65*, *rpoB*, and 16S rRNA fragments was carried out using PCR primer sets as described previously [20–22]. The PCR products of target genes were subjected to sequence analysis. The nucleotide sequences of these genes were compared with data reported by BLAST analysis (<http://www.ncbi.nlm.nih.gov>) against sequences from *M. avium* subspecies type and related strains. *M. avium* subsp. *avium* ATCC 25291, *M. avium* subsp. *hominissuis* 104, *M. avium* subsp. *paratuberculosis* K-10, and *M. avium* subsp. *silvaticum* ATCC 49884 were used as reference strains. For phylogenetic analysis, sequences were trimmed using the CLUSTAL-W multiple sequence alignment program [23]. Phylogenetic trees were obtained from DNA sequences utilizing the neighbor-joining method and Kimura's two parameter distance correction model with 1,000 bootstrap replications supported by MEGA 6.0 software [24].

hsp65 code analysis

hsp65 code analysis was performed as previously described [25]. *hsp65* gene PCR products were subjected to sequence analysis. The nucleotide sequences of the *hsp65* gene were compared with data reported by BLAST analysis (<http://www.ncbi.nlm.nih.gov>) against the *M. avium* type and related strains. *hsp65* codes were classified according to previously reported papers [25–27].

Insertion sequences element analysis

Multiplex PCR was performed to detect three target genes, IS900, IS1311, and DT1 using previously described methods [28]. A previously described primer set was used for the IS1245 insertion element [29]. The presence of ISMav6 was determined by PCR followed by sequencing analysis using a previously described primer set [26]. PCR products of insertion elements were sequenced and the existence of a specific insertion element in each strain was confirmed. DNA isolated from *M. abscessus* ATCC 19977, *M. tuberculosis* H37Rv ATCC 27294, and *M. gastri* ATCC 15754 were used as negative controls for each primer set in each PCR run.

Statistics

All data are presented as median and interquartile range for continuous variables and as number (percentage) for categorical variables. Data were compared using the Mann-Whitney *U* test for continuous variables and the chi-squared or Fisher's exact test for categorical variables. The Mantel-Haenszel test for categorical variables was used to compare each *hsp65* code or the presence of ISMav6 across resistant levels (susceptible, intermediate, resistant) of each drug [30]. Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and a *P*-value less than 0.05 was considered statistically significant.

Results

Subspecies identification of *M. avium* clinical isolates by MLSA

Isolates from 92 patients diagnosed with *M. avium* lung disease were re-identified. The 16S rRNA sequences of *M. avium* subsp. *hominissuis* are identical to those of the *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum*. All isolates were identified as *M. avium* based on 16S rRNA sequencing. The *rpoB* sequences of 76 isolates were identical to those of the *M. avium* subsp. *hominissuis* type strain (GenBank accession no. CP000479) and clinical strain (GenBank accession no. AP012555) ([S1 Fig](#)). A total of 10 isolates showed a 1-bp mismatch (99.9% similarity) in a 711-bp fragment of the *rpoB* gene of *M. avium* subsp. *hominissuis* type strain (GenBank accession no. CP000479). The other 6 isolates were identical to those of the *M. avium* subsp. *avium* type strain (GenBank accession no. GQ153306). However, the nearly complete *hsp65* sequences of all isolates, except 4 isolates, were identical only to those of the *M. avium* subsp. *hominissuis* type strain (GenBank accession no. CP000479) and clinical strains (GenBank accession nos. AP012555 and DQ284766) ([Fig 1](#)). Each pair of the 4 isolates were 99.8% (1413/1416) and 99.7% (1412/1416) similar to the *hsp65* sequences of the *M. avium* subsp. *hominissuis* type strain (GenBank accession no. CP000479). The *hsp65* sequences of *M. avium* subsp. *hominissuis* were different (6-bp mismatches) from those of the *M. avium* subsp. *avium* type strain (GenBank accession no. DQ284768). Phylogenetic analysis based on the concatenated *hsp65* and *rpoB* sequences from all isolates and from those of closely related species within the MAC showed that all isolates belong to *M. avium* subsp. *hominissuis* ([Fig 2](#)). Therefore, all isolates were identified as *M. avium* subsp. *hominissuis* using MLSA.

Distribution of *hsp65* codes in *M. avium* subsp. *hominissuis* strains

In total, the 92 isolates were classified into five different *hsp65* sequevars. There were no isolates classified to *hsp65* code 4, the *M. avium* subsp. *avium* sequevar. Four of these sequevars were well recognized as *M. avium* subsp. *hominissuis* type and clinical strains and 1 sequevar code was newly identified in this study. The new sequevar was coded N7 (following the code name given in the previous paper [N1-N3] [[26](#)] and accepted paper [N4-N6]) and two isolates were classified as code N7. The distribution of *hsp65* sequevars in the 92 isolates is shown in [Table 1](#). The major codes were 2, 15, and 16.

Detection of insertion sequence elements

All isolates were negative for IS900 (considered diagnostic for *M. avium* subsp. *paratuberculosis*) [[31](#)] and positive for IS1311 (considered diagnostic for all members of *M. avium* subspecies) [[32](#)]. One isolate belonging to the new sequevar N7 possessed DT1 (considered diagnostic for *M. intracellulare* and *M. avium* subsp. *avium*); the DT1 sequence of this isolate was similar (92% and 89% similarity) to those of the *M. intracellulare* clinical strain and *M. avium* serotype 2 (*M. intracellulare*) DT1 (GenBank accession nos. CP003491 and L04543), suggesting that horizontal transfer of DT1 might occur from *M. intracellulare* to *M. avium*. Among 92 isolates, 88 (96%) were positive for IS1245 (considered to be limited to *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, and *M. avium* subsp. *silvaticum*) [[5](#), [33](#), [34](#)] and the other 4 isolates did not harbor IS1245. Interestingly, 56 (61%) isolates possessed ISMav6. The code 15 sequevar showed a significantly higher prevalence of ISMav6 (84%) than did the other codes ([Table 1](#)).

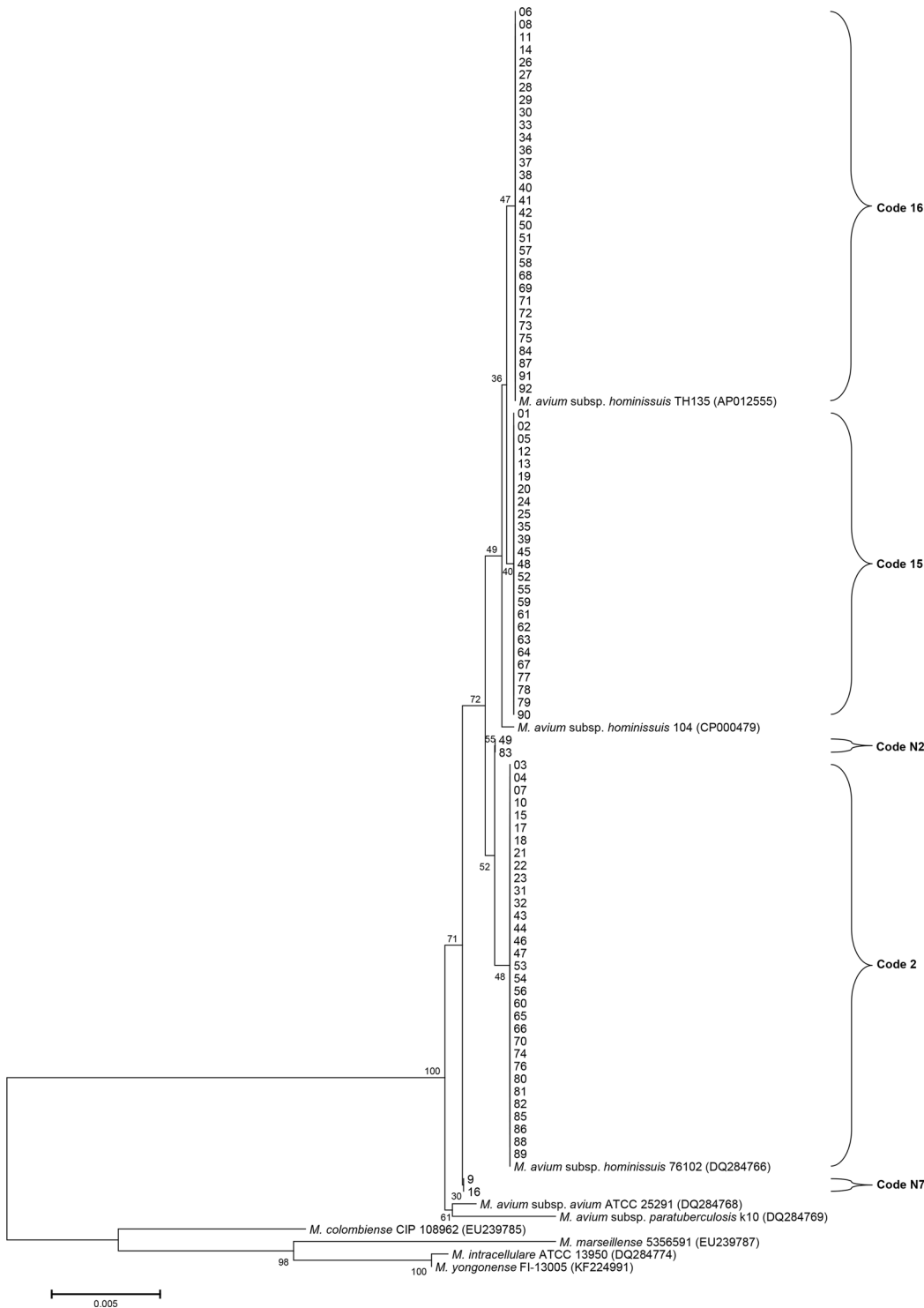


Fig 1. *hsp65* sequence-based phylogenetic tree using the neighbor-joining method with Kimura's two-parameter distance correction model. Bootstrap analyses determined from 1,000 replicates are indicated at the nodes. Bar, 0.5% difference in nucleotide sequence. GenBank accession numbers are given in parentheses.

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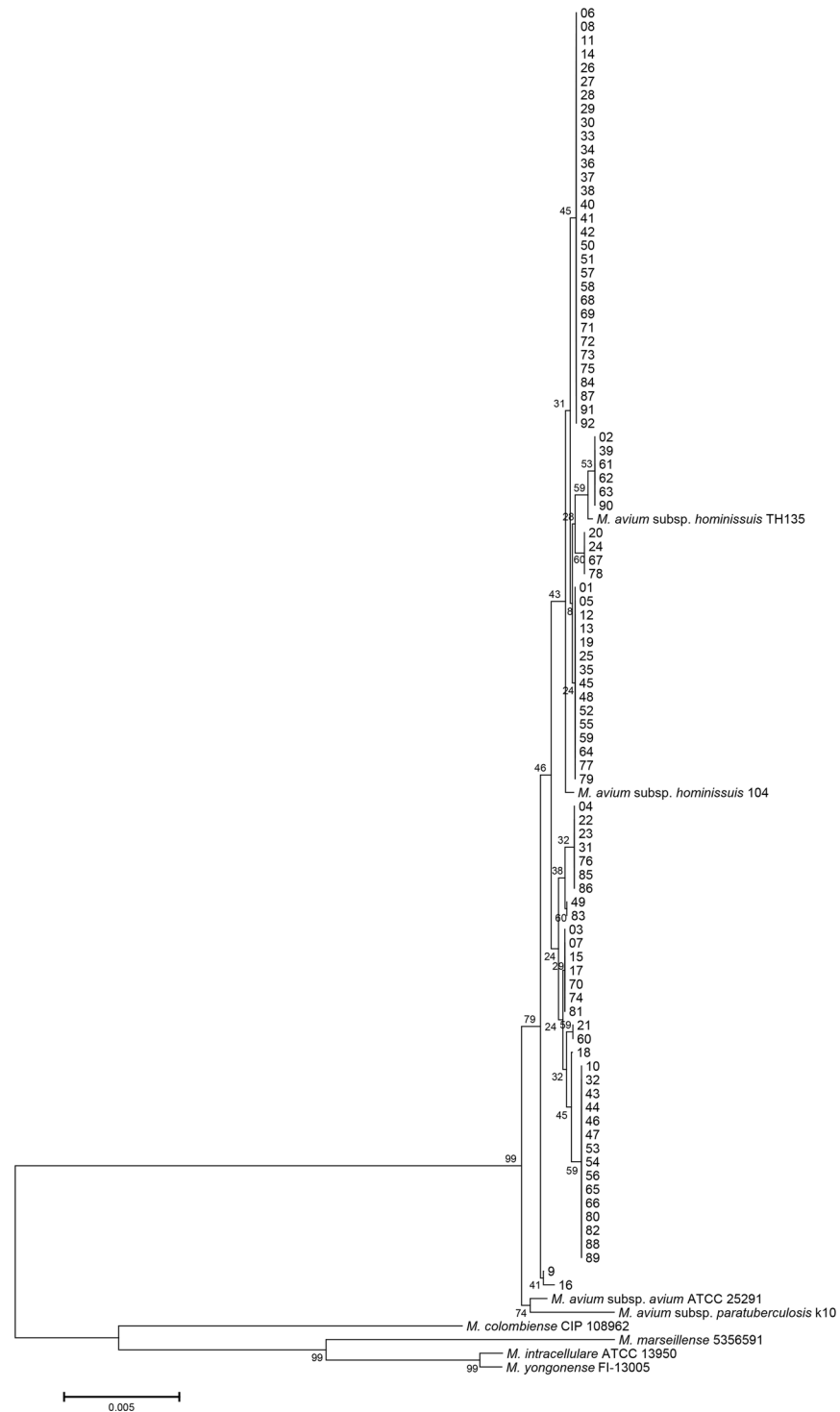


Fig 2. Phylogenetic tree based on concatenated *rpoB* and *hsp65* sequences using the neighbor-joining method with Kimura's two-parameter distance correction model. Bootstrap analyses determined from 1,000 replicates are indicated at the nodes. Bar, 0.5% difference in nucleotide sequence. GenBank accession numbers are shown in [Fig 1](#) and [S1 Fig](#).

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Table 1. Identification of the novel *hsp65* sequevar code and distribution of *hsp65* codes and insertion elements in this study.

<i>hsp65</i> code ^a	Nucleotide at the indicated base pair position (<i>hsp65</i>) ^b									<i>n</i> (%)	<i>n</i> (%) of isolates				
	324	645	927	1128	1218	1269	1272	1350	1536		IS900	IS1245	IS1311	DT1	ISMav6
Code 2	T	C	C	C	G	G	C	G	G	32 (35)	0	32	32	0	15 (47)
Code 15	C	•	T	•	A	•	•	•	A	25 (27)	0	22	25	0	21 (84)
Code 16	C	•	T	•	A	•	•	•	•	31 (34)	0	31	31	0	18 (58)
Code N2	•	•	T	•	•	•	•	•	•	2 (2)	0	1	2	0	1 (50)
Code N7 ^c	C	•	•	•	•	•	G	•	•	2 (2)	0	2	2	1	1 (50)
Total										92 (100)	0	88	92	1	56 (61)

^a Classification according to previously reported papers [25–27].

^b • indicates the same base pair as in code 2.

^c New code type found in this study designated code N7.

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Relatedness of clinical characteristics, treatment response, and drug susceptibility to *hsp65* codes and presence/absence of ISMav6

We analyzed clinical characteristics and treatment response among 3 major codes (code 2, 15, and 16). There were no significant differences in clinical features among the 3 groups (S1 and S2 Tables). We also analyzed clinical characteristics and treatment response according to the presence of ISMav6. There were no significant differences in clinical features between those with and without ISMav6 (S3 and S4 Tables). The association of genotype and the presence of ISMav6 with drug susceptibility patterns in the *M. avium* subsp. *hominissuis* isolates was evaluated for CLR and MXF. Drug susceptibility test were performed in 72 and 71 patients for CLR and MXF, respectively. None of the *hsp65* codes showed trends in drug susceptibility levels (data not shown); however, the presence of ISMav6 was correlated with greater resistance to MXF (Table 2).

Discussion

In this study, clinical isolates from 92 patients previously diagnosed with *M. avium* lung disease over a two-year period were further analyzed. Species identification was initially performed by

Table 2. Association between presence of ISMav6 and antibiotic resistance.

Drug susceptibility test	Detection of ISMav6		<i>P</i> -value for trend test ISMav6 (+) vs. ISMav6 (-)
	ISMav6 (+)	ISMav6 (-)	
CLR			0.375
S	42	27	
I	1	0	
R	2	0	
MXF			0.003
S	17	17	
I	9	9	
R	18	1	

Data are presented as numbers.

Because drug susceptibility testing was not performed in all patients, the sum of the individual categories is less than 92.

Definition of abbreviations: CLR = clarithromycin; MXF = moxifloxacin; S = susceptible; I = intermediate; R = resistant.

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a non-sequencing method and then species were re-identified using a sequencing method. Among the 92 isolates identified as *M. avium* by PRA at the time of diagnosis, all isolates were precisely identified as *M. avium* subsp. *hominissuis*.

In *hsp65* sequevar analysis, the major codes were 2, 15, and 16 (35%, 27%, and 34%, respectively). Among Japanese clinical isolates, the main codes were 2 (63/146, 43%) and 15 (50/146, 34%), followed by 16 (9/146, 6%) [26]. The prevalence of code 16 in Korea was higher than in Japan.

ISMav6 is a novel IS recently reported in the genetic characterization of Japanese human clinical isolates [27]. In the present study, the prevalence of ISMav6 in Korean patients with *M. avium* lung disease was 61%. Interestingly, more clinical isolates with *hsp65* code 15 harbored ISMav6 (84%, 21/25) than isolates with *hsp65* code 2 and code 15 (47% and 58%, respectively). Also, both *hsp65* code 15 and ISMav6 have rarely been reported in the literature except in Japan. In Germany, one *M. avium* subsp. *hominissuis* strain with *hsp65* code 15 harboring ISMav6 was reported [8]. The high proportion of ISMav6 in *M. avium* subsp. *hominissuis* strains from Korea and Japan is thought to be a specific genetic feature. Thus, both *hsp65* code 15 and ISMav6 may be related to the epidemiological diversity of *M. avium* clinical strains.

In general, DT1 is present in *M. intracellulare* and not present in *M. avium* subsp. *hominissuis*. One *M. avium* subsp. *hominissuis* isolates possessed DT1 in this study, which is a novel observation. Since a number of different IS elements have been described in various NTM species, species-specific IS elements have been revisited for MAC identification [28, 29, 35]. IS elements are mobile by nature, so there is a risk that similar elements will be found in unrelated bacteria because of mobility to or from MAC organisms. For example, natural occurrence of horizontal transfer of *M. avium*-specific IS1245 to *M. kansasii* has been reported [36]. Thus, the use of insertion sequences for species-specific markers should be more carefully conducted because it may influence molecular diagnosis and, consequently, treatment outcomes.

Kikuchi et al. reported that a variable number of tandem repeats (VNTR)-genotyping of 37 *M. avium* clinical isolates was associated with the progression of *M. avium* lung disease in Japan [37]. However, our study, which included more than 100 clinical isolates, did not identify any association between the *M. avium* VNTR genotype and disease progression of *M. avium* lung disease [38]. In the present study, disease progression was defined as when patients with *M. avium* lung disease require antibiotic treatment due to worsening symptoms, deteriorating chest radiograph features and microbiological findings within 2 years of diagnosis [39].

There was no difference in clinical characteristics and treatment response according to *hsp65* sequevar codes and ISMav6, in agreement with previous VNTR-based observations that there was no association between the genotype and clinical characteristics of Korean patients [38]. Interestingly, the presence of ISMav6 was associated with drug resistance to MXF in this study. Tatano et al. reported an association between the VNTR genotype and susceptibility to quinolones and EMB [40]. Dvorska et al. found no relationship between IS1311 and IS1245-based RFLP genotypes and drug susceptibility in MAC isolates [41]. These findings suggest that some genetic factors may influence the acquisition of drug resistance and ISMav6 may be a genetic factor associated with drug resistance. As far as we know, this is the first study to suggest an association between genotypes according to *hsp65* codes and ISMav6 and clinical features with drug susceptibility. Our results indicate that specific genotypes among *M. avium* subsp. *hominissuis* organisms are not predominantly responsible for *M. avium* lung disease in Korea and further analysis of ISMav6 (*i.e.* the location of ISMav6 in the genome of *M. avium* isolates) will help identify relationships between genetic features and drug susceptibility.

The present study has some limitations. First, this study was conducted at a single center and was performed on a referral basis with final analysis of only a small number of Korean patients; therefore, caution should be used when attempting to generalize our findings. Second,

this study was preliminary because we did not investigate the specific genes associated with drug resistance. Thus, further precise drug resistance typing of *rpoB* and *gyrA/B* with a large number of isolates will provide a better understanding of the association between *M. avium* subsp. *hominissuis* genotypes and drug resistance.

Nevertheless, to the best of our knowledge, this is the first report to investigate the link between IS*Mav6* and drug resistance to MXF in *M. avium* subsp. *hominissuis* strains from Korean patients. Future studies of informative and valuable genetic factors related to *M. avium* lung disease should be conducted in both the pathogen and host.

Supporting Information

S1 Fig. *rpoB* sequence-based phylogenetic tree using the neighbor-joining method with Kimura's two-parameter distance correction model. Bootstrap analyses determined from 1,000 replicates are indicated at the nodes. Bar, 0.5% difference in nucleotide sequence. GenBank accession numbers are given in parentheses.

(TIF)

S1 Table. Clinical characteristics according to *hsp65* sequevar codes.

(DOC)

S2 Table. Treatment response according to *hsp65* sequevar codes.

(DOC)

S3 Table. Clinical characteristics according to the presence or absence of IS*Mav6*.

(DOC)

S4 Table. Treatment response according to the presence or absence of IS*Mav6*.

(DOC)

Author Contributions

Conceived and designed the experiments: SJS WJK. Performed the experiments: SYK SJH. Analyzed the data: SYK BHJ HYP. Contributed reagents/materials/analysis tools: BHJ HYP KJ WJK. Wrote the paper: SYK BHJ HYP KJ SJH SJS WJK.

References

1. Brode SK, Daley CL, Marras TK. The epidemiologic relationship between tuberculosis and non-tuberculous mycobacterial disease: a systematic review. *Int J Tuberc Lung Dis*. 2014; 18: 1370–1377. doi: [10.5588/ijtld.14.0120](https://doi.org/10.5588/ijtld.14.0120) PMID: [25299873](https://pubmed.ncbi.nlm.nih.gov/25299873/)
2. Koh WJ, Chang B, Jeong BH, Jeon K, Kim SY, Lee NY, et al. Increasing recovery of nontuberculous mycobacteria from respiratory specimens over a 10-year period in a tertiary referral hospital in South Korea. *Tuberc Respir Dis (Seoul)*. 2013; 75: 199–204.
3. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007; 175: 367–416. PMID: [17277290](https://pubmed.ncbi.nlm.nih.gov/17277290/)
4. Thorel MF, Krichevsky M, Levy-Frebault VV. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov., and *Mycobacterium avium* subsp. *silvaticum* subsp. nov. *Int J Syst Bacteriol*. 1990; 40: 254–260. PMID: [2397193](https://pubmed.ncbi.nlm.nih.gov/2397193/)
5. Mijs W, de Haas P, Rossau R, Van der Laan T, Rigouts L, Portaels F, et al. Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* for bird-type isolates and '*M. avium* subsp. *hominissuis*' for the human/porcine type of *M. avium*. *Int J Syst Evol Microbiol*. 2002; 52: 1505–1518. PMID: [12361252](https://pubmed.ncbi.nlm.nih.gov/12361252/)

6. Despierres L, Cohen-Bacrie S, Richet H, Drancourt M. Diversity of *Mycobacterium avium* subsp. *hominissuis* mycobacteria causing lymphadenitis, France. *Eur J Clin Microbiol Infect Dis*. 2012; 31: 1373–1379. doi: [10.1007/s10096-011-1452-2](https://doi.org/10.1007/s10096-011-1452-2) PMID: [22042560](https://pubmed.ncbi.nlm.nih.gov/22042560/)
7. Tran QT, Han XY. Subspecies identification and significance of 257 clinical strains of *Mycobacterium avium*. *J Clin Microbiol*. 2014; 52: 1201–1206. doi: [10.1128/JCM.03399-13](https://doi.org/10.1128/JCM.03399-13) PMID: [24501026](https://pubmed.ncbi.nlm.nih.gov/24501026/)
8. Kolb J, Hillemann D, Mobius P, Reetz J, Lahiri A, Lewin A, et al. Genetic characterization of German *Mycobacterium avium* strains isolated from different hosts and specimens by multilocus sequence typing. *Int J Med Microbiol*. 2014; 304: 941–948. doi: [10.1016/j.ijmm.2014.06.001](https://doi.org/10.1016/j.ijmm.2014.06.001) PMID: [25091874](https://pubmed.ncbi.nlm.nih.gov/25091874/)
9. Schweickert B, Goldenberg O, Richter E, Gobel UB, Petrich A, Buchholz P, et al. Occurrence and clinical relevance of *Mycobacterium chimaera* sp. nov., Germany. *Emerg Infect Dis*. 2008; 14: 1443–1446. doi: [10.3201/eid1409.071032](https://doi.org/10.3201/eid1409.071032) PMID: [18760016](https://pubmed.ncbi.nlm.nih.gov/18760016/)
10. Wallace RJ Jr., Iakhiaeva E, Williams MD, Brown-Elliott BA, Vasireddy S, Vasireddy R, et al. Absence of *Mycobacterium intracellulare* and presence of *Mycobacterium chimaera* in household water and bio-film samples of patients in the United States with *Mycobacterium avium* complex respiratory disease. *J Clin Microbiol*. 2013; 51: 1747–1752. doi: [10.1128/JCM.00186-13](https://doi.org/10.1128/JCM.00186-13) PMID: [23536397](https://pubmed.ncbi.nlm.nih.gov/23536397/)
11. Dai J, Chen Y, Dean S, Morris JG, Salfinger M, Johnson JA. Multiple-genome comparison reveals new loci for *Mycobacterium* species identification. *J Clin Microbiol*. 2011; 49: 144–153. doi: [10.1128/JCM.00957-10](https://doi.org/10.1128/JCM.00957-10) PMID: [21048007](https://pubmed.ncbi.nlm.nih.gov/21048007/)
12. Macheras E, Roux AL, Bastian S, Leao SC, Palaci M, Sivadon-Tardy V, et al. Multilocus sequence analysis and *rpoB* sequencing of *Mycobacterium abscessus* (sensu lato) strains. *J Clin Microbiol*. 2011; 49: 491–499. doi: [10.1128/JCM.01274-10](https://doi.org/10.1128/JCM.01274-10) PMID: [21106786](https://pubmed.ncbi.nlm.nih.gov/21106786/)
13. Hashemi-Shahraki A, Bostanabad SZ, Heidarieh P, Titov LP, Khosravi AD, Sheikhi N, et al. Species spectrum of nontuberculous mycobacteria isolated from suspected tuberculosis patients, identification by multi locus sequence analysis. *Infect Genet Evol*. 2013; 20: 312–324. doi: [10.1016/j.meegid.2013.08.027](https://doi.org/10.1016/j.meegid.2013.08.027) PMID: [24070831](https://pubmed.ncbi.nlm.nih.gov/24070831/)
14. Jang MA, Koh WJ, Huh HJ, Kim SY, Jeon K, Ki CS, et al. Distribution of nontuberculous mycobacteria by multigene sequence-based typing and clinical significance of isolated strains. *J Clin Microbiol*. 2014; 52: 1207–1212. doi: [10.1128/JCM.03053-13](https://doi.org/10.1128/JCM.03053-13) PMID: [24501029](https://pubmed.ncbi.nlm.nih.gov/24501029/)
15. Rindi L, Garzelli C. Genetic diversity and phylogeny of *Mycobacterium avium*. *Infect Genet Evol*. 2014; 21: 375–383. doi: [10.1016/j.meegid.2013.12.007](https://doi.org/10.1016/j.meegid.2013.12.007) PMID: [24345519](https://pubmed.ncbi.nlm.nih.gov/24345519/)
16. Koh WJ, Jeong BH, Jeon K, Lee NY, Lee KS, Woo SY, et al. Clinical significance of the differentiation between *Mycobacterium avium* and *Mycobacterium intracellulare* in *M. avium* complex lung disease. *Chest*. 2012; 142: 1482–1488. doi: [10.1378/chest.12-0494](https://doi.org/10.1378/chest.12-0494) PMID: [22628488](https://pubmed.ncbi.nlm.nih.gov/22628488/)
17. Koh WJ, Jeong BH, Jeon K, Lee NY, Lee KS, Woo SY, et al. Clinical significance of the differentiation between *Mycobacterium avium* and *Mycobacterium intracellulare* in *M. avium* complex lung disease. *Chest*. 2012; 142: 1482–1488. doi: [10.1378/chest.12-0494](https://doi.org/10.1378/chest.12-0494) PMID: [22628488](https://pubmed.ncbi.nlm.nih.gov/22628488/)
18. Jeong BH, Jeon K, Park HY, Kim SY, Lee KS, Huh HJ, et al. Intermittent antibiotic therapy for nodular bronchiectatic *Mycobacterium avium* complex lung disease. *Am J Respir Crit Care Med*. 2015; 191: 96–103. doi: [10.1164/rccm.201408-1545OC](https://doi.org/10.1164/rccm.201408-1545OC) PMID: [25393520](https://pubmed.ncbi.nlm.nih.gov/25393520/)
19. Clinical and Laboratory Standards Institute. Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard-Second Edition (M24-A2). Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
20. Adekambi T, Colson P, Drancourt M. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. *J Clin Microbiol*. 2003; 41: 5699–5708. PMID: [14662964](https://pubmed.ncbi.nlm.nih.gov/14662964/)
21. Ben Salah I, Adekambi T, Raoult D, Drancourt M. *rpoB* sequence-based identification of *Mycobacterium avium* complex species. *Microbiology*. 2008; 154: 3715–3723. doi: [10.1099/mic.0.2008/020164-0](https://doi.org/10.1099/mic.0.2008/020164-0) PMID: [19047739](https://pubmed.ncbi.nlm.nih.gov/19047739/)
22. Harmsen D, Dostal S, Roth A, Niemann S, Rothganger J, Sammeth M, et al. RIDOM: comprehensive and public sequence database for identification of *Mycobacterium* species. *BMC Infect Dis*. 2003; 3: 26. PMID: [14611664](https://pubmed.ncbi.nlm.nih.gov/14611664/)
23. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. *Bioinformatics*. 2007; 23: 2947–2948. PMID: [17846036](https://pubmed.ncbi.nlm.nih.gov/17846036/)
24. Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol*. 2013; 30: 2725–2729. doi: [10.1093/molbev/mst197](https://doi.org/10.1093/molbev/mst197) PMID: [24132122](https://pubmed.ncbi.nlm.nih.gov/24132122/)
25. Turenne CY, Semret M, Cousins DV, Collins DM, Behr MA. Sequencing of *hsp65* distinguishes among subsets of the *Mycobacterium avium* complex. *J Clin Microbiol*. 2006; 44: 433–440. PMID: [16455896](https://pubmed.ncbi.nlm.nih.gov/16455896/)
26. Iwamoto T, Nakajima C, Nishiuchi Y, Kato T, Yoshida S, Nakanishi N, et al. Genetic diversity of *Mycobacterium avium* subsp. *hominissuis* strains isolated from humans, pigs, and human living environment. *Infect Genet Evol*. 2012; 12: 846–852. doi: [10.1016/j.meegid.2011.06.018](https://doi.org/10.1016/j.meegid.2011.06.018) PMID: [21745597](https://pubmed.ncbi.nlm.nih.gov/21745597/)

27. Ichikawa K, Yagi T, Moriyama M, Inagaki T, Nakagawa T, Uchiya K, et al. Characterization of *Mycobacterium avium* clinical isolates in Japan using subspecies-specific insertion sequences, and identification of a new insertion sequence, ISMav6. *J Med Microbiol*. 2009; 58: 945–950. doi: [10.1099/jmm.0.008623-0](https://doi.org/10.1099/jmm.0.008623-0) PMID: [19502362](https://pubmed.ncbi.nlm.nih.gov/19502362/)
28. Shin SJ, Lee BS, Koh WJ, Manning EJ, Anklam K, Sreevatsan S, et al. Efficient differentiation of *Mycobacterium avium* complex species and subspecies by use of five-target multiplex PCR. *J Clin Microbiol*. 2010; 48: 4057–4062. doi: [10.1128/JCM.00904-10](https://doi.org/10.1128/JCM.00904-10) PMID: [20810779](https://pubmed.ncbi.nlm.nih.gov/20810779/)
29. Johansen TB, Djonne B, Jensen MR, Olsen I. Distribution of IS1311 and IS1245 in *Mycobacterium avium* subspecies revisited. *J Clin Microbiol*. 2005; 43: 2500–2502. PMID: [15872295](https://pubmed.ncbi.nlm.nih.gov/15872295/)
30. Bewick V, Cheek L, Ball J. Statistics review 8: Qualitative data—tests of association. *Crit Care*. 2004; 8: 46–53. PMID: [14975045](https://pubmed.ncbi.nlm.nih.gov/14975045/)
31. Englund S, Ballagi-Pordany A, Bolske G, Johansson KE. Single PCR and nested PCR with a mimic molecule for detection of *Mycobacterium avium* subsp. *paratuberculosis*. *Diagn Microbiol Infect Dis*. 1999; 33: 163–171. PMID: [10092965](https://pubmed.ncbi.nlm.nih.gov/10092965/)
32. Roiz MP, Palenque E, Guerrero C, Garcia MJ. Use of restriction fragment length polymorphism as a genetic marker for typing *Mycobacterium avium* strains. *J Clin Microbiol*. 1995; 33: 1389–1391. PMID: [7615764](https://pubmed.ncbi.nlm.nih.gov/7615764/)
33. Guerrero C, Bernasconi C, Burki D, Bodmer T, Telenti A. A novel insertion element from *Mycobacterium avium*, IS1245, is a specific target for analysis of strain relatedness. *J Clin Microbiol*. 1995; 33: 304–307. PMID: [7714183](https://pubmed.ncbi.nlm.nih.gov/7714183/)
34. Oliveira RS, Sircili MP, Oliveira EM, Balian SC, Ferreira-Neto JS, Leao SC. Identification of *Mycobacterium avium* genotypes with distinctive traits by combination of IS1245-based restriction fragment length polymorphism and restriction analysis of *hsp65*. *J Clin Microbiol*. 2003; 41: 44–49. PMID: [12517823](https://pubmed.ncbi.nlm.nih.gov/12517823/)
35. Bartos M, Hlozek P, Svastova P, Dvorska L, Bull T, Matlova L, et al. Identification of members of *Mycobacterium avium* species by Accu-Probes, serotyping, and single IS900, IS901, IS1245 and IS901-flanking region PCR with internal standards. *J Microbiol Methods*. 2006; 64: 333–345. PMID: [16061296](https://pubmed.ncbi.nlm.nih.gov/16061296/)
36. da Silva Rabello MC, de Oliveira RS, Silva RM, Leao SC. Natural occurrence of horizontal transfer of *Mycobacterium avium*-specific insertion sequence IS1245 to *Mycobacterium kansasii*. *J Clin Microbiol*. 2010; 48: 2257–2259. doi: [10.1128/JCM.02256-09](https://doi.org/10.1128/JCM.02256-09) PMID: [20392924](https://pubmed.ncbi.nlm.nih.gov/20392924/)
37. Kikuchi T, Watanabe A, Gomi K, Sakakibara T, Nishimori K, Daito H, et al. Association between mycobacterial genotypes and disease progression in *Mycobacterium avium* pulmonary infection. *Thorax*. 2009; 64: 901–907. doi: [10.1136/thx.2009.114603](https://doi.org/10.1136/thx.2009.114603) PMID: [19553234](https://pubmed.ncbi.nlm.nih.gov/19553234/)
38. Kim SY, Lee ST, Jeong BH, Jeon K, Kim JW, Shin SJ, et al. Clinical significance of mycobacterial genotyping in *Mycobacterium avium* lung disease in Korea. *Int J Tuberc Lung Dis*. 2012; 16: 1393–1399. doi: [10.5588/ijtld.12.0147](https://doi.org/10.5588/ijtld.12.0147) PMID: [23107637](https://pubmed.ncbi.nlm.nih.gov/23107637/)
39. Shin SJ, Choi GE, Cho SN, Woo SY, Jeong BH, Jeon K, et al. Mycobacterial genotypes are associated with clinical manifestation and progression of lung disease caused by *Mycobacterium abscessus* and *Mycobacterium massiliense*. *Clin Infect Dis*. 2013; 57: 32–39. doi: [10.1093/cid/cit172](https://doi.org/10.1093/cid/cit172) PMID: [23511298](https://pubmed.ncbi.nlm.nih.gov/23511298/)
40. Tatano Y, Sano C, Yasumoto K, Shimizu T, Sato K, Nishimori K, et al. Correlation between variable-number tandem-repeat-based genotypes and drug susceptibility in *Mycobacterium avium* isolates. *Eur J Clin Microbiol Infect Dis*. 2012; 31: 445–454. doi: [10.1007/s10096-011-1326-7](https://doi.org/10.1007/s10096-011-1326-7) PMID: [21748484](https://pubmed.ncbi.nlm.nih.gov/21748484/)
41. Dvorska L, Bartos M, Ostadal O, Kaustova J, Matlova L, Pavlik I. IS1311 and IS1245 restriction fragment length polymorphism analyses, serotypes, and drug susceptibilities of *Mycobacterium avium* complex isolates obtained from a human immunodeficiency virus-negative patient. *J Clin Microbiol*. 2002; 40: 3712–3719. PMID: [12354870](https://pubmed.ncbi.nlm.nih.gov/12354870/)