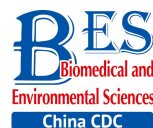


## Original Article



# Comparing the Genotype and Drug Susceptibilities between *Mycobacterium avium* and *Mycobacterium intracellulare* in China\*

ZHENG Hui Wen<sup>1,2</sup>, PANG Yu<sup>2</sup>, HE Guang Xue<sup>3</sup>, SONG Yuan Yuan<sup>2</sup>, and ZHAO Yan Lin<sup>2,#</sup>

1. National institute for communicable disease control and prevention, Chinese center for disease control and prevention, Beijing 102206, China; 2. National Tuberculosis Reference Laboratory, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 3. Science and Technology Department, Chinese Center for Disease Control and Prevention, Beijing 102206, China

## Abstract

**Objective** *Mycobacterium avium* (*M. avium*) and *Mycobacterium intracellulare* (*M. intracellulare*) are the major causative agents of nontuberculous mycobacteria (NTM)-related pulmonary infections. However, little is known about the differences in drug susceptibility profiles between these two species.

**Methods** A total of 393 NTM isolates were collected from Shanghai Pulmonary Disease Hospital. Sequencing of partial genes was performed to identify the strains at species level. The minimum inhibitory concentration (MIC) was used to evaluate the drug susceptibility against 20 antimicrobial agents. Variable number of tandem repeat (VNTR) typing was conducted to genotype these two species.

**Results** A total of 173 (44.0%) *M. avium* complex (MAC) isolates were identified, including 41 (10.4%) *M. avium* isolates and 132 (33.6%) *M. intracellulare* isolates. Clarithromycin and amikacin were the two most effective agents against MAC isolates. The Hunter-Gaston Discriminatory Index (HGDI) values for VNTR typing of *M. avium* and *M. intracellulare* isolates were 0.993 and 0.995, respectively. Levofloxacin resistance was more common among the unclustered strains than among the clustered strains of *M. intracellulare*.

**Conclusion** *M. intracellulare* was the most common NTM species in China. Clarithromycin and amikacin had high antimicrobial activities against MAC. VNTR typing of MAC isolates revealed a high discriminatory power. Levofloxacin resistance was associated with unclustered strains of *M. intracellulare*.

**Key words:** Nontuberculous mycobacterium; Phenotype; Genotype; Susceptibility; Resistance

Biomed Environ Sci, 2017; 30(7): 517-525

doi: 10.3967/bes2017.068

ISSN: 0895-3988

www.besjournal.com (full text)

CN: 11-2816/Q

Copyright ©2017 by China CDC

## INTRODUCTION

Nontuberculous mycobacteria (NTM) are widely distributed in soil, water, and animals<sup>[1-3]</sup>. Recently, the high emergence of NTM diseases in humans has attracted

increased attention worldwide. Previous epidemiological data show that the prevalence of NTM infections has been increasing in several countries<sup>[4-6]</sup>. National surveys in China have reported that the proportion of NTM infections has increased from 11.1% in 1990 to 22.9% in 2010,

\*This research was supported by a grant from the National Basic Research Program of China 2014CB744403; and the National Science and Technology Major Project 2014ZX100030002.

#Correspondence should be addressed to Dr. ZHAO Yan Lin, E-mail: zhaoyanlin@chinatb.org

Biographical note of the first author: ZHENG Hui Wen, female, born in 1987, PhD candidate, majoring in pathogenic biology.

phenotypes and to provide guideline for the instruction of empirical clinical medication.

## MATERIALS AND METHODS

Patients diagnosed with an NTM lung disease were enrolled in this study between 2012 and 2014 from Shanghai Pulmonary Disease Hospital. All the strains isolated from sputum samples of these patients were identified as NTM using paranitrobenzoic acid and thiophene-2-carboxylic acid hydrazide (TCH) in solid media<sup>[17]</sup>. Sequencing of partial genes, including 16S rRNA, *hsp65*, *rpoB*, and the 16S-23S rRNA internal transcribed spacer, was performed to identify the strains at species level according to previous reports<sup>[18-19]</sup>. The protocols used in this study were approved by the Ethics Committee of the Chinese Center for Disease Control and Prevention. Informed consent was obtained from all patients whose sputum specimens were used in studies.

Antimicrobial susceptibility testing was performed at the National Tuberculosis Reference Laboratory against 20 antimicrobial agents, including clarithromycin (CLA), amikacin (AMK), moxifloxacin (MOX), linezolid (LZD), rifampin (RIF), rifabutin (RFB), ethambutol (EMB), tobramycin (TOB), meropenem (MEM), imipenem (IMP), cefoxitin (CFX), capreomycin (CAP), azithromycin (AZM), levofloxacin (LFX), gatifloxacin (GAT), minocycline (MIN), tigecycline (TIG), sulfamethoxazole (SFX), streptomycin (SM), and clofazimine (CFM). All the above mentioned agents were purchased from Sigma-Aldrich.

The minimal inhibitory concentration (MIC) of the antimicrobial agents was determined by broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The bacteria on the solid culture media were transferred to cation-adjusted Mueller-Hinton broth (CAMHB) containing 0.02% Tween 80. The suspension was mixed vigorously using a vortexmixer until the bacterial colonies were dispersed homogeneously. After keeping undisturbed for 15 min, the suspension was diluted to the density of a 0.5-McFarland standard using saline. The CAMHB medium (pH 7.3-7.4) supplemented with 5% OADC (oleic acid, albumin, dextrose, and catalase medium) was used to prepare

the final inoculum (with an organism density of approximately  $5 \times 10^5$  CFU/mL), and then 100  $\mu$ L bacterial suspension was added to the 96-well microtiter plates containing successive two-fold dilutions of the antimicrobial agents. The breakpoints for the following antimicrobial agents were recommended by the CLSI: clarithromycin  $\geq 32$   $\mu$ g/mL, moxifloxacin  $\geq 4$   $\mu$ g/mL, and linezolid  $\geq 32$   $\mu$ g/mL. The breakpoints for some other antimicrobial agents were in accordance with previous studies as follows: rifampicin  $\geq 8$   $\mu$ g/mL<sup>[20]</sup>, ethambutol  $\geq 8$   $\mu$ g/mL<sup>[21]</sup>, amikacin  $\geq 32$   $\mu$ g/mL<sup>[22]</sup>, and capreomycin  $\geq 16$   $\mu$ g/mL<sup>[23]</sup>. For other drugs, the breakpoints were according to Zhang<sup>[24]</sup>. *M. avium* ATCC700898 was used as quality control. The drug concentrations required to inhibit the growth of 50% and 90% of the tested strains were expressed as MIC<sub>50</sub> and MIC<sub>90</sub>, respectively.

### VNTR Analysis

The primers and method recommended by Kenji et al. were used to genotype the *M. avium* and *M. intracellulare* isolates, respectively<sup>[15-16]</sup>. PCR mixtures were prepared using 2.5  $\mu$ L genomic DNA, 2.5  $\mu$ L 10  $\times$  PCR buffer, and 12.5  $\mu$ mol/L of each primer, and then sterilized purified water was added to a total volume of 25  $\mu$ L. The PCR conditions were as follows: initial denaturation at 95 °C for 10 min, 38 cycles of denaturation at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, followed by 7 min at 72 °C for final extension. The PCR products were subjected to 2% agar gel to determine their size. The allelic diversity was calculated according to Selander's formula<sup>[25]</sup>, and the discriminatory powers of different VNTR loci typing were calculated by the Hunter-Gaston Discriminatory Index (HGDI)<sup>[26]</sup>. The VNTR data were analyzed using BioNumerics software. In addition, cluster analysis was performed in BioNumerics using the UPGMA coefficient.

### Statistical Analysis

SPSS v.14.0 (SPSS Inc.) was used to perform  $\chi^2$  analysis, and  $P < 0.05$  was defined as statistically significant.

## RESULTS

### Identification of NTM Species

A total of 393 NTM isolates were identified at species level using multilocus sequence analysis. *M. intracellulare* (132, 33.6%) was the most abundant

organism, followed by the *M. abscessus* group (100, 25.4%), *M. kansasii* (82, 20.9%), *M. avium* (41, 10.4%), *M. goodii* (26, 6.6%), and *M. fortuitum* (12, 3.1%). In total, 173 MAC isolates were used for further study, including 132 *M. intracellulare* and 41 *M. avium* isolates.

### Antimicrobial Susceptibility Profiles of Clinical MAC

Antimicrobial susceptibility testing was performed on *M. avium* and *M. intracellulare* isolates. The range of MICs of each antimicrobial agent for *M. avium* and *M. intracellulare* is shown in Table 1. Clarithromycin and amikacin were the two most effective agents against both *M. avium* (97.6% and 97.6%, respectively) and *M. intracellulare* isolates (96.2% and 94.7%, respectively), with no significant difference between the species ( $P < 0.05$ ). Compared with *M. intracellulare* isolates, lower rates of drug resistance to LZD, RIF, MEM, IMP, CFX, GAT, and SFX were observed in *M. avium* isolates (48.8% vs. 63.2%,  $P = 0.018$  for LZD; 41.5% vs. 78.7%,  $P < 0.001$  for RIF; 75.6% vs. 97.7%,  $P < 0.001$  for MEM; 78.0% vs. 99.2%,  $P = 0.005$  for IMP; 73.0% vs. 99.2%,  $P = 0.007$  for CFX; 41.5% vs. 74.2%,  $P < 0.001$  for GAT, and 48.8% vs. 87.1%,  $P < 0.001$  for SFX), with a significant difference between these two species ( $P < 0.05$ ). In addition, MOX (34.1% vs. 33.1%), AZM (70.7% vs. 60.6%), and RFB (36.6% vs. 26.5%) exhibited higher activity against *M. intracellulare* than against *M. avium* isolates, but there was no statistical difference between these two species ( $P > 0.05$ ).

### VNTR Genotyping and Cluster Analysis

A total of 41 *M. avium* strains were genotyped by 13 *M. avium* tandem repeat (MATR) loci combinations recommended by Kenji, except MATR-9 and MATR-12, which cannot be amplified for most DNA extracts, even when different conditions were attempted, and these two loci were excluded for further analysis. Using this method, the 41 isolates were differentiated into six clusters (two isolates per cluster) and 29 unique genotypes. As shown in Figure 1, of the 13 MATR loci, six loci (MATR-2, -3, -4, -6, -7, and -8) had a high diversity index ( $h \geq 0.5$ ); five loci (MATR-1, -5, -11, -13, and -16) achieved a medium diversity index ( $0.5 > h \geq 0.1$ ); and MATR-14 and -15 had a low diversity index ( $h < 0.1$ ). The cumulative cluster rate was 14.6%, and the HGDI value for the VNTR typing of *M. avium* isolates was 0.993 as shown in Table 2, indicating that the VNTR analysis was capable of high

discriminatory power.

The 132 *M. intracellulare* isolates were classified into 88 genotypes by 16-locus MIRU-VNTR as recommended by Kenji, including 48 isolates from 19 clusters (2-5 isolates per cluster) and 84 isolates with unique patterns; the cumulative cluster rate was 22.0%, and the HGDI value was 0.995 as shown in Table 2. As shown in Figure 2, of the 16 VNTR loci, VNTR-7 and VNTR-11 were classified as lowly discriminating ( $h < 0.1$ ), and the remaining 14 loci were

defined as highly discriminating ( $h \geq 0.5$ ).

Association between VNTR Genotypes and Drug Susceptibility

As shown in Table 3, of the 12 clustered and 29 unclustered isolates of *M. avium*, the proportion of isolates resistant to CAP (41.7% vs. 37.9%), AZM (75.0% vs. 69.0%), LFX (100.0% vs. 75.9%), SM (91.7% vs. 62.1%), MEM (83.3% vs. 72.4%), and CFX (75.0% vs. 72.4%) was higher than that of unclustered isolates,

Table 1. In Vitro Antibiotic Susceptibility of 41 *M. avium* and 132 *M. intracellulare* Isolates by Broth Microdilution Method

Antimicrobial Agent	<i>M. avium</i> (n = 41)			<i>M. intracellulare</i> (n = 132)			$\chi^2$	P
	MIC <sub>50</sub> <sup>a</sup> (µg/mL)	MIC <sub>90</sub> <sup>a</sup> (µg/mL)	%Resistant Strains <sup>b</sup>	MIC <sub>50</sub> <sup>a</sup> (µg/mL)	MIC <sub>90</sub> <sup>a</sup> (µg/mL)	%Resistant Strains <sup>b</sup>		
CLA	2	16	2.4	1	16	3.8	0.096	0.756
AMK	16	32	2.4	16	32	5.3	0.673	0.412
MOX	2	4	34.1	2	4	33.1	1.186	0.276
LZD	16	64	48.8	32	64	63.2	5.570	0.018
RIF	4	64	41.5	8	64	78.7	29.360	0.000
EMB	4	64	36.6	4	16	45.6	1.668	0.196
CAP	4	32	39.0	4	64	50.0	1.511	0.219
TOB	4	16	36.6	4	32	41.7	0.655	0.418
MEM	64	256	75.6	256	256	97.7	15.111	0.000
IMP	256	256	78.0	256	256	99.2	7.800	0.005
CFX	256	256	73.0	256	256	99.2	7.352	0.007
AZM	32	64	70.7	32	64	60.6	1.376	0.241
LFX	16	32	82.9	16	32	90.9	1.182	0.277
GAT	2	8	41.5	4	8	74.2	12.623	0.000
MIN	8	16	73.1	16	32	82.6	1.808	0.179
TIG	64	64	97.6	64	64	99.2	0.774	0.379
SFX	32	256	48.8	128	256	87.1	26.829	0.000
SM	16	64	90.2	16	64	92.4	0.095	0.758
CFM	1	16	39.0	1	32	43.2	0.222	0.638
RFB	0.125	32	36.6	0.25	32	26.5	1.544	0.214

**Note.** <sup>a</sup>MIC<sub>50</sub> represents the concentration required to inhibit the growth of 50% of the strains; MIC<sub>90</sub> represents the concentration required to inhibit the growth of 90% of the strains. <sup>b</sup>The breakpoints to establish susceptibility and resistance were followed as recommended by the Clinical and Laboratory Standards Institute (CLSI-M24-A2).

Table 2. Discriminatory Index and Clustering Rate of VNTR Applied to MAC Strains

Organism	Total Isolates (n)	Clustered Isolates (n)	Isolates in Each Cluster (n)	Clustering Rate/%	HGDI <sup>o</sup>
<i>M. avium</i>	41	12	2	14.6	0.993
<i>M. intracellulare</i>	132	48	2-5	22.0	0.995

**Note.** <sup>o</sup>HGDI = Hunter-Gaston Discriminatory Index.

but there was no significant difference ( $P > 0.05$ ). The resistance rate of other drugs in unclustered isolates was higher than that in clustered isolates, and the difference was not significant ( $P > 0.05$ ).

As shown in Table 4, among the 48 clustered and 84 unclustered strains of *M. intracellulare*, CLA (4.2% vs. 3.6%), LZD (75.0% vs. 57.1%), GAT (77.1% vs. 72.6%), SM (95.8% vs. 90.5%), and CFM (50.0% vs. 39.3%) showed higher resistance rates in clustered isolates than that in unclustered isolates, with no significant difference ( $P > 0.05$ ). Statistical analysis revealed that the percentage of LFX-resistant isolates was significantly higher in unclustered isolates than that in clustered isolates ( $P = 0.004$ ).

DISCUSSION

MAC, the most frequently isolated NTM in clinical samples, serves as the major pathogen for chronic obstructive pulmonary disease, cystic fibrosis,

and immunodeficiency diseases<sup>[27]</sup>. In the USA, Japan, and South Korea, MAC was reported to be the most common pathogen associated with NTM lung diseases<sup>[5,28-29]</sup>. According to a series of studies, MAC was also the most common cause of NTM lung diseases in China<sup>[30]</sup>. The overall rate of NTM isolated from mycobacterial culture-positive patients in Shanghai showed a significantly increasing trend from 3.0% in 2008 to 8.5% in 2012, and the second frequently identified organism was *M. intracellulare*<sup>[31]</sup>. The treatment failure rate of MAC was as high as 20%-40%<sup>[32]</sup>, which may be attributed to the low level of response to conventional antimicrobial agents. Therefore, antimicrobial susceptibility testing is essential for effective treatment of nontuberculosis diseases. In this study, we performed the drug susceptibility testing of clinical MAC isolates against 20 antimicrobial agents and evaluated the potential association between VNTR genotypes and drug susceptibility profiles.

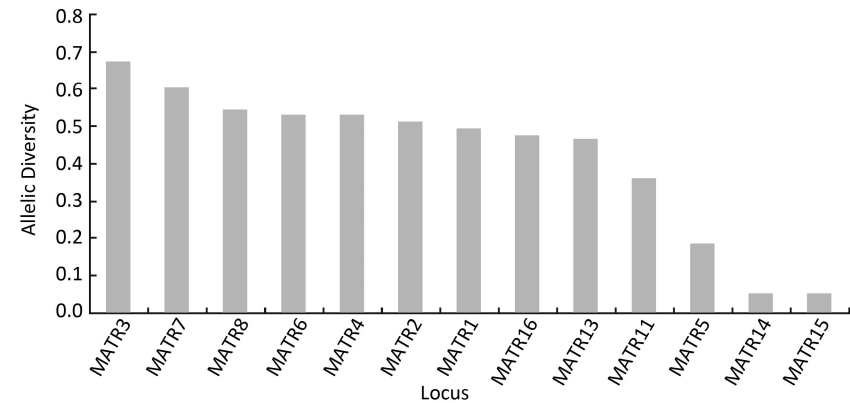


Figure 1. VNTR allelic distribution in 41 *M. avium* clinical isolates.

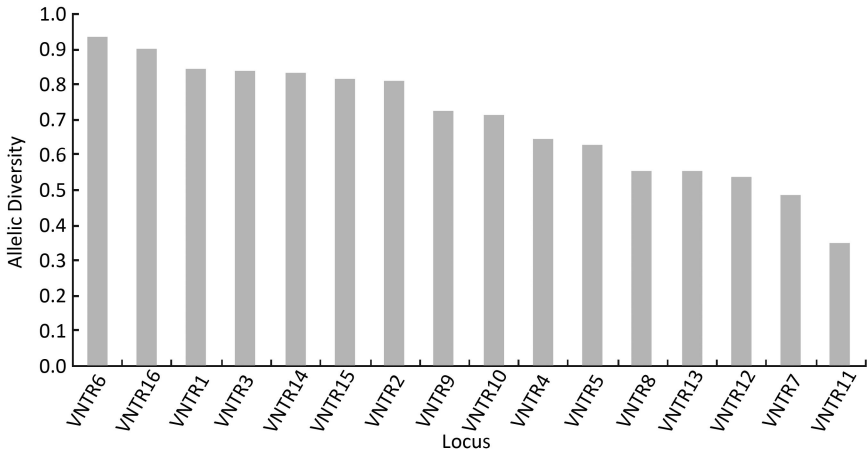


Figure 2. VNTR allelic distribution in 132 *M. intracellulare* clinical isolates.

**Table 3.** Drug Susceptibility Profiles of Clustered and Unclustered Strains of *M. avium*

Drug	Clustered (12 isolates)		Unclustered (29 isolates)		$\chi^2$	P
	No.(%) of Sensitive Strains	No.(%) of Resistant Strains	No.(%) of Sensitive Strains	No.(%) of Resistant Strains		
CLA	10 (83.3)	0 (0.0)	23 (79.3)	1 (3.4)	0.429	0.512
AMK	10 (83.3)	0 (0.0)	26 (89.7)	1 (3.4)	0.381	0.537
MOX	4 (33.3)	3 (25.0)	10 (34.5)	11 (37.9)	0.190	0.663
LZD	4 (33.3)	4 (33.3)	9 (31.0)	16 (55.2)	0.498	0.481
CAP	7 (58.3)	5 (41.7)	18 (62.1)	11 (37.9)	0.050	0.823
AZM	3 (25.0)	9 (75.0)	9 (31.0)	20 (69.0)	0.149	0.699
LFX	0 (0.0)	12 (100.0)	4 (33.3)	22 (75.9)	2.063	0.151
GAT	4 (33.3)	4 (33.3)	10 (34.5)	13 (44.8)	0.102	0.750
SM	1 (8.3)	11 (91.7)	11 (37.9)	18 (62.1)	3.592	0.058
CFM	9 (75.0)	3 (25.0)	16 (55.2)	13 (44.8)	1.402	0.236
TOB	6 (50.0)	4 (33.3)	10 (34.5)	11 (37.9)	0.416	0.519
MEM	0 (0.0)	10 (83.3)	4 (33.3)	21 (72.4)	1.806	0.179
IMP	1 (8.3)	6 (50.0)	1 (3.4)	26 (89.7)	1.124	0.289
CFX	3 (25.0)	9 (75.0)	8 (27.6)	21 (72.4)	0.029	0.865
MIN	0 (0.0)	8 (66.7)	2 (6.9)	22 (75.9)	0.711	0.399
RFB	8 (66.7)	4 (33.3)	18 (62.1)	11 (37.9)	0.077	0.781
SFX	9 (75.0)	3 (25.0)	12 (41.4)	17 (58.6)	3.840	0.050
RIF	8 (66.7)	4 (33.3)	16 (55.2)	13 (44.8)	0.462	0.497
EMB	8 (66.7)	4 (33.3)	18 (62.1)	11 (37.9)	0.077	0.781

**Table 4.** Drug Susceptibility Profiles of Clustered and Unclustered Strains of *M. intracellulare*

Drug	Clustered (48 isolates)		Unclustered (84 isolates)		$\chi^2$	P
	No.(%) of Sensitive Strains	No.(%) of Resistant Strains	No.(%) of Sensitive Strains	No.(%) of Resistant Strains		
CLA	45 (93.8)	2 (4.2)	77 (91.7)	3 (3.6)	0.020	0.888
AMK	40 (83.3)	1 (2.1)	66 (78.6)	6 (7.1)	1.562	0.211
MOX	9 (18.8)	15 (31.3)	18 (21.4)	29 (34.5)	0.004	0.948
LZD	4 (8.3)	36 (75.0)	16 (19.0)	48 (57.1)	3.566	0.059
CAP	29 (60.4)	19 (39.6)	37 (44.0)	47 (56.0)	3.274	0.070
AZM	21 (43.8)	27 (56.3)	31 (36.9)	53 (63.1)	0.599	0.439
LFX	6 (12.5)	39 (81.3)	1 (1.2)	81 (96.4)	8.187	0.004
GAT	6 (12.5)	37 (77.1)	12 (14.3)	61 (72.6)	0.127	0.721
SM	2 (4.2)	46 (95.8)	8 (9.5)	76 (90.5)	1.252	0.263
CFM	24 (50.0)	24 (50.0)	51 (60.7)	33 (39.3)	1.429	0.232
TOB	15 (31.3)	20 (41.7)	27 (32.1)	35 (41.7)	0.004	0.947
CFX	0 (0.0)	48 (100.0)	1 (1.2)	83 (98.8)	0.576	0.448
MIN	0 (0.0)	42 (87.5)	2 (2.4)	67 (80.0)	1.240	0.266
RFB	35 (72.9)	13 (27.1)	62 (73.8)	22 (26.2)	0.012	0.911
SFX	6 (12.5)	42 (87.5)	11 (13.1)	73 (86.9)	0.010	0.922
RIF	14 (29.2)	34 (70.8)	13 (15.5)	71 (84.5)	3.519	0.061
EMB	28 (58.3)	20 (41.7)	42 (50.0)	42 (50.0)	0.852	0.356

The results of our study provided new information on the candidate antimicrobial agents against MAC. As the major therapeutic agent for the treatment of MAC lung diseases, the macrolides have shown excellent *in vitro* activity against MAC isolates<sup>[33-34]</sup>. Consistent with previous studies<sup>[33-34]</sup>, clarithromycin showed better *in vitro* activity against MAC. Of the three quinolones tested in this study, moxifloxacin exhibited better antimicrobial activity against MAC than the other drugs. This finding is in agreement with several other investigations regarding the stronger activity of moxifloxacin<sup>[35]</sup>. Regarding the antimicrobial activity of three injectable agents, we found that amikacin had better antimicrobial activity than those of capreomycin and streptomycin against MAC, which is also in line with previous research<sup>[36]</sup>. Clarithromycin, combined with ethambutol and rifampicin, is the treatment regimen for MAC diseases as recommended by the ATS/IDSA<sup>[37]</sup>. In our study, the percentages of ethambutol-resistant and rifampicin-resistant strains among *M. avium* were lower than those among *M. intracellulare*, in accordance with the study of Zhang et al.<sup>[38]</sup>. However, Guthertz's study showed that *M. intracellulare* was more susceptible than *M. avium* to ethambutol and rifampicin<sup>[39]</sup>. The different observations may be due to the test methods or the applied breakpoint concentrations. Moreover, our study demonstrated that rifabutin was more active than rifampicin against MAC, which was similar to a previous research<sup>[38]</sup>, indicating that MAC-infected patients may achieve a more effective therapeutic result by replacing rifampicin with rifabutin during the treatment.

Our study showed that the 13-loci VNTR typing of *M. avium* and the 16-loci VNTR analysis of *M. intracellulare* had a high discriminatory power with HGDI values of 0.993 and 0.995, respectively, exceeding those previously observed in Japanese isolates (HGDI = 0.990 and 0.994, respectively). The allelic diversity for most loci of *M. avium* was similar to that in a previous report<sup>[15-16]</sup>; however, the discriminatory indexes of MATR-4 and -14 were different, with diversity indexes of 0.096 and 0.48 in Japan versus 0.532 and 0.049 in China, respectively. Except for VNTR-4, -7, and -11, the remaining loci had a higher discriminatory power in China than that in Japan. One possible explanation was that the strains were incongruent in different regions, and the distinguishing ability was different for some loci.

To our knowledge, there are fewer reports from China comparing the association between the

genotypes and drug resistance phenotypes for *M. avium* and *M. intracellulare* strains with such a large sample size. We observed that LFX resistance was more common among the unclustered strains than among the clustered strains of *M. intracellulare*. In a previous study, Wei et al. reported that rifampicin resistance was more common among the unclustered strains than among the clustered strains of *M. avium*<sup>[14]</sup>. Although NTM are regarded as opportunistic bacteria that cause infections in immunocompromised or immunocompetent people with some predisposing factors<sup>[40]</sup>, the resistance to LFX may be related to the pathogenicity and host preference for *M. intracellulare* isolates, leading to the bias distribution of LFX resistance in *M. intracellulare* isolates.

This study has several limitations. First, as the number of the isolates was relatively small, it may be not sufficient to detect the differences between clusters. Second, the synergy effect among different drugs was not tested in this study. Third, the relationship between specific single nucleotide polymorphisms and drug resistance of MAC was not analyzed.

In conclusion, the results of the present study illustrated that *M. intracellulare* was the most common NTM species in China. Clarithromycin and amikacin had high antimicrobial activities against MAC, and resistance to LZD, RIF, MEM, IMP, FOX, GAT, and SFX was more common among *M. intracellulare* than among *M. avium* isolates. In addition, the 13-loci and 16-loci VNTR typing of *M. avium* and *M. intracellulare* revealed a high discriminatory power with HGDI values of 0.993 and 0.995, respectively. LFX resistance was more common among the unclustered strains than among the clustered strains of *M. intracellulare*. The large variations in the drug resistance spectrum within the MAC isolates to currently available antimicrobial agents imply that a differentiation of subspecies should be performed to optimize the empirical treatment.

## ACKNOWLEDGMENTS

We thank Dr. CHEN Jin (Shanghai Pulmonary Disease Hospital) for providing *M. avium* complex clinical strains. We also thank all the staff of the National Tuberculosis Reference Laboratory of China for their technical assistance.

Received: March 6, 2017;

Accepted: June 5, 2017

REFERENCES

1. Bauer J, Andersen AB, Askgaard D, et al. Typing of clinical *Mycobacterium avium* complex strains cultured during a 2-year period in Denmark by using IS1245. *J Clin Microbiol*, 1999; 37, 600-5.

2. Dvorska L, Bartos M, Ostadal O, et al. 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, 3712-9.

3. De Groote MA, Pace NR, Fulton K, et al. Relationships between *Mycobacterium* isolates from patients with pulmonary mycobacterial infection and potting soils. *Appl Environ Microbiol*, 2006; 72, 7602-6.

4. Daley CL, Griffith DE. Pulmonary non-tuberculous mycobacterial infections. *Int J Tuberc Lung Dis*, 2010; 14, 665-71.

5. Simons S, van Ingen J, Hsueh PR, et al. Nontuberculous mycobacteria in respiratory tract infections, eastern Asia. *Emerg Infect Dis*, 2011; 17, 343-9.

6. Kim SY, Lee ST, Jeong BH, et al. Genotyping of *Mycobacterium intracellulare* isolates and clinical characteristics of lung disease. *Int J Tuberc Lung Dis*, 2013; 17, 669-75.

7. Xu HB, Jiang RH, Li L. Treatment outcomes for *Mycobacterium avium* complex: a systematic review and meta-analysis. *Eur J Clin Microbiol Infect Dis*, 2014; 33, 347-58.

8. Wang L, Zhang H, Ruan Y, et al. Tuberculosis prevalence in China, 1990-2010; a longitudinal analysis of national survey data. *Lancet*, 2014; 383, 2057-64.

9. Maekura R, Okuda Y, Hirotsu A, et al. Clinical and prognostic importance of serotyping *Mycobacterium avium-Mycobacterium intracellulare* complex isolates in human immunodeficiency virus-negative patients. *J Clin Microbiol*, 2005; 43, 3150-8.

10. Marras TK, Daley CL. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin Chest Med*, 2002; 23, 553-67.

11. Han XY, Tarrand JJ, Infante R, et al. Clinical significance and epidemiologic analyses of *Mycobacterium avium* and *Mycobacterium intracellulare* among patients without AIDS. *J Clin Microbiol*, 2005; 43, 4407-12.

12. Koh WJ, Jeong BH, Jeon K, et al. Clinical significance of the differentiation between *Mycobacterium avium* and *Mycobacterium intracellulare* in *M. avium* complex lung disease. *Chest*, 2012; 142, 1482-8.

13. Glassroth J. Pulmonary disease due to nontuberculous mycobacteria. *Chest*, 2008; 133, 243-51.

14. Wei G, Huang M, Wang G, et al. Antimicrobial susceptibility testing and genotyping of *Mycobacterium avium* isolates of two tertiary tuberculosis designated hospital, China. *Infect Genet Evol*, 2015; 36, 141-6.

15. Ichikawa K, Yagi T, Inagaki T, et al. Molecular typing of *Mycobacterium intracellulare* using multilocus variable-number of tandem-repeat analysis: identification of loci and analysis of clinical isolates. *Microbiology*, 2010; 156, 496-504.

16. Inagaki T, Nishimori K, Yagi T, et al. Comparison of a variable-number tandem-repeat (VNTR) method for typing *Mycobacterium avium* with mycobacterial interspersed repetitive-unit-VNTR and IS1245 restriction fragment length polymorphism typing. *J Clin Microbiol*, 2009; 47, 2156-64.

17. Pang Y, Zhou Y, Zhao B, et al. Spoligotyping and drug resistance analysis of *Mycobacterium tuberculosis* strains from national survey in China. *PLoS One*, 2012; 7, e32976.

18. Adekambi T, Drancourt M. Dissection of phylogenetic relationships among 19 rapidly growing *Mycobacterium* species by 16S rRNA, hsp65, sodA, recA and rpoB gene sequencing. *Int J Syst Evo Micr*, 2004; 54, 2095-105.

19. Park H, Jang H, Kim C, et al. Detection and identification of mycobacteria by amplification of the internal transcribed spacer regions with genus- and species-specific PCR primers. *J Clin Microbiol*, 2000; 38, 4080-5.

20. Rakoff-Nahoum S, Kuebler PJ, Heymann JJ, et al. Detection of T lymphocytes specific for human endogenous retrovirus K (HERV-K) in patients with seminoma. *AIDS Res Hum Retroviruses*, 2006; 22, 52-6.

21. Kobashi Y, Yoshida K, Miyashita N, et al. Relationship between clinical efficacy of treatment of pulmonary *Mycobacterium avium* complex disease and drug-sensitivity testing of *Mycobacterium avium* complex isolates. *J Infect Chemother*, 2006; 12, 195-202.

22. Brown-Elliott BA, Iakhiaeva E, Griffith DE, et al. In vitro activity of amikacin against isolates of *Mycobacterium avium* complex with proposed MIC breakpoints and finding of a 16S rRNA gene mutation in treated isolates. *J Clin Microbiol*, 2013; 51, 3389-94.

23. Heifets L. MIC as a quantitative measurement of the susceptibility of *Mycobacterium avium* strains to seven antituberculosis drugs. *Antimicrob Agents Ch*, 1988; 32, 1131-6.

24. Zhang Z. Drug susceptibility testing and molecular mechanism of resistance to clarithromycin in *Mycobacterium abscessus* complex. Medical school of Chinese People's Liberation Army, 2015. (In Chinese)

25. Selander RK, Caugant DA, Ochman H, et al. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Appl Environ Microbiol*, 1986; 51, 873-84.

26. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol*, 1988; 26, 2465-6.

27. Dailloux M, Abalain ML, Laurain C, et al. Respiratory infections associated with nontuberculous mycobacteria in non-HIV patients. *Eur Respir J*, 2006; 28, 1211-5.

28. Alvarez-Uria G. Lung disease caused by nontuberculous



- mycobacteria. *Curr Opin Pulm Med*, 2010; 16, 251-6.
- 29.Satta G, McHugh TD, Mountford J, et al. Managing pulmonary nontuberculous mycobacterial infection. time for a patient-centered approach. *Ann Am Thorac Soc*, 2014; 11, 117-21.
- 30.Xu K, Bi S, Ji Z, et al. Distinguishing nontuberculous mycobacteria from multidrug-resistant *Mycobacterium tuberculosis*, China. *Emerg Infect Dis*, 2014; 20, 1060-2.
- 31.Wu J, Zhang Y, Li J, et al. Increase in nontuberculous mycobacteria isolated in Shanghai, China: results from a population-based study. *PLoS One*, 2014; 9, e109736.
- 32.Zheng C, Fanta CH. Non-tuberculous mycobacterial pulmonary infection in the immunocompetent host. *QJM*, 2013; 106, 307-15.
- 33.Brown-Elliott BA, Nash KA, Wallace RJ Jr. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. *Clin Microbiol Rev*, 2012; 25, 545-82.
- 34.Griffith DE, Brown BA, Cegielski P, et al. Early results (at 6 months) with intermittent clarithromycin-including regimens for lung disease due to *Mycobacterium avium* complex. *Clin Infect Dis*, 2000; 30, 288-92.
- 35.Kohno Y, Ohno H, Miyazaki Y, et al. In vitro and in vivo activities of novel fluoroquinolones alone and in combination with clarithromycin against clinically isolated *Mycobacterium avium* complex strains in Japan. *Antimicrob Agents Ch*, 2007; 51, 4071-6.
- 36.Heifets L, Lindholm-Levy P. Comparison of bactericidal activities of streptomycin, amikacin, kanamycin, and capreomycin against *Mycobacterium avium* and *M. tuberculosis*. *Antimicrob Agents Ch*, 1989; 33, 1298-301.
- 37.McGrath EE, Anderson PB. The therapeutic approach to non-tuberculous mycobacterial infection of the lung. *Pulm Pharmacol Ther*, 2010; 23, 389-96.
- 38.Zhang Z, Pang Y, Wang Y, et al. Differences in risk factors and drug susceptibility between *Mycobacterium avium* and *Mycobacterium intracellulare* lung diseases in China. *Int J Antimicrob Agents*, 2015; 45, 491-5.
- 39.Guthertz LS, Damsker B, Bottone EJ, et al. *Mycobacterium avium* and *Mycobacterium intracellulare* infections in patients with and without AIDS. *J Infect Dis*, 1989; 160, 1037-41.
- 40.van Ingen J, Boeree MJ, Dekhuijzen PN, et al. Environmental sources of rapid growing nontuberculous mycobacteria causing disease in humans. *Clin Microbiol Infect*, 2009; 15, 888-93.