Letter to the Editor

Molecular Characteristics and Drug Susceptibility of *Mycobacterium tuberculosis* Isolates from Patients Co-infected with Human Immunodeficiency Virus in Beijing, China*

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70 clinical *Mycobacterium tuberculosis* strains isolated from AIDS patients in two HIV/AIDS referral hospitals in Beijing were used in this study. *M. tuberculosis* and non-tuberculosis mycobacterium (NTM) were identified by using multi-locus PCR. *M. tuberculosis* was genotyped by using 15-locus MIRU-VNTR technique and spoligotyping afterwards. Meanwhile, the drug susceptibilities of the strains to the four first-line anti TB drugs (rifampin, isoniazid, streptomycin, and ethambutol) and the four second-line anti-TB drugs (capreomycin, kanamycin, ofloxacin, and ethionanide) were tested with proportional method. In this study, *M. tuberculosis* and NTM strains isolated from AIDS patients with TB-like symptoms were identified and genotyping analysis indicated that Beijing genotype was the predominant genotype. In addition, the prevalence of drug-resistant TB, especially the prevalence of XDR-TB, was higher than that in TB patients without HIV infection.

Tuberculosis (TB) is the most common opportunistic infection among people infected with human immune deficiency virus (HIV), including those antiretroviral treatment patients. It was estimated that 1.1 million (13%) of the 8.6 million people who developed TB in 2012 were HIV-positive[1]. TB is also the leading cause of death among people infected with HIV and almost one in three HIV related deaths is due to TB. The situation is also serious in China and China is now facing a grim challenge of drug resistant tuberculosis. The first nationwide drug resistance survey in China revealed that 5.7% of new TB cases and 25.6% of treated TB cases were multi-drug resistant (MDR), and it was estimated that about 100,000 MDR-TB cases occurred in China every year[2] resulting in great difficulty to control TB-HIV co-infection. Yet, no research on MDR-TB and extensively drug resistant (XDR)-TB in patients co-infected with TB and HIV have been reported in China.

Until now, the control and treatment of HIV patients co-infected with TB becomes more complicated by the emergence of non-tuberculous mycobacterium (NTM)[5]. *Mycobacterium* mainly includes *Mycobacterium tuberculosis* complex (MTBC) and NTM. The non-tuberculosis species *Mycobacterium avium* and *Mycobacterium intracellulare* (collectively known as *Mycobacterium. avium* complex or MAC) and *Mycobacterium kansasii* are the important causes of pulmonary and disseminated diseases in HIV-infected patients[3]. Although the symptoms of NTM infection is often confused with TB, the treatment is not directly analogous to that of TB.

In this study, HIV-infected patients from two HIV/AIDS referral hospitals in Beijing were enrolled from 2009 to 2012 and two liquid methods (BACTEC MGIT 960 and BACTEC 9120) and also PCR assays were performed in order to isolate and identify *Mycobacterium* stains. Of 627 HIV-infected participants, 74 were culture-confirmed to be infected with *Mycobacterium*. 70 cases (62 males and 8

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females aged 1-67 years) with completed background information were included in the present study. Among them, 26 cases were infected with HIV through sexual contact, 9 cases were infected through blood transfusion or blood donation, 2 cases were infected through injection drug use, 1 case was infected through mother to infant transmission. The infection mode of other cases were unknown.

We chosed the following specific DNA fragments in this study: *16SrRNA*, *Rv0577*, *IS1561*, *Rv1510*, *Rv1970*, *Rv3877/8*, and *Rv3120 and* by multi-locus PCR[9], 64 *M. tuberculosis* strains and 6 NTM strains were identified. After the sequencing of *hsp65*, the 6 NTM strains were further identified, i.e. 4 strains of *Mycobacterium avium* Complex (MAC) and 2 strains of *Mycobacterium kansasii.* The results of multi-locus PCR showed the same as that of the sequencing of *hsp65*. In our study, among the 70 *Mycobacterium* strains identified, 6 were NTM strains, accounting for 8.57%, and 4 in the 6 NTM strains were strains of MAC, accounting for 66.67%, being substantially higher than that of detected in United States also[7]. However, the limitation of this study is the relatively small number of NTM cases. It is likely that a larger patient cohort would make a more accurate estimation. To date, the initial treatment for TB is based on smear results rather than culturing and *Mycobacterium* identification. Therefore, patients infected with NTM (who have pulmonary manifestations similar to TB and might respond to empirical treatment with anti-TB drugs) might not be appropriately diagnosed.

We used the $VNTR15_{-China}$ Scheme[4] comprised the following markers: ETR-A, ETR-B, ETR-C, ETR-D (MIRU04), ETR-E (MIRU31), MIRU10, MIRU16, MIRU23, MIRU26, MIRU27, MIRU39, MIRU40, Mtub21, Mtub30, and Mtub39. In total, 15-loci MIRU-VNTR method differentiated 41 genotypes among the 64 strains of *M. tuberculosis*, in which 32 had unique patterns and the remaining 32 formed 9 clusters (2 to 15 isolates per cluster).

Spoligotyping of the isolates was performed as described in the previous study[4]. By spoligotyping, only 6 distinct spoligotypes were recognized among the 64 *M. tuberculosis* strains (Table 1). Typing analysis revealed that 59 strains belonged to one genotype, while the other 5 strains belonged to 5 unique spoligotypes.

By comparison of the spoligotyping results with SpolDB4.0 database and application of the published definition of the Beijing lineage (hybridized to at least three of the spacers 35 to 43 in the genomic direct-repeat region and showed an absence of hybridization to spacers 1 to 34)[4], 59 isolates belonged to Beijing genotype and 1 isolate belonged to MANU family, whereas four isolates belonged to 4 unknown new types (Table 1). All the 59 Beijing genotype isolates belonged to the classical Beijing genotype with a pattern that depicted the absence of the first 34 spacer oligonucleotides and the presence of spacers 35 to 43[4].

The genotyping results indicated that the population structure of *M. tuberculosis* isolates from HIV positive patients was highly homogeneous, as 59 (92.19%) isolates belonged to the typical Beijing genotype. The predominance of Beijing genotype in general population has been well described in the previous studies. And in our study, Beijing genotype also appeared to be the predominant genotype of the *M. tuberculosis* strains from the HIV positive participants.

As showed in Figure 1, there was a consistency between the MIRU-VNTR analysis and spoligotyping of the Beijing family. Owing that Beijing family strains accounted for 92.19% of the total *M. tuberculosis* strains in this study (52/64), we compared the results

Table 1. Spoligotypes of 64 *M. tuberculosis* Isolates

No.	Spoligotype	SIT[a]	Family[b]	*N*(%)[c]
1	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■	1	Beijing	59 (92.19)
2	■□■■■■■■■■■■■■■■■■■■■■■■■■■■□□■■■■■■■■■	1096	MANU2	1 (1.56)
3	■□■■■■■■■■■■■■■□■■■■■■■■■■■■■□□□□■■■■■■■	NEW	Unknown	1 (1.56)
4	■■■■■■■■■■■■■■■■■■■■■■■■■■□■■□□□□□□□□■■■	NEW	Unknown	1 (1.56)
5	■□■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□□■■■□■■■	NEW	Unknown	1 (1.56)
6	■■□■■■■□■■■■■■■□■■■■■■■■■■■■■□□□□■■■□□□□	NEW	Unknown	1 (1.56)

Note. [a]SIT number from the SpolDB4.0 database. SIT, spoligotype international type; [b]Spoligotype families as assigned in SpolDB4.0; [c]The number of the isolates with a common SIT.

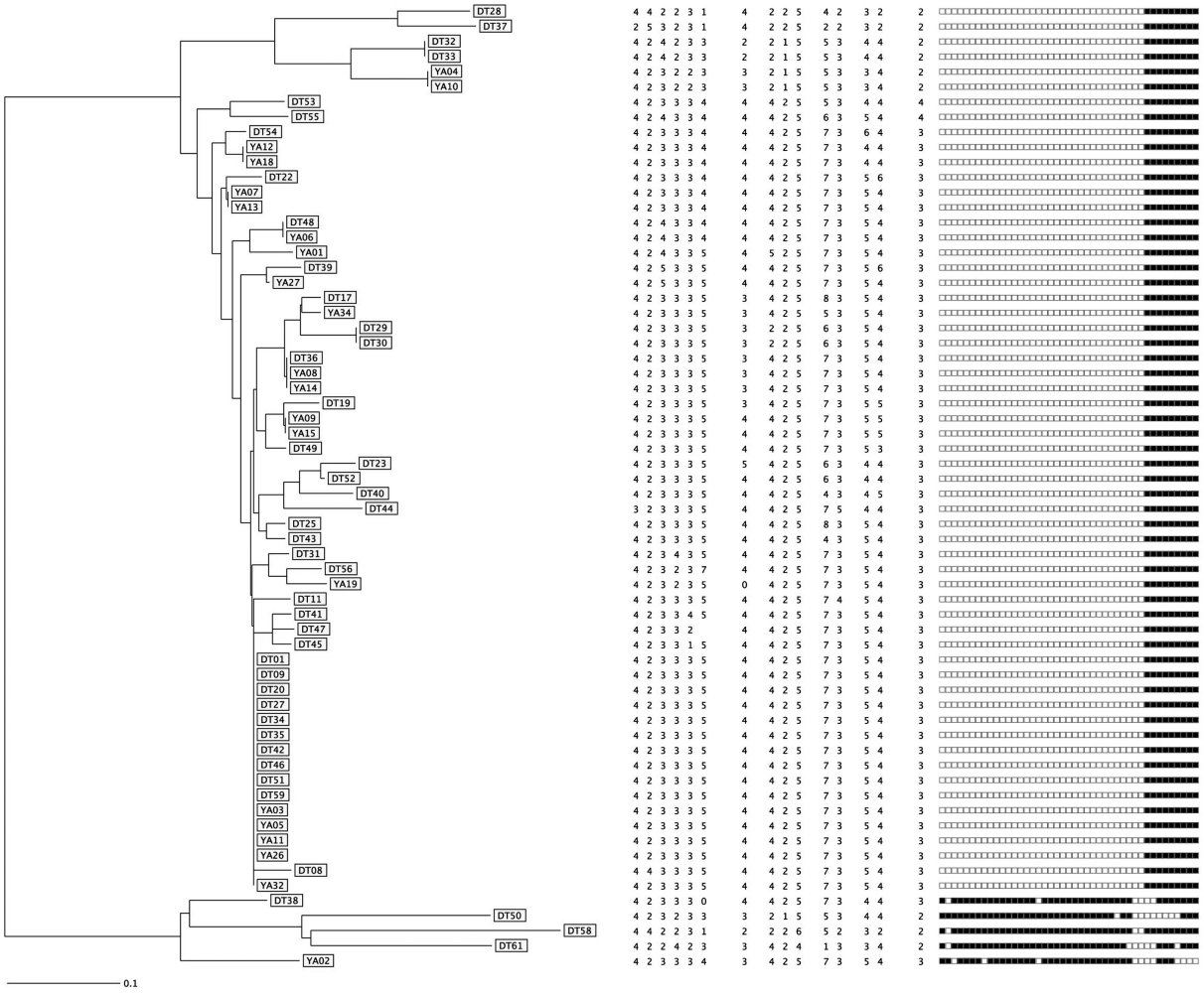

Figure 1. UPGMA phylogenic tree (MIRU-VNTR) based on spoligotyping and MIRU-VNTR analysis of *M. tuberculosis* isolates. From left to right: UPGMA dendrogram generated by VNTR15-$_{China}$; strain No; the repeat number in each VNTR-loci; spoligotyping patterns.

from the two methods in clustering of Beijing family strains. As shown in Table 1, 59 Beijing family strains had the same fingerprint. Meanwhile, by the 15-loci MIRU-VNTR analysis, there were 32 genotypes and 9 clusters in the 59 strains, with an HGDI score of 0.951. Apparently, the discriminatory power of the 15-loci MIRU-VNTR method was higher than that of spoligotyping in analyzing the Beijing family strains. A combination of the two methods can most likely improve the classification of *M. tuberculosis* strains.

Drug susceptibility tests were conducted with proportion method on Lowenstein-Jensen media for the collected strains against the four first-line drugs (rifampin, isoniazid, streptomycin, and ethambutol) and the four second-line drugs (capreomycin, kanamycin, ofloxacin, and ethionanide) according to the standard procedure recommended by Chinese Antituberculosis Association[6]. The drug concentrations used were as followed: 40 μg/mL for rifampin (RFP), 0.2 μg/mL for isoniazid (INH), 4 μg/mL for streptomycin (SM), 2 μg/mL for ethambutol (EMB), 40 μg/mL for capreomycin (CPM), 30 μg/mL for kanamycin (KAN), 2 μg/mL for ofloxacin (OFLX) and 40 μg/mL for ethionanide (ETH). H37Rv was used as a control with each batch of drug susceptibility test. Table 2 shows the drug-resistance of 64 *M, tuberculosis* isolates. Among the 64 *M, tuberculosis* isolates, 50 (78.13%) were resistant to at least one drug, 7 (10.93%) were resistant to at least RFP and INHand they were MDR strains. Two in 7 MDR strains were resistant to all the first-line drugs as well as one

Table 2. Results of Drug Susceptibility Test

Drug	Result	All MTB (*n*=64)	Beijing (*n*=59)	Non-Beijing (*n*=5)
INH	R (%)	20 (31.25%)	19 (32.20%)	1 (20%)
	S (%)	44 (68.75%)	40 (67.80%)	4 (80%)
RFP	R (%)	13 (20.31%)	13 (22.03%)	0 (0)
	S (%)	51 (79.69%)	46 (77.97%)	5 (100%)
SM	R (%)	29 (45.31%)	27 (45.76%)	2 (40%)
	S (%)	35 (54.69%)	32 (54.24%)	3 (60%)
EMB	R (%)	8 (12.50%)	8 (13.56%)	0 (0)
	S (%)	56 (87.5%)	51 (86.44%)	5 (100%)
CPM	R (%)	25 (39.06%)	24 (40.68%)	1 (20%)
	S (%)	39 (60.94%)	35 (59.32%)	4 (80%)
OFLX	R (%)	11 (17.19%)	10 (16.95%)	1 (20%)
	S (%)	53 (82.81%)	49 (83.05%)	4 (80%)
KAN	R (%)	4 (6.25%)	4 (6.78%)	0 (0)
	S (%)	60 (93.75%)	55 (93.22%)	5 (100%)
ETH	R (%)	5 (7.81%)	5 (8.47%)	0 (0)
	S (%)	59 (92.19%)	54 (91.53%)	5 (100%)

kind of fluoroquinolones and at least one of the second-line anti-TB injectable drugs and therefore they were XDR strains. All the MDR and XDR strains belonged to Beijing family and all of these strains were isolated from the patients undergoing re-treatment.

The majority of the isolates were resistant to at least one drug and the resistant rate was 78.13%, being much higher than that reported previously[3]. The prevalence of MDR-TB (10.94%) and XDR-TB (3.13%) among the HIV-infected participants in the present study were similar to those from the previous study in China[8]. And all the MDR strains or XDR strains were isolated from the patients undergoing re-treatment and the inappropriate and excessive use of the first-line anti-TB drugs might be one of the influencing factors. Early comprehensive treatment of MDR/XDR TB is important and drug susceptibility testing is essential for appropriate patient management[10]. The treatment of drug-resistant TB patients co-infected with HIV is more complicated and no relevant guidelines are available yet. It is therefore necessary to conduct drug susceptibility test before drug administration. In this study, we also found that the Beijing genotype was predominant in XDR isolates and this is in consistent with previous study[8].

In summary, *M. tuberculosis* and NTM were identified from AIDS patients with TB-like symptoms in this study. Genotype analysis demonstrated Beijing genotype appeared to be the predominant genotype of the *M. tuberculosis* strains. In addition, the prevalence of drug-resistant TB, especially the prevalence of XDR-TB, was higher than that in TB patients without HIV infection.

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