Molecular Characterization and Drug Susceptibility of *Mycobacterium Bovis* Isolated from Cattle in Xinjiang, China

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To the Editor: Bovine tuberculosis (TB) is a zoonotic disease caused by *Mycobacterium bovis*. It not only seriously influences the raising of livestock but also threatens the health of human beings and other economically important animals. Therefore, the prevention and control of *M. bovis* has great significance for public and animal health. Spoligotyping is the most widely used method for genotyping of *M. bovis* and investigates transmission across different geographical regions. However, its discriminatory power varies widely among countries. Variable number of tandem repeats (VNTR) analysis has emerged as an alternative method for genotyping of bacterial species isolates. Despite the similar biological characteristics of *M. bovis* and *Mycobacterium tuberculosis*, the two neighboring species show different drug-resistance profiles. Overall, *M. bovis* is naturally resistant to pyrazinamide and usually susceptible to most antibiotics used to treat human TB. In China, the excessive use or abuse of veterinary antibiotics is common to prevent bacterial infections among livestock, especially among self-employed, cattle-breeding households. Thus, it is interesting to investigate whether the overuse of antibiotics is affecting the drug susceptibility of *M. bovis* in China, where knowledge of drug susceptibility is limited. This study presented a version of mycobacterial interspersed repetitive units (MIRU)-VNTR analysis based on three triplex polymerase chain reactions with automatic allelic assignation of the products analyzed in capillary electrophoresis (CE) to find which loci are most discriminative in the *M. bovis* of China, and assessed whether extensive transmission of *M. bovis* has occurred among cattle in Xinjiang. It also evaluated the susceptibility of *M. bovis* isolates to two first-line tuberculosis drugs.

It was found that single intradermal comparative cervical tuberculin and interferon-γ tests were both positive for 44 of 341 cattles from five regions within the Xinjiang in April 2014. Twenty-six mycobacterium strains were isolated from these 44 cattles. A commercially available DNA strip assay (GenoType MTBC; Hain Lifescience GmbH, Nehren, Germany) was used to identify *M. bovis*. Molecular species identification confirmed that all 26 isolates belonged to *M. bovis*, indicating an infection rate of 7.6% (26/341).

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CE-VNTR analysis found that the 26 strains could be classified into 15 different MIRU-VNTR types, whereas spoligotyping identified eight different types [Figure 1]. The results of spoligotyping showed four unique genotypes containing only one strain, and the largest genotype contained 10 strains that were isolated from the same region (region 3). Among 15 VNTR patterns, 10 unique cases and 15 clustering cases belonging to 5 clusters (cluster sizes ranging from 2 to 8 cases) were identified. This study further analyzed results from regions with cattles that were infected with the clustered strains. Interestingly, the 8 cases in the largest cluster were all isolated from region 3. The other four clusters were also from the same region.

Special repeat numbers of three loci (MIRU-4, VNTR 1955, and MIRU 39) were discovered [Table 1]. Five loci (5/24) used in the regular MIRU-VNTR method showed no polymorphism (MIRU02, MIRU 10, MIRU23, MIRU40, VNTR53). Two loci (ETR-D and VNTR 1955) were defined as highly discriminative and eight loci (ETR-B, VNTR 52, QUB11b, QUB26, VNTR49, MIRU16, ETR-A, MIRU 24) as moderately discriminative [Table 1].

Drug susceptibility of *M. bovis* isolates was determined by both GenoType MTBDRplus assay and a conventional proportion method. Pyrazinamide susceptibility was determined with a BACTEC MGIT 960 system. All 26 strains were sensitive to isoniazid and rifampin but resistant to pyrazinamide as assessed by BACTEC MGIT 960 system.

In this study, the prevalence of *M. bovis* infection in cattle was higher than that reported in previous studies in Xinjiang.[1] This might be due to an outbreak of *M. bovis* infection in Xinjiang. Based

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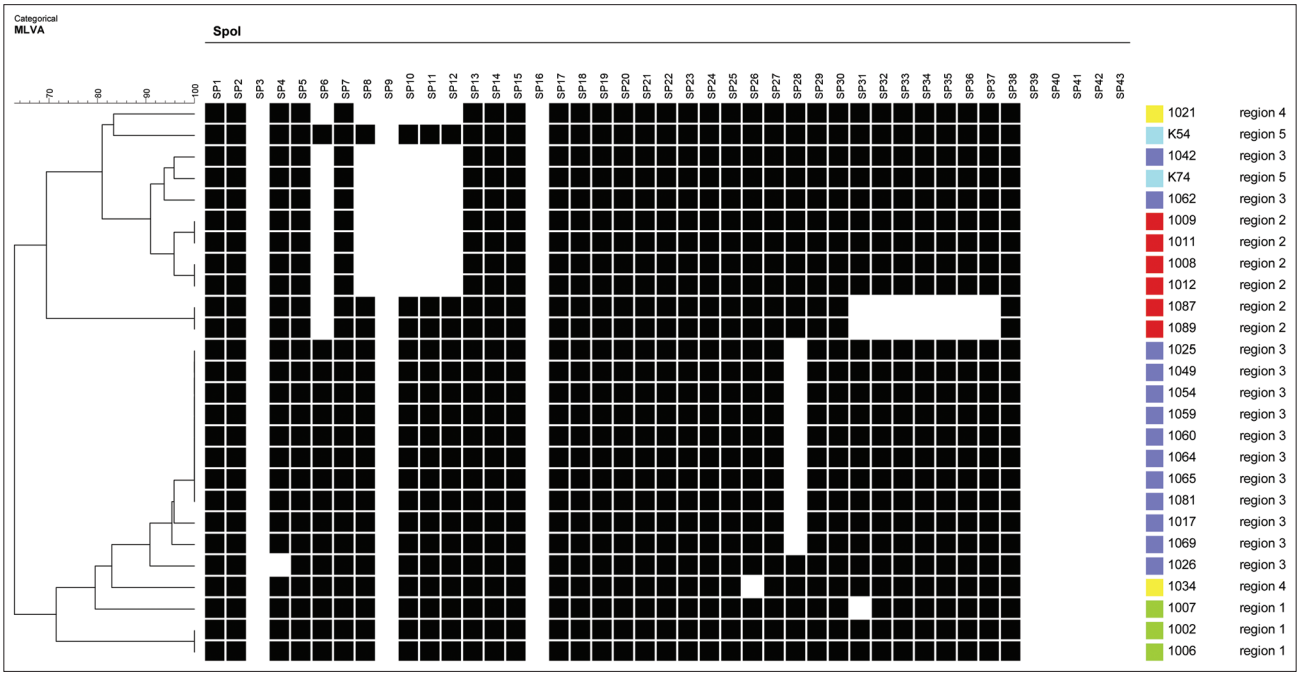

Figure 1: CE-VNTR and spoligotyping patterns of isolates obtained from different farms in Xinjiang Uygur Autonomous Region of China. The dendrogram at the left was based on CE-VNTR profiles. Isolates 1002, 1006, and 1007 were from region 1; 1008, 1009, 1011, 1012, 1087, and 1089 were isolated from region 2; 1017, 1025, 1026, 1042, 1049, 1054, 1059, 1060, 1062, 1064, 1065, 1069, and 1081 were isolated from region 3; 1021 and 1034 were isolated from region 4; and K54 and K74 were isolated from region 5. CE: Capillary electrophoresis; VNTR: Variable number of tandem repeat.

Table 1: Twenty-four loci used for CE-VNTR in this study

Mix	MIRU-VNTR locus	MIRU-VNTR alias	Repeat copy numbers and frequency, *n* (%)	Diversity index
Mix 1	580	MIRU04/ETR-D	3 (42), 4 (38), 5 (19)*	0.662
	2996	MIRU26	2 (4), 3 (4), 5 (92)	0.151
	802	MIRU40	2 (100)	0.000
Mix 2	960	MIRU10	2 (100)	0.000
	1644	MIRU16	2 (35), 3 (65)	0.471
	3192	MIRU31/ETR-E	2 (4), 3 (96)	0.077
Mix 3	424	VNTR 0424	0 (8), 2 (92)	0.148
	577	VNTR43/ETR-C	3 (8), 5 (92)	0.148
	2165	ETR-A	5 (27), 6 (4), 7 (69)	0.465
Mix 4	2401	VNTR47	2 (8), 4 (92)	0.148
	3690	VNTR52	2 (50), 3 (50)	0.520
	4156	VNTR53/QUB-4156c	1 (100)	0.000
Mix 5	2163b	QUB-11b	2 (4), 3 (69), 4 (19), 5 (8)	0.495
	1955	VNTR 1955	1 (8), 3 (38), 4 (8)*, 5 (46)*	0.652
	4052	QUB-26	2 (62), 4 (38)	0.492
Mix 6	154	MIRU 2	2 (100)	0.000
	2531	MIRU 23	4 (100)	0.000
	4348	MIRU 39	1 (8)*, 2 (92)	0.148
Mix 7	2059	MIRU 20	1 (4), 2 (96)	0.077
	2687	MIRU 24	1 (27), 2 (73)	0.409
	3007	MIRU 27/QUB-5	2 (8), 3 (92)	0.148
Mix 8	2347	VNTR46	3 (100)	0.000
	2461	VNTR48/ETR-B	3 (4), 4 (58), 5 (38)	0.538
	3171	VNTR49	2 (38), 3 (62)	0.492

*The tandem repeat copy numbers or allelic diversities in those loci were different from the former reports. MIRU: Mycobacterium interspersed repetitive unit; VNTR: Variable number of tandem repeat; ETR: Exact tandem repeats; QUB: Queen's university Belfast; CE: Capillary electrophoresis.

on CE-VNTR testing, 15 of 26 strains clustered into five groups of indistinguishable strains. All clusters were small, comprising two cases, with the exception of the largest cluster, which had eight cases. In addition, all five clusters were isolated from the

same region. This might reflect recent cattle-to-cattle transmission among clustered cases because the indistinguishable cases came from the same region, and this transmission likely contributed to the subsequent outbreak.

The QUB 11b, QUB 26, and ETR-A loci used for CE-VNTR showed more polymorphism than did other loci. A new combination of 10 MIRU-VNTR loci (ETR-D, VNTR 1955, ETR-B, VNTR 52, QUB11b, QUB26, VNTR49, MIRU16, ETR-A, MIRU 24) was most appropriate for first-line typing of *M. bovis* clones from China. It was interesting to note that the copy numbers of tandem repeat in the loci of MIRU 4, VNTR 1955, and MIRU 39 were different from those in previous studies.[2-4] Few genotypes were identified, possibly because of the limited number of cattle in this study.

There is increasing concern over the observation of drug-resistant isolates of *M. bovis* and *M. tuberculosis*.[5] The main control of *M. bovis* is through a tuberculin test and slaughter strategy, but because of the financial burden of this strategy, uncontrolled and unregistered use of antibiotics, mainly isoniazide, is frequently occurring for treatment of cattle with signs of infection.[6] Although monotherapy, mainly with isoniazid, might result in the generation of drug-resistant *M. bovis*, this study did not find any strains resistant to isoniazid and rifampin.

In conclusion, CE-VNTR method using 10 loci was discriminative for *M. bovis* in Xinjiang, and there was evidence of recent zoonotic transmission in the farms of Xinjiang. Future studies are needed to definitively assess the prevalence of drug-resistant *M. bovis* in China.

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Conflicts of interest

There are no conflicts of interest.

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