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Population structure of drug-resistant *Mycobacterium tuberculosis* in Central Asia

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Abstract

Background: Drug-resistant tuberculosis (TB) is a major public health concern threathing the success of TB control efforts, and this is particularily problematic in Central Asia. Here, we present the first analysis of the population structure of *Mycobacterium tuberculosis* complex isolates in the Central Asian republics Uzbekistan, Tajikistan, and Kyrgyzstan.

Methods: The study set consisted of 607 isolates with 235 from Uzbekistan, 206 from Tajikistan, and 166 from Kyrgyzstan. 24-loci MIRU-VNTR (Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats) typing and spoligotyping were combined for genotyping. In addition, phenotypic drug suceptibility was performed.

Results: The population structure mainly comprises strains of the Beijing lineage (411/607). 349 of the 411 Beijing isolates formed clusters, compared to only 33 of the 196 isolates from other clades. Beijing 94–32 ($n = 145$) and 100–32 ($n = 70$) formed the largest clusters. Beijing isolates were more frequently multidrug-resistant, pre-extensively resistant (pre-XDR)- or XDR-TB than other genotypes.

Conclusions: Beijing clusters 94–32 and 100–32 are the dominant MTB genotypes in Central Asia. The relative size of 100–32 compared to previous studies in Kazakhstan and its unequal geographic distribution support the hypothesis of its more recent emergence in Central Asia. The data also demonstrate that clonal spread of resistant TB strains, particularly of the Beijing lineage, is a root of the so far uncontroled MDR-TB epidemic in Central Asia.

Keywords: Molecular typing, MIRU, MDR-TB, Beijing, Cluster

Background

Drug-resistant tuberculosis (TB) is a public health concern threathing the success of TB control efforts. This is particularily problematic in the Central Asian Republics (CARs) Uzbekistan, Tajikistan, and Kyrgyzstan where multidrug-resistant (MDR)-TB rates reach 24%, 22%, 27%, and 63%, 45% and 60% among new and previously treated cases, respectively [1].

Little is known about the population structure of drug resistant *Myocbacterium tuberculosis* (MTB) strains in Central Asia. Available reports are limited by either outdated genotyping techniques, sampling of special populations and/or small sample sizes [2–4]. Here, we provide a snapshot of the population structure of MTB strains based on World Health Organization resistance surveys performed in Kyrgyzstan, Tajikistan, and Uzbekistan representing two thirds of the Central Asian population.

For genotyping we combined 24-loci MIRU-VNTR typing (Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats) with spoligotyping. MIRU-VNTR provides high-resolution discrimination of strains for epidemiological studies and phylogenetic classification [5]. MIRU-VNTR data allow also to generate parsimonious phylogenetic networks such as minimum-spanning trees (MST) [6]. These results were combined with phenotypic drug susceptibility data to determine the presence of highly resistant dominant clones.

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Methods

Seven hundred and seven clinical MTB isolates (306 from Uzbekistan, 216 from Tajikistan, and 185 from Kyrgyzstan) were collected in the framework of cross-sectional national drug resistance surveys (DRS) [7, 8] and included in the study. The study population was biased towards resistant strains since only isolates that required re-testing for quality control purposes as per the WHO DRS protocol which was valid at the time were included [8]. This approach artificially increased the proportion of MDR-,pre-extensively drug-resistant (pre-XDR) and XDR-TB strains. Ethical approvals for the DRSs were granted by the respective national ethical committees.

DNA was extracted by heat lysis of bacteria from solid culture on LJ medium. 24-loci MIRU-VNTR genotyping and spoligotyping was performed as previously described [5, 9]. Genotypes were identified on the MIRU-VNTR*plus* web database (www.miruvntrplus.org) [5]. Spoligotyping data were used to confirm strain relationships and for genotype classification. Typing data was analysed with Bionumerics (v7.5; Applied Maths, Sint-Martens-Latem, Belgium). MST analysis based on

Table 1 Demographic data, genotypes, haplotypes and phenotypes of the Central Asian *M. tuberculosis* complex study population

	All countries			Uzbekistan			Tajikistan			Kyrgystan		
	No. strains	%	SD	No. strains	%	SD	No. strains	%	SD	No. strains	%	SD
Samples	607			235	38.7		206	33.9		166	27.3	
Gender												
Male		359	59.1	139	59.1		120	58.3		100	60.2	
Female		239	39.4	96	40.9		85	41.3		58	34.9	
Unknown		9		0			1			8		
Age[a]												
Mean	37.94		15.67	42.91		16.6	34.53		14.36	34.18		13.49
Treatment												
New	333	54.9		156	66.4		93	45.1		84	50.6	
Previous	221	36.4		79	33.6		113	54.9		29	17.5	
Unknown	53			0			0			53		
MIRU lineage												
Beijing	411	67.7		136	57.9		154	74.8		121	72.9	
Dehli/CAS	6	1		6	2.6							
H37Rv-like	60	9.9		31	13.2		12	5.8		17	10.2	
LAM	36	5.9		22	9.4		5	2.4		9	5.4	
NEW-1	31	5.1		16	6.8		10	4.9		5	3	
URAL	28	4.6		10	4.3		11	5.3		7	4.2	
Haarlem	26	4.3		7	3		13	6.3		6	3.6	
S-type	3	0.5		2	0.9					1	0.6	
TUR	1	0.2					1	0.5				
X-type	1	0.2		1	0.4							
Undefined	4	0.7		4	1.7							
MLVA MtbC15–9												
94–32 (Beijing)	145	23.9		58	24.7		49	23.8		38	22.9	
100–32 (Beijing)	70	11.5		12	5.1		51	24.8		7	4.2	
Resistance pattern												
Pan-susceptible	35	5.8		21	8.9		2	1		12	7.2	
Other resistances	279	46		127	54		66	32		86	51.8	
MDR-TB	172	28.3		47	20		71	34.5		54	32.5	
Pre XDR-TB	94	15.5		34	14.5		50	24.3		10	6	
XDR-TB	27	4.4		6	2.6		17	8.3		4	2.4	

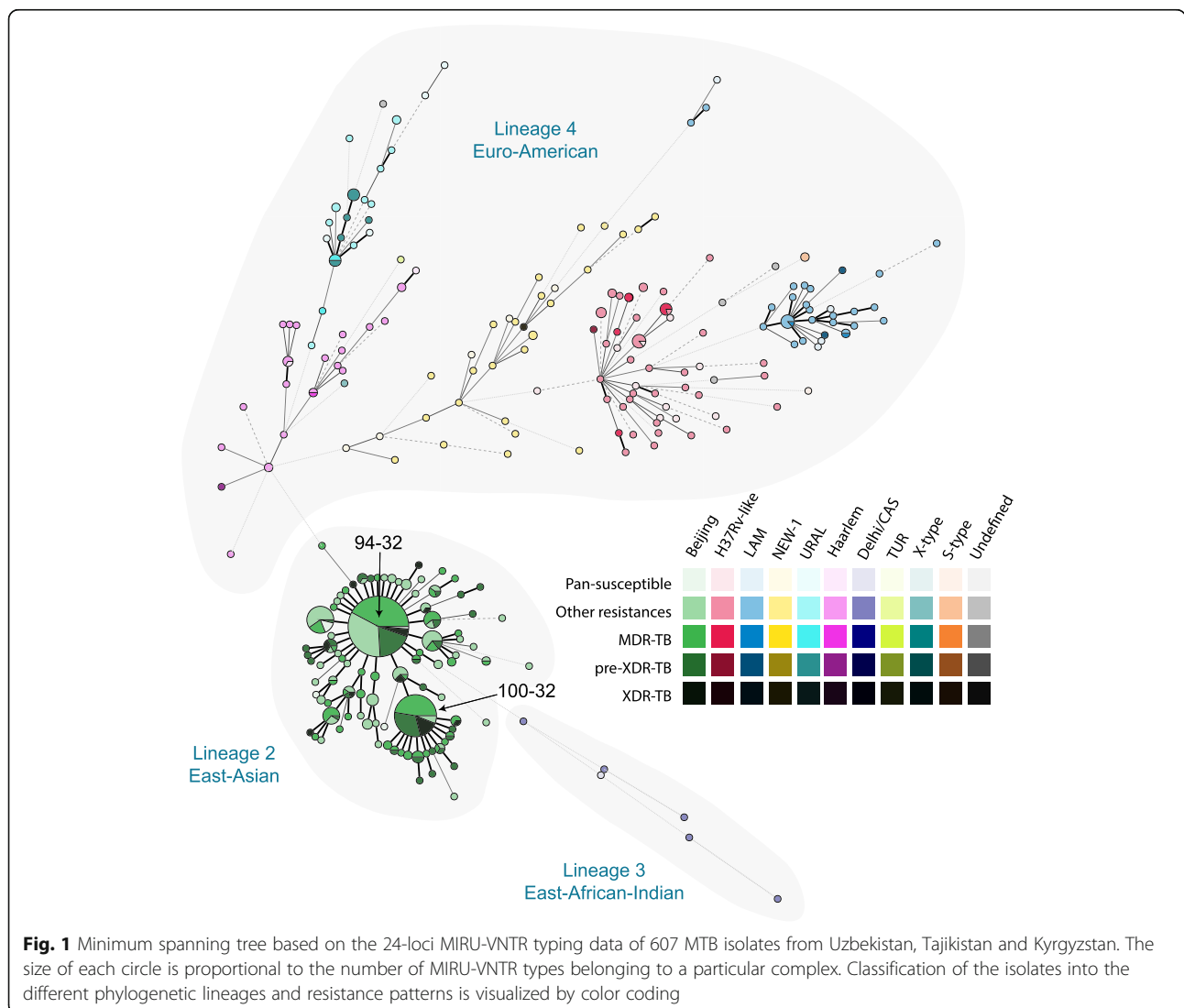[a]Unkown for Tajikistan and 23 unknown for Kyrgystan
SD standard deviation

MIRU-VNTR data was performed using the categorical coefficient. For each 24-loci MIRU-VNTR pattern a unique multiple loci VNTR analysis (MLVA) MtbC15–9 haplotype was assigned by using the MIRU-VNTR*plus* nomenclature [10]. A cluster was defined as a minimum of two isolates harboring identical genotyping patterns from different patients. Patients with mixed molecular typing patterns were excluded. Drug susceptibility testing was performed by the resazurin microtiter assay [11].

Results

Twenty four-loci MIRU-VNTR typing and spoligotyping were successfully performed for 607 isolates, while 76 isolates (62 from Uzbekistan, seven from Tajikistan, and seven from Kyrgyzstan) yielded indeterminate results. For 23 isolates (9 from Uzbekistan, three from Tajikistan, and 11 from Kyrgyzstan), mixed genotyping patterns were observed. One isolate from Kyrgyzstan had incomplete phenotypic data.

The final study set consisted of 607 isolates of which 235 (38.7%) originated from Uzbekistan, 206 (33.9%) from Tajikistan, and 166 (27.3%) from Kyrgyzstan (Table 1). The male to female ratio of patients was 1.5 and ages ranged from 12 to 85 years with a mean of 37.9 years (SD ±15.7). Across the three countries, 333 (54.9%) were new and 221 (36.4%) previously treated cases (for 53 patients information regarding previous treatments was missing; Table 1). Thirty five isolates (5.8%) were pan-susceptible, 293 (48.3%) MDR-, pre-XDR, or XDR-TB, while the remaining 279 isolates (46.0%) had any other resistance pattern. MDR- and XDR-TB were more prominent among Kyrgyz (33%) and Tajik (35%) than among Uzbek isolates (20%), which was mainly attributable to different re-testing algorithms in the DRS protocols.

Based on the MIRU-VNTR profiles and spoligotyping patterns, 603 isolates were classified into previously

Fig. 1 Minimum spanning tree based on the 24-loci MIRU-VNTR typing data of 607 MTB isolates from Uzbekistan, Tajikistan and Kyrgyzstan. The size of each circle is proportional to the number of MIRU-VNTR types belonging to a particular complex. Classification of the isolates into the different phylogenetic lineages and resistance patterns is visualized by color coding

described lineages (Table 1). 411 (67.7%) isolates belonging to lineage 2 (East-Asian; Beijing genotype) [12]. Six (1.0%) isolates belonged to lineage 3 (East-African-Indian; Delhi/CAS genotype), and 186 (30.6%) isolates belonged to lineage 4 (Euro-American) with the predominiating genotypes H37Rv-like (60; 9.9%), LAM (36; 5.9%), NEW-1 (31; 5.1%), URAL (28; 4.6%), and Haarlem (26; 4.3%). Four (0.7%) isolates could not be linked to a previously described lineage and were classified as "undefined". An MST was calculated (Fig. 1) confirming the UPGMA (unweighted pair group method with arithmetic mean) tree-based genotype classification of MIRU-VNTR*plus* (data not shown).

Cluster analysis revealed that 382 (62.9%) of the 607 isolates shared a genotyping pattern with at least one other isolate. They were grouped in 38 clusters ranging from two to 145 isolates. The two largest clusters had haplotypes 94–32 ($n = 145$; 23.9%) and 100–32 ($n = 70$; 11.5%) (Fig. 1). The prevalence of 94–32 was equally distributed over the CARs whereas cluster 100–32 was particularly prominent in Tajikistan (51 strains; 72.9%). 349 (84.9%) of the 411 Beijing isolates formed clusters, compared to only 33 (16.8%) of the 196 isolates from other clades.

Beijing strains were more frequently MDR-, pre-XDR- or XDR-TB (265/411; 64.5%) than strains of other genotypes (28/196; 14.3%; no XDR-TB) (Fig. 2). Beijing cluster 94–32 showed the same drug resistance pattern as other Beijing strains, whereas cluster 100–32 was almost completely (66/70; 94.3%) MDR, pre-XDR, or XDR.

Discussion

High prevalence of Beijing strains has previously been reported from Central Asia [4]. The star-like shape of the lineage 2/Beijing population in Fig. 1 with two central clones surrounded by layers of single and multi-locus variants is a typical pattern for emerging and expanding MTB populations [13]. The two largest clusters 94–32 and 100–32 were found in all three Central Asian countries and represented more than a third of all study isolates indicating strong population expansion across borders. Our findings confirm the results of a global Beijing population study which reported that 94–32 and 100–32 were predominantly prominent in Uzbekistan, Russia and Eastern European countries and had particularly high clustering rates [13]. The smaller size of 100–32 and its unequal distribution to the three CARs suggest however its more recent arrival in Central Asia and a shorter regional expansion time than of 94–32. This hypothesis is further supported by Mokrousov [14] and Skibe et al. [15] who have already reported 100–32 as minor group (< 4%) of Beijing isolates in Central Asia while it reached proportions of over 10% and over 20% in Eastern Europe and Russia, respectively, and while Skibe et al. observed 94–32 as dominant cluster in Kazakhstan. The extent of cross-border spread and locally restricted transmisison networks can however not be ultimately resolved with MIRU-VNTR cluster data and require further investigation with higher resolution.

High percentages of MDR among Beijing strains has also been reported from other Eastern European countries, like Georgia, Kazakhstan, and Uzbekistan [4, 15, 16]. Higher proportions of MDR- and XDR-TB were also observed within the 100–32 genotype by Merker and colleagues [13]. The authors analyzed the full genomes of MDR-TB strains belonging to MLVA-VNTR haplotypes 94–32 and 100–32 and showed that the maximal divergences were 17 and 23 SNPs, respectively, within genomes of those clusters [13].

Our data indicate that spread of resistant MTB strains, particularly of lineage 2/Beijing, is an important root of
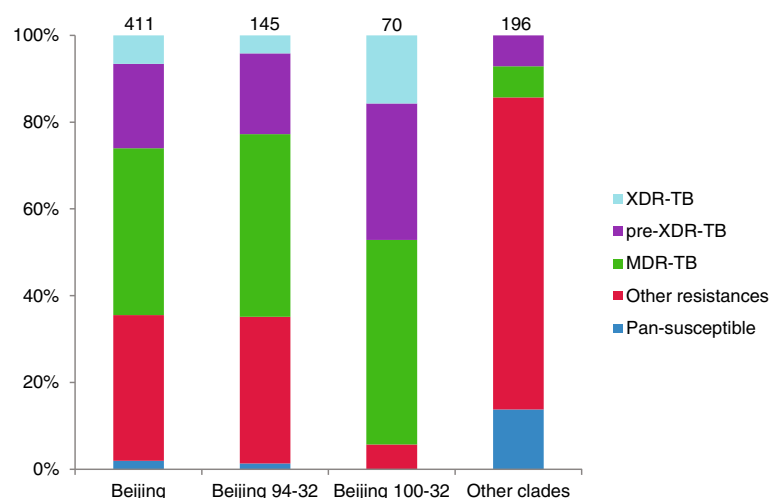

Fig. 2 Proportions of isolates based on drug resistance patterns. The number of isolates with a given genotype/MLVA MtbC15–9 haplotype is given above the corresponding column

the ongoing MDR-TB epidemic in Central Asia. MIRU-VNTR is a powerful tool for investigating population structures and transmission dynamics, however, advances in the field of whole genome sequencing have resulted in molecular methods that can give better resolution and should ideally be used to elucidate specific transmission pathways. Analyzing the full genomes of this or an unbiased and thus even more representative study population could provide an even deeper insight into the MTB population structure in CAR.

Conclusions

Clusters 94–32 and 100–32 are the dominant genotypes driving population expansion of Beijing strains in the CARs Uzbekistan, Tajikistan, and Kyrgyzstan. The data demonstrate that spread of resistant TB strains, particularly of the Beijing lineage and its clusters 94–32 and 100–32, is an important root of the ongoing MDR-TB epidemic in Central Asia. Measures to combat this epidemic need to be improved.

Abbreviations

CAR: Central Asian Republic; DRS: Drug resistance surveys; MDR: Multi-drug-resistant / resistance; MIRU-VNTR: Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats; MLVA: Multiple loci VNTR analysis; MST: Minimum-spanning tree; MTB: *Myocbacterium tuberculosis*; TB: Tuberculosis; UPGMA: Unweighted pair group method with arithmetic mean; XDR: Extensively drug resistant / extensive drug resistance

Authors' contributions

SN and HH designed the study, AE performed the experiments, AE, UA, AK, GK, OK, NP, AR, ES, ZS, and HH collected data, AE, KK, SN and HH analyzed the data, AE, KK, MM, SN and HH wrote the manuscript. All authors read and approved the final manuscript.

Authors' information

None

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable, but see please see methods section and references 8 and 9.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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