Tuberculosis 93 (2013) 501–507

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

MOLECULAR ASPECTS

Molecular snapshot of *Mycobacterium tuberculosis* population structure and drug-resistance in Kyrgyzstan

Igor Mokrousov [a,b,*], Jainagul Isakova [c,**], Violeta Valcheva [b,d], Almaz Aldashev [c], Nalin Rastogi [b]

[a] *Laboratory of Molecular Microbiology, St. Petersburg Pasteur Institute, St. Petersburg, Russia*
[b] *WHO Supranational TB Reference Laboratory, Unité de la Tuberculose et des Mycobactéries, Institut Pasteur de Guadeloupe, Abymes Cedex, Guadeloupe, France*
[c] *Institute of Molecular Biology and Medicine, Bishkek 720040, Kyrgyzstan*
[d] *The Stephan Angeloff Institute of Microbiology BAS, Sofia, Bulgaria*

ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form
27 May 2013
Accepted 30 May 2013

Keywords:
Molecular epidemiology
Tuberculosis
Drug resistance
Kyrgyzstan

SUMMARY

Kyrgyzstan is a post-Soviet country in Central Asia marked with high incidence and mortality rates of tuberculosis (TB). The present study provided first assessment of *Mycobacterium tuberculosis* population structure and drug-resistance in civilian population here. The collection included 103 *M. tuberculosis* DNA samples subjected to the analysis of rifampin and isoniazid resistance mutations and spoligotyping. The major spoligotype-defined families were Beijing ($n = 62$), T ($n = 14$), LAM ($n = 9$), Ural-2 ($n = 6$) and Ural-1 ($n = 3$). Genotypically, 20 isolates were RIF-resistant, 28 were INH-resistant, 17 were multidrug-resistant. Drug resistant isolates were more prevalent among Beijing than non-Beijing groups ($P = 0.03$). The predominance of the mainly "Russian" spoligotypes among the non-Beijing strains (LAM-RUS and Ural-1) in this study along with previously demonstrated prevalence of the Russia-specific subtype of the Beijing family in Kyrgyz prison (Mokrousov et al., 2009) suggest that the current population structure of *M. tuberculosis* in Kyrgyzstan has been mainly formed within the course of the 20th century when the country was a part of the Russian Empire and Soviet Union. On the other hand, a prevalence of the Asia-specific Ural-2 type in the oldest age group (68–85 years old; $P < 0.0001$) may present a heritage of the more distant historical events. In summary, we suggest: (i) a clear shift of the local *M. tuberculosis* population structure during the last 100 years and (ii) a critical impact of the Beijing genotype on the current situation with drug resistant TB in Kyrgyzstan.

1. Introduction

Kyrgyzstan is a mainly high-altitude post-Soviet country in Central Asia. Although cardiovascular diseases constitute the major cause of mortality, among prevailing infectious diseases tuberculosis (TB) is listed as one of the most important diseases here [1]. As in many post-Soviet countries, and in spite of the different measures implemented in the national TB control program, situation with TB in Kyrgyzstan remains marked with high rates of incidence and mortality: 97.4 and 8.6 per 100,000 civilian population, respectively (95.1 and 8.7 per 100,000 total population including penitentiary system) in 2011 [2].

While a number of publications on molecular epidemiology of TB in Kyrgyzstan's neighbours have been published [3–5], only one paper reported results of *Mycobacterium tuberculosis* molecular typing in Kyrgyzstan although in the special, penitentiary population [6]. In addition, a number of studies described particular gene mutations associated with resistance to the main anti-TB drugs, rifampin (RIF) and isoniazid (INH) in Kyrgyz isolates but they were published in Russian journals and are of limited access to the international community [7,8].

For this reason, the present study was undertaken in order to gain understanding of the population structure of *M. tuberculosis* in Kyrgyzstan, compare different strain/patient features across different genotypes, and view the situation in this country in regional and global context through comparison with international databases and other available sources of information.

* Corresponding author. Laboratory of Molecular Microbiology, St. Petersburg Pasteur Institute, 197101 St. Petersburg, Russia. Tel.: +7 812 2332149; fax: +7 812 2329217.
** Corresponding author. Institute of Molecular Biology and Medicine, 3 Togolok Moldo Str., Bishkek 720040, Kyrgyzstan. Tel.: +996 (312) 66 38 56; fax: +996 (312) 66 03 87.
E-mail addresses: imokrousov@mail.ru, igormokrousov@yahoo.com (I. Mokrousov), jainagul@mail.ru (J. Isakova).

1472-9792/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tube.2013.05.008

2. Material and methods

2.1. Study sample

The study enrolled 133 adult HIV-negative newly-diagnosed pulmonary TB patients admitted at the hospital of the National Center of Phthisiatry, Bishkek, Kyrgyzstan, from August to November 2008. They constituted 70% of all newly-diagnosed patients with pulmonary TB admitted at this hospital within the timeframe of the study. Sputum specimens were collected once from each patient on the 1st day of their admittance and sent to the laboratory after removal of patient identifiers. All patients were found microscopy positive for presence of *M. tuberculosis* which was confirmed by TB-Biochip assay (Biochip-IMB, Moscow, Russia). Ethical approval was obtained from the Bioethics Committee of Kyrgyz Republic. Informed consent was obtained from each study participant.

2.2. Resistance mutations detection

DNA was extracted from microscopy-positive sputum and was used for *M. tuberculosis* species identification and detection of mutations in *katG315* and *inhA* promoter region associated with INH resistance and mutations in *rpoB* rifampin resistance determining region (RRDR, *rpoB* codons 507–533) associated with RIF resistance by using TB-Biochip kit (Biochip-IMB, Moscow, Russia) as described previously [7].

2.3. Genotyping

Spoligotyping of isolates was performed as previously described [9]. The spoligoprofiles were entered into Excel spreadsheets and compared with SITVIT2, an international spoligotype database in Institut Pasteur de Guadeloupe, which is a most recent update of the published SITVITWEB database [10]. An insufficient quality and quantity of DNA extracted from sputum samples prevented from further genotyping, e.g., using VNTR loci.

2.4. Statistical analysis

Hunter Gaston index (HGI) was calculated as described previously [11] and was used to evaluate diversity of a marker/population. A 2 × 2 χ^2 test was used to detect any significant difference between the two groups. Yates corrected χ^2 and *P*-values were calculated with 95% confidence interval using EpiCalc 2000 version 1.02 software [12].

3. Results

3.1. Sample description

A total of 133 sputum specimens were taken from 133 different HIV-negative adult (17–85 years) patients newly diagnosed as having pulmonary TB and admitted to the TB hospital in Bishkek. The samples were proven positive for *M. tuberculosis* by microscopy and IS*6110*-PCR using TB-Biochip kit. Of 133 DNA samples, 103 gave interpretable results under spoligotyping and further analysis was done for those 103 isolates.

All 103 patients were permanent residents of the Kyrgyz Republic, of them 97 were ethnically Kyrgyz, and other were Russian, Uyghur, Korean – 2 patients each. By origin, patients represented different provinces (*oblast*) of the country. Two Russian patients were from Bishkek and Chuy, two Uyghur patients – from Bishkek and Chuy, and two Korean patients - from Jalal-Abad and Chuy, i.e. non-Kyrgyz patients represented both capital city Bishkek and north-central part of the country as a whole (Figure 1).

Age range of the studied population was from 17 to 85 years old, mean age was 40.5 ± 15.2. The mean age in the male group was 42.2 ± 13.1 and in the female group 38.5 ± 17.4. The study sample included 56 male and 47 female patients although this proportion was different in different age groups: female were in higher rate in the youngest (24 [64.9%] of 37 aged less than 31 years old) and oldest (6 of 10 aged more than 60 years old) age groups.

3.2. Molecular characterization

3.2.1. Genotyping

Spoligotyping of 103 samples identified 31 patterns, including 7 groups ("clusters") shared by 2–61 isolates (in total, 79 "clustered" isolates), and 24 singletons (Table 1). The profiles were further compared to the international databases. Comparison to the SITVIT2 database assigned 98 isolates to the described or newly created shared types. Nine isolates were not previously described in SITVIT2. Four of them formed newly created SIT (3434 and 3435) while five remained orphans.

The profiles were further compared to the MIRU-VNTRplus database [13] and some previous publications in order to clarify their family/lineage position. Spoligotypes defined as T5-RUS1 in SITVIT were redefined as Latin-American Mediterranean (LAM) family based on the previous findings [14–16]. Russian spoligotypes of the LAM family including prototype/ancestral SIT42 and a number of derived SIT (defined as T5-RUS1) were previously shown to belong to the sublineage within the LAM family characterized by the specific IS*6110* insertion in the *plcA* gene [14,15]. In this study, we assumed that all LAM strains (SIT42 and former T5-RUS1) indeed belong to the LAM-RUS sublineage. Interestingly, two LAM types SIT2956 and SIT3434 were found only in Kyrgyzstan.

Spoligotypes within SITVIT-defined Haarlem subclade (H4 in the previous version SpolDB4 and H3 in the current SITVITWEB databases) were redefined as Ural according to the MIRU-VNTRplus database and previous findings of the Ural and Haarlem specific SNPs and spoligotype signatures [17–19]. Those with present signal #2 were further defined as Ural-1 (=Ural in MIRU-VNTRplus database). Spoligotypes within H4 with absent signal #2 were ascribed to the sublineage tentatively named "Ural-2" [19]. These two groups Ural-1 and Ural-2 were analysed separately in this study.

As a whole, based on spoligotyping data, the largest groups in this study were Beijing ($n = 62$), T ($n = 14$) and LAM ($n = 9$) families. The spoligotype-based diversity for the entire collection evaluated as HGI was 0.65. Formally 'moderate', this value is low as 60% of

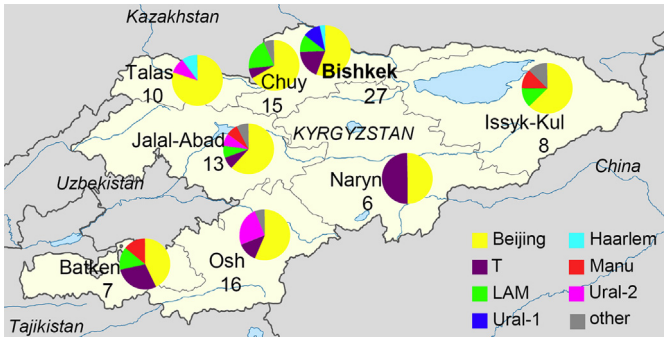

Figure 1. Regional distribution of the genetic families identified in 103 *M. tuberculosis* strains from Kyrgyzstan. Exact number of strains per location is shown under the city's name. In bold: national capital. Map of Kyrgyzstan: http://ftpmirror.your.org/pub/wikimedia/images/wikipedia/commons/archive/7/7b/.