Molecular Epidemiology of Multidrug Resistant and Extensively Drug Resistant Tuberculosis in country of Georgia

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INTRODUCTION

Introduction of modern molecular approaches added a new opportunities to the classical epidemiology of TB. Molecular Epidemiology enhances understanding of transmission dynamics of TB, investigates spread of related strains in community, and determines frequency of specific strains for given geographical regions. Knowledge of the complete genome sequence of *M. tuberculosis* provided the most powerful tool for the epidemiology of TB. Despite of these advances, the emergence of drug-resistant TB, in particular, multidrug-resistant tuberculosis - MDR TB and extensively drug- resistant tuberculosis - XDR TB, seriously challenges the success of TB control worldwide.

MDR/XDR TB has been detected in every region of the world, with the highest rates found in countries of the former Soviet Union, including Georgia. Although there has been a small decline in TB rates in Georgia over the last few years, TB remains an huge public health problem.

MATERIALS AND METHODS

Patients and Bacterial Isolates:

M.tuberculosis MDR/XDR isolates (n=224) recovered from patients with pulmonary tuberculosis (TB) were collected countrywide in the period between February 2014 and December 2015.

Susceptibility testing to the first line antituberculosis drugs (isoniazid, rifampin, ethambutol, streptomycin) was performed by the method of absolute concentrations in 7H9 broth in Bactec MGIT 960 system;

M. tuberculosis isolates that showed to be MDR TB were subjected to the second-line DST (Ethionamide, Ofloxacin, p-aminosalicilic acid, Capreomycin, Kanamicin, Amikacin) by the proportion method on solid LJ media;

M. tuberculosis MDR/XDR isolates were subcultured and delivered to NCDC Lugar Center for molecular investigations.

Spoligotyping;

The chromosomal DNA from the strains of *M.tuberculosis* was prepared by the internationally standardized method of chloroform-isoamyl alcohol extraction (1).

Spoligotyping was performed according to the described methodology (2), procedures were carried out by using commercial kit (Ocimun Biosolutions).

Extracted DNA was subjected to PCR (initial denaturation at 96°C for 3 min, 25 cycles of 1 min each at 96°C, 1 min at 55°C and 30 seconds at 72°C, and extension at 72°C for 5 min), PCR products were hybridized to a membrane containing 43 oligonucleotides by reverse line blotting. *M. tuberculosis* H37Rv and *M. bovis* BCG were used as positive controls in each run.

Absence and presence of spacer oligonucleotides were documented in the form of binary code that was converted into octal code.

24-locus MIRU-VNTR typing:

85 *M.tuberculosis* isolates were genotyped by standardized 24-loci MIRU-VNTR typing as described (3).PCR amplification of the original 24 MIRU-VNTR loci was performed by multiplex PCR with the following conditions: (initial denaturation at 95°C for 15 min, 30 cycles of 1 min each at 94°C, 1 min at 59°C and 1 min 30s at 72°C, and final cycle of extension at 72°C for 10 min). PCR products were run with Genescan 1200LIZ size standard (Applied Biosystems,) on ABI 3130xl sequencer.

Sizing of the PCR fragments and assignment of MIRU-VNTR alleles were done by Gene Mapper software version 4.1 (Applied Biosystems) according to the instructions of the manufacturer.

RESULTS

- Typing of 224strains of *M.tuberculosis* clinical isolates resulted in 47 different spoligotype patterns
- 185 *M.tuberculosis* isolates distributed in 6 different clusters, 39 (17.4%) strains displayed unique profiles. Clustering rate was 82, 6%. The distribution of clusters is shown in Table 1
- The Beijing lineage representing 60.3% (n=135) of the total MTB isolates was found to be the most dominant spoligotype followed by Beijing-like–16,5%.
- 85 strains of *M tuberculosis* were genotyped by 24 locus MIRU-VNTR typing.
- Selection of the strains was based on their previously identified spoligotype. 37 strains were from Beijing family, 11 – from Beijing like, 4 strains belonged to two different clusters; 33 were non-clustered strains.
- All 85 isolates typed were found to have unique MIRU-VNTR profiles, there was absence of any clustering

Table 1. Distribution of clusters of MTB Spoligotypes

Octal number	Ratio in the 224 strains	
	N	%
000000000003771	135	60.3
000000000003371	37	16.5
000000000003661	3	1.3
777777777760771	4	1.8
774777777420771	4	1.8
774767777630771	2	0.9

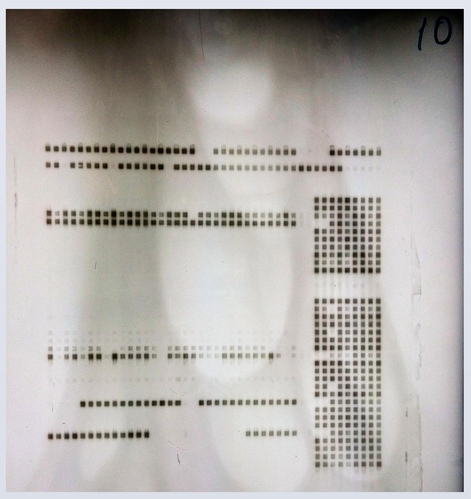

Figure 1. Spoligotyping autoradiograph; line--1 *M. tuberculosis* H37Rv; line-2 *M. bovis* BCG

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