Typing of *Mycobacterium tuberculosis* Strains Resistant to Rifampicin and Isoniazid by Molecular Biological Methods

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Mutations in the *rpoB*, *katG*, *inhA*, *oxyR/ahpC* genes in rifampicin- and isoniazid-resistant *M. tuberculosis* strains isolated from residents of Moscow, Astrakhan', and Moldova Republic were studied by molecular biological methods (heteroduplex analysis, single strand conformational polymorphism, biochips). Twenty-five combinations of mutations were detected. Some differences in the type distribution of detected mutations were found. The use of biochips is the most perspective method for determining the type of mutation.

Key Words: *Mycobacterium tuberculosis; mutations; rifampicin; isoniazid; biochip*

The use of modern methods of molecular biology for detecting mutations responsible for drug resistance is limited by the number of mutations detected in one analysis.

The use of new technologies of biological microchips markedly simplifies identification of mutations. After two-step PCR and chip hybridization we detected the presence (or absence) of mutations in four genes determining the resistance to two drugs. Simultaneous detection of the resistance to rifampicin and isoniazid is of special importance, because this resistance is a marker of multiple drug resistance of *M. tuberculosis* (MBT) strains [5]. On the other hand, the use of microchips allows identification of only those mutations, for which appropriate discriminating probes were synthesized.

We investigated the repertoire of mutations in the *rpoB*, *katG*, *inhA*, *oxyR/ahpC* genes in rifampicin- and isoniazid-resistant MBT strains isolated from residents of Moscow, Astrakhan', and Moldova Republic using biological microchips.

Identification of mutations responsible for MBT resistance to rifampicin and isoniazid is important from the epidemiological viewpoint and for effective drug therapy of tuberculosis.

MATERIALS AND METHODS

The study was carried out at Moscow Tuberculosis Control Center, Public Health Committee of Moscow. Biological fluids from the lungs (sputum and bronchoalveolar lavage fluid) were collected from patients with various forms of lung tuberculosis. In addition, clinical strains of MBT grown in solid Lowenstein—Jensen medium or in 7H9 liquid medium were studied (the material was collected from tuberculous patients in Moscow and Moscow region, Astrakhan', and Moldova Republic).

Primary treatment of the material, isolation of DNA, and PCR were carried out as described previously [1].

Mutations in the *rpoB* gene (codons 507-533) responsible for rifampicin resistance of MBT strains

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TABLE 1. Distribution of Mutations in Genes Responsible for Rifampicin and Isoniazid Resistance in MBT Strains Isolated from Patients with Pulmonary Tuberculosis

Regions	Genes responsible for resistance to			
	rifampicin	isoniazid		
	rpoB	katG	inhA	oxyR/ahpC
Moscow (*n*=81)	68.8	72.2	44.4	11.1
Astrakhan' (*n*=18)	81.8	54.5	9.0	45.5
Moldova Republic (*n*=11)	54.5	91.0	18.2	—

Note. Strains with mutation in at least one of the studied genes were taken for 100%.

were detected by heteroduplex analysis (HDA) using a universal heteroduplex generator (a plasmid containing the studied *rpoB* gene site with four 3-nucleotide deletions and three 2-nucleotide substitutions) [2].

Isoniazid-resistant MBT strains were detected by single strand conformational polymorphism (SSCP) analysis by detecting mutations in *katG* (codons 315-335), *inhA* (promotor and structural regions of the gene), *oxyR/ahpC* (intergene regulatory area of *ahpC* gene and translation regulator), *kasA* genes (nucleotide sequence 31632-31426 region). This method allows detection of isoniazid resistance caused by mutations in any of the four genes in the same sample under the same amplification conditions and followed by amplicon analysis after their denaturing and separation in polyacrylamide gel.

Simultaneous detection of MBT resistance to rifampicin and isoniazid was carried out using biological microchips (IMB-Biochip). Discriminating oligonucleotides detecting mutations in *rpoB* (for 95% of rifampicin-resistant strains), *katG*, *inhA*, *oxyR/ahpC* genes (for 80% of isoniazid-resistant strains) were immobilized on the biochip [3].

Sequencing for identification of new mutations was carried out on an AB1 373A device (Applied Biosystem).

RESULTS

The study of four genes of MBT strains on chips with subsequent sequencing showed that the number of mutations in each gene was different in different regions (Table 1). DNA mutations in the studied strains were identified in codons (Table 2).

Strains with the most prevalent mutations (for example, in codon 531 of *rpoB* gene and in codon 315 of *katG* gene) predominated, but some regional differences were revealed. For example, *inhA* gene of MBT strains from Astrakhan' did not possess such mutation variety as *inhA* gene in mycobacteria isolated from Moscow residents. On the other hand, the number of mutations in *oxyR* gene in strains isolated in Astrakhan was higher than in Moscow strains. Mutations in genes of MBT strains from Moldova were detected in codons typical of the majority of studied mycobacteria, but not in *inhA* and *oxyR/ahpC* genes, which can be attributed to low number of samples.

Our findings suggest that the majority of rifampicin- and isoniazid-resistant MBT strains had mutations in two genes, a lesser number of strains had mutations in three genes, and the least number in one gene. A total of 25 combinations of mutations were distinguished. We detected MBT strains resistant to isoniazid alone, but none strains resistant to rifampicin alone.

Information on precisely identified mutations in drug-resistant MBT strains can be very useful for epidemiological studies. For example, the results of identification of strains with rare mutations, double mutations in one gene, or mutations involving several genes can be used for detecting the source of infection, routes of its transmission and, hence, arresting further trans-

TABLE 2. Distribution of Mutations in *rpoB*, *katG*, *inhA*, *oxyR/ahpC* DNA Gene Codons in Different Strains of *M. tuberculosis*

Regions	Genes (codon Nos.)			
	rpoB	katG	inhA	oxyR/ahpC
Moscow	511, 516, 522, 526, 531, 533	315, 328	8, 9, 15	6, 10, 12
Astrakhan'	511, 512, 531	315	15	9, 10, 12
Moldova Republic	516, 531	315	—	—

mission. Such a study was previously impossible, because the procedure of precise identification took several weeks and the data were difficult to use for description of the transmission process. Creation of a database based on the results of identification of mutations in MBT strains allowed us to evaluate the relationship between the type of mutation and the minimum inhibitory concentration of antibiotics [4-6].

Thus, the need for new molecular genetic methods precisely identifying the type of mutation responsible for rifampicin and isoniazid resistance is obvious. We believe that the method of biochips is the most perspective due to its simple technology, the possibility to obtain the results within 2 days, and low costs; moreover, it allows simultaneous identification of several mutations. In addition, this method can be used for screening studies, when it is important to detect the resistance of MBT strains to both rifampicin and isoniazid in many examinees. However, it is desirable to use other molecular methods for genetic identification of strains along with biochips, because the available biochips do not yet allow us to detect the entire spectrum of DNA mutations in MBT strains isolated from tuberculosis patients.

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