Tanaffos (2007) 6(3), 11-19

Mutations in Codon 315 of the *katG* Gene Associated with High-level Resistance to Isoniazid

Saeed Zaker Bostanabad [1,2], Ahmad Reza Bahrmand [1], Shahin Poorazar [1], Farid Abdolrahimi [1], Ali Nur-Nemattollahi [1], Morteza Massomi [1], Leonid Petrovich Titov [2,3]

[1]Pasteur Institute of Iran, Tehran-Iran, [2] Belarusian State Medical University, [3] Belarusian Research Institute for Epidemiology and Microbiology, Minsk-Belarus.

ABSTRACT

Background: *The aim of this study was to investigate the significance of mutation in codon 315 of katG gene and its correlation with high-level of resistance to isoniazid, nuclotide and amino acid changes in mycobacterium tuberculosis (MTB) isolates randomly collected from sputums of 42 patients with active pulmonary tuberculosis in different regions of Belarus.*

Materials and Methods: *Drug susceptibility testing was determined using the CDC standard conventional proportional method. DNA Extraction, katG gene amplification, and DNA sequencing analysis were performed.*

Results: *Six isolates (14%) bearing multi-mutations in three codons (309,315 and 316), 26 Isolates (61.9%) demonstrated multi-mutations in all or two of the above codons, and 8 (19%) were found to have a single mutation in 315. Four types of mutations were identified in codons 315: AGC→ACC (n=36)85%, AGC→AGG (n=1) 2.3%, AGC→AAC (n=2) 4.7%, AGC→GGC (n=1) 2.3%, one type of mutation in 316: GGC→AGC (n=18)41.4%, and four types of mutations in 309: GGT→GGT (n=7)16.1%, GGT→GCT (n=4)9.2%, GGT→GTC (n=3)6.9%, GGT→GGG (n=1)2.7%. In 2 (4.7%) isolates mutations were identified in codons 463, 357, and in codons 454, 357 respectively.*

Conclusion: *MTB in patients from Belarus were found to have high-level resistance to isoniazid in the isolates with mutations in codon 315 (> 10 μg/mL).* ***(Tanaffos 2007; 6(3): 11-19)***

Key words: *Mutation, Codon 315, Resistance, katG gene, Isoniazid*

INTRODUCTION

Isoniazid is the first line chemotherapeutic drug used in tuberculosis (TB) therapy (1-4).

Resistance to isoniazid is associated with a variety of mutations affecting one or more genes such as those encoding catalase-peroxidase *(katG)* (5-9). *KatG* gene is the most commonly targeted gene with the majority of mutations occurring in codon 315 in 30–90% of isoniazid-resistant strains depending on geographical distribution (10-13). The observation that most isoniazid-resistant *M. tuberculosis* strains did not have high numbers of *katG* deletions, suggested the need to more precisely analyze the

Correspondence to: Zaker Bostanabad S
Address: Pasteur Institute of Iran, Pasteur St., 13164, Tehran, Iran.
Email address: saeedzaker20@yahoo.com
Received: 10 Feb 2007
Accepted: 1 Sep 2007

structure of *katG* in resistant organisms. Further studies revealed that *katG* gene deletions are very rare (1, 12, 14, 15) and this requires more detailed analysis of its structure (1, 6, 9, 16). Several groups have recently reported that many isoniazid-resistant strains contain missense and other types of mutations (1, 4, 17, 18). Mutations at the Ser315 codon of *katG* have been reported to be associated with a high-level of isoniazid resistance (van Soolingen et al., 2003). Resistance to isoniazid has the second degree of magnitude in Belarus (19), and combinations of mutations conferring *M. tuberculosis* resistance to isoniazid have been reported to be more common in the multiple drug resistant (MDR) than in mono-resistant isolates, suggesting that isolates develop resistance to isoniazid by a stepwise accumulation of mutations (11, 16, 20, 21, 22).

Another frequent target is the regulatory region of the inhA gene, where mutations have been reported in up to 32% of INH-resistant isolates (15, 17, 23, 24). Mutations in other genomic regions, such as the promoter of ahpC gene and in the kasA gene, have been reported in 12–24% and 10–14% of INH-resistant strains, respectively (23, 25, 26). Genotypic assays that detect mutations within such regions are predictive of drug resistance and have the potential to provide rapid detection of resistance in mycobacterial isolates. During the past few years several genotypic assays have been developed for detection of the mutations responsible for INH resistance, particularly those at codon 315 of the katG gene (1, 6, 12, 17, 27). This work was to determine mutations in codons causing high level resistant to isoniazid in the gene of catalase–peroxidase MTB strains in Belarusians.

MATERIALS AND METHODS

Mycobacterial strains. One hundred sixty three MTB strains randomly isolated from sputum of patients with active pulmonary tuberculosis from different geographic regions of Belarus (Minsk 11, Mogilev 9, Gomel 3, Grodho 3, Brest 8, Vitebsk 8) from December 2004 to May 2005. All 163 tuberculosis patients had proven registration of clinical diagnostic exanimations, such as chest X-ray, PPD, cough, weight loss, etc. Patient sputum samples were cultured on Lowenstein– Jensen solid medium and colonies were identified to the species level using TCH (2-thiophene carboxylic acid) and PN99B (paranitrobenzoic acid) selective media or by standard biochemical procedures. Four sensitive isolates were used as negative control.

Susceptibility, methods and MICs. Drug susceptibility tests were performed using proportional method (isoniazid 1 μg/mL, rifampin 40 μg/mL, streptomycin 10 μg/mL, or ethambutol 2 μg/mL)with absolute concentration of antituberculosis agent on slants using H37RV strain as positive control and additionally using the BACTEC system . In the absolute concentration method, resistance was defined as growth on solid media containing graded concentrations of drugs with more than 20 CFU at a specific drug concentration. The breakpoints for isoniazid were 1, 2 and 10 μg/ml on Lowenstein-Jensen medium and 0.1 μg/ml on the BACTEC system and for rifampicin were 40.0 μg/ml on Lowenstein-Jensen medium and 2.0 μg/ml on the BACTEC system.

Standard PCR identification and *katG* gene amplification. DNA extraction was performed using the manufacturer's procedure Fermentas kits (K512). DNA extracted from a *standard* strain of *Mycobacterium H37RV* was used as a control. A 210 bp and 750bp segments of the *katG* gene was amplified by PCR using the following synthetic oligonucleotide primers *katG F* 5-GAAACAGCGGCGCTGGATCGT-3, *katG R* 5-GTTGTCCCATTTCGTCGGGG-3 for 210bp and *katG* F 5-CGGGATCCGCTGGAGCAGATGGGC-3 and *katG* R 5-

CGGAATTCCAGGGTGCGAATGACCT-3 for 750bp [14, 19]. The following thermocycler parameters were applied with initial denaturation at 95□C for 5 min; 36 cycles of denaturation at 94ºC for 1 min; primer annealing at 56ºC for 1 min; extension at 72ºC for 1 min; and a final extension at 72ºC for 10 min. The PCR product was amplified and purified again and controlled on gel electrophoresis. The final purified DNA obtained was used for sequencing.

DNA sequencing. The 209-bp and 750bp fragments of *katG* gene was amplified by PCR using forward and reverse primers; 33 cycles of denaturation at 94°C for 30sec; primer annealing at 48°C for 45 sec; extension at 60°C for 4 min. *katG* gene fragments were sequenced using the Amersham auto sequencer and Amersham Pharmacia DYEnamic ET Terminator Cycle Sequencing Premix Kits. The purified *katG* gene fragments (4 sensitive and standard strains of *Mycobacterium H37RV*) were sequenced and used as control.

Analyzing DNA sequencing. Alignment of the DNA fragments *(katG)* was carried out with the help of MEGA and DNAMAN software (Gen bank_ PUBMED/BLAST) and was compared with standard strains of CDC1551, H37RV and *M. tuberculosis 210*. The Blast 2 sequencing computer program was used for DNA sequence comparisons (http://www.ncbi.nlm.nih.gov/BLAST/). Alignment of the DNA fragments (*katG*) was carried out with the help of MEGA 3.1 software (www.megasoftware.net/mega3.1/) and obtained data were analyzed and edited with DNAMAN software.

RESULTS

Mycobacterial strains and susceptibility. From 163 isolates, 42 were identified as resistant to isoniazid (100%), rifampicin (90%), streptomycin (90%), and 8 (28%) to etambutol. Mono-resistance to isoniazid was observed in 4 isolates (9.5%). 42 isoniazid resistant, and 121 sensitive isolates were identified and four sensitive isolates were used as controls in the sequencing method.

Mutations were not detected for four sensitive isolates to isoniazid in 209 bp and 750 bp regions of the *katG* gene. Mutations were observed in affected codons 305, 306, 307, 309, 314, 315, 316, 321, 328 in 209 bp and in 357, 454, 463 of 750 bp fragments of *katG* gene. Six isolates (14%) were found to have multi-mutations in three codons (309,315 and 316), 26 (61.9%) demonstrated multi-mutations in all and or two of the three codons (309, 315, 316); and 8 (19%) were found to have single mutations in 315; 95% resistant isolates (n=40) demonstrated to have mutations in codon 315, whereas 40% of all mutations conferring different types were observed in codon 315: AGC→ACC (Ser→Thr) 36%, AGC→AGG (Ser→Arg) 0.9%, AGC→AAC (Ser→Asn) 1.8%, and AGC→GGC (Ser→Gly) 0.9%. Four types of mutations were detected in codon 309: GGT→GTT (Cys→Phe) 6.3%, GGT→GCT (Cys→Ser) 3.6%, GGT→GTC (Cys→Phe) 2.7%, GGT→GGG (Cys→Thr) 0.9% and one in 316 GGC→AGC (Gly→Ser) 14.4%.

In this study, 75% of all isolates demonstrated mutations in codons 309 (n=15, 34%) and 316 (n=18, 41.4%) which might represent the importance of mutations in isolates from Belarus. In 40 isolates four types of mutations were identified in codon 315: AGC→ACC (n=36)85%, AGC→AGG (n=1) 2.3%, AGC→AAC (n=2) 4.7%, AGC→GGC (n=1) 2.3%. One type of mutation was found in codon 316: GGC→AGC (n=18)41.4%, and in 15 isolates four types of mutations were demonstrated in codon 309: GGT→GGT (n=7)16.1%, GGT→GCT (n=4)9.2%, GGT→GTC (n=3)6.9%, GGT→GGG (n=1)2.7% (Tables 1 and 2).

Table 1. Frequency of amino acid and nucleotide changes of different codons in katG gene of 42 isoniazid-resistant strains of M. Tuberculosis isolated in Belarus

Codon	Frequency	Amino acid change	Nucleotide change	Isolates
			1 Mutation	
		Ser→Thr	AGC→ACC	489, 446, 94, 85, 894, 932
315	9	Ser→Asn	AGC→AAC	471
		Ser→Gly	AGC→GGC	446 (MDR)
		Ser→Arg	AGC→AGG	457
		2 Mutations		
305	1	Gly→Ala	GGC→GCC	411
315		Ser→Thr	AGC→ACC	
309	3	Gly→Cys, Phe, Ala	GGT→GTT, GTC, GCT	455, 2331, 469
315		Ser→Thr	AGC→ACC	
314	1	Thr→Asn	ACC→AAC	118 (MDR)
315		Ser→Thr	AGC→ACC	
311	2	Asn→Phe, Tyr	GAC→TTC, TAC	414, 384 (MDR)
315		Ser→Thr	AGC→ACC	
315	8	Ser→Thr, Arg	AGC→ACC, AGG	TUB2, 3255, 24276, 443, 571, 3246, 2262 (MDR), 23623 (MDR)
316		Gly→Ser	GGC→AGC	
357	1	Asp→His	GAC→CAC	402
463		Arg→Leu	CGG→CTG	
357	1	Asp→Asn	GAC→AAC	74
454		Glu→Arg	GAG→CGA	
			3 Mutations	
309		Gly→Ala	GGT→GCT	
311	1	Asn→Phe	GAC→TTC	139
315		Ser→Thr	AGC→ACC	
307		Gly→Arg	GGA→CGA	
309	1	Gly→Gly	GGT→GGG	2331-2 (MDR)
315		Ser→Thr	AGC→ACC	
305		Gly→Ala	GGC→GCC	
315	1	Ser→Thr	AGC→ACC	7285
321		Trp→Leu	TGG→TTG	
305		Gly→Ala	GGC→GCC	
309	1	Gly→Ser	GGT→GCT	1416 (MDR)
315		Ser→Thr	AGC→ACC	
315		Ser→Thr	AGC→ACC	
316	1	Gly→Ser	GGC→AGC	2738
328		Trp→Cys	TGG→TGT	
315		Ser→Thr	AGC→AAC	
316	2	Gly→Ser	GGC→AGC	407, 412
321		Trp→Ser, STOP	TGG→TCC, TAG	
309		Gly→Cys	GGT→GTT	
315	2	Ser→Thr	AGC→ACC	139-2, 447 (MDR)
316		Gly→Ser	GGC→AGC	
			4 Mutation	
309		Gly→Cys	GGT→GTT	
311	2	Asp→Phe	GAC→TTC	369, 370 (MDR)
315		Ser→Thr	AGC→ACC	
316		Gly→Ser	GGC→AGC	
309		Gly→Phe	GGT→GTC	
315	1	Ser→Thr	AGC→ACC	1217
316		Gly→Ser	GGC→AGC	
328		Trp→Cys	TGG→TGT	
309		Gly→Cys	GGT→GTT	
315	1	Ser→Thr	AGC→ACC	453 (MDR)
321		Trp→Leu	TGG→TTG	
328		Trp→Cys	TGG→TGT	
			5 Mutation	
309		Gly→Phe	GGT→GTC	
311		Asp→Tyr	GAC→TAC	
315	1	Ser→Thr	AGC→ACC	2715 (MDR)
316		Gly→Ser	GGC→AGC	
321		Trp→STOP	TGG→TAG	
307		Gly→Ala	GGA→GCA	
309		Gly→Ser	GGT→GCT	
311	2	Asp→Tyr	GAC→TAC	388, 368 (MDR)
314		Thr→Thr	ACC→ACG	
315		Ser→Thr	AGC→ACC	
306		Pro→Pro	CCG→CCC	
315		Ser→Thr	AGC→ACC	
316	1	Gly→Ser	GGC→AGC	2831 (MDR)
321		Trp→Cys	TGG→TGT	
328		Trp→Cys	TGG→TGT	

Table 2. Correlation of mutations with high-level resistance, nucleotide and amino acid changes among 42 isoniazid resistant isolates of M. tuberculosis collected from tuberculosis patients in Belarus

Isolates	Mutation	µg/ml	Nucleotide change	Amino acid change
402	357, 463	< 2	GAC→CAC, CGG→CTG	Asp→His, Arg→Leu
74	357, 454		GAC→AAC, GAG→CGA	Asp→Asn, Glu→Arg
2331	309, 315	< 5	GGT→GTC, AGC→ACC	Gly→Phe, Ser→Thr
24276,23623, tub2, 3255, 443, 571, 3246,2262	315, 316		AGC→ACC or AGG, GGC→AGC	Ser→Thr or Arg, Gly→Ser
118	314, 315		ACC→AAC, AGC→ACC	Thr→Asn, Ser→Thr
7285	305, 315, 321		GGC→GCC, AGC→ACC TGG→TTG	Gly→Ala, Ser→Thr Trp→Leu
139	309, 311, 315		GGT→GCT, GAC→TTC AGC→ACC	Gly→Ala, Asn→Phe Ser→Thr
369, 370	309, 311, 315, 316		GGT→GTT, GAC→TTC AGC→ACC, GGC→AGC	Gly→Val, Asp→Phe Ser→Thr, Gly→Ser
94, 932, 489, 85, 894	315	< 10	AGC→ACC	Ser→Thr
457	315		AGC→AGG	Ser→Arg
446	315		AGC→GGC	Ser→Gly
471	315		AGC→AAC	Ser→ Asn
384	311, 315		GAC→TAC or TTC, AGC→ACC	Asp→Tyr or Phe, Ser→Thr
455, 2331, 469	309, 315		GGT→GTT, AGC→AAC	Gly→Val, Ser→Asn
2331-2	307, 309, 315		GGA→CGA, GGT→GGG AGC→ACC	Gly→Arg, Gly→Gly Ser→Thr
1416	305, 309, 315		GGC→GCC, GGT→GCT AGC→ACC	Gly→Ala, Gly→Ala Ser→Thr
2738	315, 316, 328		AGC→ACC, GGC→AGC TGG→TGT	Ser→Thr, Gly→Ser, Trp→Cys
139-2, 447	309, 315, 316		GGT→GTT, AGC→ACC, GGC→AGC	Gly→Cys, Ser→Thr Gly→Ser
411	305, 315		GGC→GCC, AGC→ACC	Gly→Ala, Ser→Thr
407, 412	315, 316, 321		AGC→AAC, GGC→AGC TGG→TAG, TCC	Ser→Asn, Gly→Ser Trp→STOP, Ser
1217	309, 315, 316, 328		GGT→GTC, AGC→ACC GGC→AGC, TGG→TGT	Gly→Phe, Ser→Thr Gly→Ser, Trp→Cys
453	309, 315, 321, 328		GGT→GTT, AGC→ACC TGG→TTG, TGG→TGT	Gly→Cys, Ser→Thr Trp→Leu, Trp→Cys
2715	309, 311, 315, 316, 321		GGT→GTC, GAC→TAC AGC→ACC, GGC→AGC TGG→TAG	Gly→Val, Asp→Tyr Ser→Thr, Gly→Ser Trp→STOP
388, 368	307, 309, 311, 314, 315		GGA→GCA, GGT→GCT GAC→TAC, ACC→ACG AGC→ACC	Gly→Alal, Gly→Ser Asp→Tyr, Thr→Thr Ser→Thr
2831	306, 315, 316, 321, 328		CCG→CCC, AGC→ACC GGC→AGC, TGG→TGT TGG→TGT	Pro→ Pro Ser→Thr, Gly→Ser, Trp→Cys Trp→Cys

Table 3. Strains isolated from patients with primary and secondary tuberculosis from different regions of Belarus

Patients with primary tuberculosis	Patients with secondary tuberculosis
Amount of isolates (%)	Amount of isolates (%)
10 (24%)	32 (76%)
Isolates numbers	Isolates numbers
118, 388,446,369,447, 1217,2715,370,139,368	74,932,384,2331-2, 23623,407,443,tub2,489,139-2,469, 2262,414,85,411,2831,2458,7285,1416, 2331,453 24276,442,455,412,402,94,894,3255,3246,571,471

In two isolates 2 types of mutations were found in codon 357 GAC→CAC and GAC→AAC, in addition to two mutations which were observed in codons 463 CGG→CTG and 454 GAG→CGA. Nine isolates bearing single mutations 19%, 17 – double mutations 40.46%, 9 – three mutations 21.42%, four – four mutations 9.5% and 4 – five mutations 9.5% were also observed in all 42 resistant isolates (Table 2).

Mutations in codon 315, 309 and 316 were found to have high resistance to izoniazid (>10 μg/ml). Mutations in codons 357, 454, 463 and other codons have not demonstrated to have high resistance to isoniazid (1–2 μg/ml, Table 1). The 40 isolates revealed to have high level of resistant to isoniazid (>5-10 μg/ml) 95% (n=40) demonstrated to have mutations located in codons 315, 309 (34%), and 316 (41.4%, Table 1).

Silent mutations. Three silent mutations were identified in four isolates in codons 306 (CCG→CCC), 309 (GGT→GGG) and 314 (ACC→ACG). These silent mutations revealed to have no effect on the susceptibility testing pattern (Tables 1 and 2).

DISCUSSION

The majority of hot mutations in *katG* gene of *Mycobacterium tuberculosis* have been reported in codon 315 (Ser →Thr) and less in other codons (3, 8, 10, 14). Unlike most resistance-conferring mutations, codon 315 (Ser →Thr) was found to result with near-normal catalase-proxidase activities and somewhat higher levels of virulence conferring resistance to isoniazid. This mechanism of isoniazid resistance is not usually associated with a large reduction in virulence and is an exception to the rule that antibiotic-resistance conferring mutation carry a significant cost (11, 25).

The known genes related to INH-r are katG, inhA, ahpC, and kasA (14, 17, 23). Different articles reported resistance of *M. tuberculosis* to isoniazid corresponding to changes in codon 315 (5, 10, 11, 12, 14, 21). The most common mutations reported by other authors are in codon 315 (AGC→ACC (Thr→Ser)) and 463 (CGG→CTG (Arg→Leu)) (5, 10, 11, 12, 14, 21). We observed different types of mutations: four in codons 315: AGC→ACC (Ser→Thr) 36%, AGC→AGG (Ser→Arg) 0.9%, AGC→AAC (Ser→Asn) 1.8 %, AGC→GGC (Ser→Gly) 0.9% (Table 2), one in 463 CGG→CTG (Arg→Leu) 0.9%, four in 309: GGT→GTT (Cys→Phe) 6.3%, GGT→GCT (Cys→Ser) 3.6%, GGT→GTC (Cys→Phe) 2.7%, GGT→GGG (Cys→Thr) 0.9% and one in 316 GGC→AGC (Gly→Ser) 14.4%. Our data indicate that the highest number of mutations are observed in 1: 315AGC→ACC (Ser→Thr) 36%, 2 and 316 GGC→AGC (Gly→Ser) 14.4%. Similar results were reported in Latvia in which mutations in codon 315 of katG 95% corresponding to AGC→ACC (Ser→Thr) (90%) that resemble our finding but AGC→ACA (Ser→Thr) (10%) were not observed in our study(2). In Poland 90% of mutations were in codon 315AGC which corresponds to 5 types of mutations (ACC, ACT, ACA, AAC, ATC) resembling similar patterns of changes with our data on nucleotide ACC and AAC. However in this research we did not observe nucleotide changes to ACT, ACA and ATC among Belarusian strains studied (28). In Russia 70% of reported nucleotide changes were in codons 315 AGC→ACC and in 463 CGC→CTG which also shows similarity to our data (12, 13, 14). Other mutations found in Belarusian strains can be important and effective causes of resistance because 60% of strains showed mutation in codons 316 and 309. Mutations detected in one strain in codons 463 and 357, 454 and 357, may indicate that this type of mutation in Belarus has less degree of concern when compared with neighbouring countries (1, 12, 13, 16, 21). Other studies mentioned

that silent mutations were never detected in katG (9, 17, 23, 28). However, in this research, two silent mutations were obtained which indicate no effect on drug resistant pattern. We sequenced katG and rpoB genes of a standard strain H37RV used as control. But nonsense mutations were not observed in the control strain. We also conclude that sequencing is more sensitive for determination of mutations among M.D.R. tuberculosis strains when compared with other methods such as spilotyping, SSCP and PCR–RFLP(24, 25).

The high percentage of double mutations found among the isolates in Belarus differed clearly from the lower prevalence of double mutations in other studies. A characteristic prominent finding of this study was the high frequency of double (40.47%) and triple (21.42%), quartile (9.5%) and five mutations (9.5%) occurring in separate codons (Table 2). Were detected 2 (1.9%) nonsense mutations in 2 different (codon 306 and codon 309) isolates which demonstrated no effect in drug resistant patterns to isoniazid (Table 1). All isoniazid resistance isolates studied had mutations of different types in katG region. However, we don't have any knowledge of which combination of specific types of mutations are associated with isoniazid resistance. Thirty-two (76%) were isolated from secondary cases (we don't have data on reactivation cases). Interestingly, all single and double mutations which were found in isolates of sputum samples taken from patients with secondary infection. From 42 isoniazid resistance isolates 10 (24%, Table 3) were from sputum of patients with primary infection which consisted of different types of mutations, in 4 isolates we found five mutations, in 4 isolates quartile and in 2 isolates triple. All sputum of patients with primary infection were detected mutation in codon 315. In 10 (24%) of patients with primary infection there were predominant mutations in codon 315, in 5(11.9%) in codon 316 and in 9 in codon 309. For patients with secondary infection 32 (76%) we found mutations in codon 315, 12(19%) in codon 316 and 7(11.1%) in codon 309 (Table 3). Isolates of *M. tuberculosis* isolated in Belarus had a wide spectrum of the important mutations and might belong to the epidemic of widespread clones.

Belarus had the highest frequency of common mutation shearing in primary and secondary infections occurring in codon 315.

Acknowledgment

We thank our colleagues in Belarusian Institutes of Pulmonology and Tuberculosis for sample collections.

REFERENCES

1. Abate G, Hoffner SE, Thomsen VO, Miörner H. Characterization of isoniazid-resistant strains of Mycobacterium tuberculosis on the basis of phenotypic properties and mutations in katG. ***Eur J Clin Microbiol Infect Dis*** 2001; 20 (5): 329- 33.
2. Bakonyte D, Baranauskaite A, Cicenaite J, Sosnovskaja A, Stakenas P. Mutations in the rpoB gene of rifampicin-resistant Mycobacterium tuberculosis clinical isolates from Lithuania. ***Int J Tuberc Lung Dis*** 2005; 9 (8): 936- 8.
3. Young DB. Ten years of research progress and what's to come. ***Tuberculosis (Edinb)*** 2003; 83 (1-3): 77- 81.
4. Espinal MA. The global situation of MDR-TB. ***Tuberculosis (Edinb)*** 2003; 83 (1-3): 44- 51.
5. Herrera-León L, Molina T, Saíz P, Sáez-Nieto JA, Jiménez MS. New multiplex PCR for rapid detection of isoniazid-resistant Mycobacterium tuberculosis clinical isolates. ***Antimicrob Agents Chemother*** 2005; 49 (1): 144- 7.
6. Mokrousov I, Otten T, Filipenko M, Vyazovaya A, Chrapov E, Limeschenko E, et al. Detection of isoniazid-resistant Mycobacterium tuberculosis strains by a multiplex allele-specific PCR assay targeting katG codon 315 variation. ***J Clin Microbiol*** 2002; 40 (7): 2509- 12.
7. Eltringham IJ, Drobniewski FA, Mangan JA, Butcher PD, Wilson SM. Evaluation of reverse transcription-PCR and a

bacteriophage-based assay for rapid phenotypic detection of rifampin resistance in clinical isolates of Mycobacterium tuberculosis. ***J Clin Microbiol*** 1999; 37 (11): 3524- 7.

8. Kim JY, Mukherjee JS, Rich ML, Mate K, Bayona J, Becerra MC. From multidrug-resistant tuberculosis to DOTS expansion and beyond: making the most of a paradigm shift. ***Tuberculosis (Edinb)*** 2003; 83 (1-3): 59-65.
9. Kim BJ, Hong SK, Lee KH, Yun YJ, Kim EC, Park YG, et al. Differential identification of Mycobacterium tuberculosis complex and nontuberculous mycobacteria by duplex PCR assay using the RNA polymerase gene (rpoB). ***J Clin Microbiol*** 2004; 42 (3): 1308- 12.
10. van Soolingen D, de Haas PE, van Doorn HR, Kuijper E, Rinder H, Borgdorff MW. Mutations at amino acid position 315 of the katG gene are associated with high-level resistance to isoniazid, other drug resistance, and successful transmission of Mycobacterium tuberculosis in the Netherlands. ***J Infect Dis*** 2000; 182 (6): 1788-90.
11. Leung ET, Kam KM, Chiu A, Ho PL, Seto WH, Yuen KY, et al. Detection of katG Ser315Thr substitution in respiratory specimens from patients with isoniazid-resistant Mycobacterium tuberculosis using PCR-RFLP. ***J Med Microbiol*** 2003; 52 (Pt 11): 999- 1003.
12. Mokrousov I, Narvskaya O, Otten T, Limeschenko E, Steklova L, Vyshnevskiy B. High prevalence of KatG Ser315Thr substitution among isoniazid-resistant Mycobacterium tuberculosis clinical isolates from northwestern Russia, 1996 to 2001. ***Antimicrob Agents Chemother*** 2002; 46 (5): 1417- 24.
13. Mokrousov I, Otten T, Filipenko M, Vyazovaya A, Chrapov E, Limeschenko E, et al. Detection of isoniazid-resistant Mycobacterium tuberculosis strains by a multiplex allele-specific PCR assay targeting katG codon 315 variation. ***J Clin Microbiol*** 2002; 40 (7): 2509- 12.
14. Zheltkova EA, Chernousova LN, Smirnova TG, Andreevskaia SN, Yates M, Drobniewskiĭ F. Molecular genotyping of Mycobacterium tuberculosis strains isolated from patients in the Samara region by the restriction DNA fragment length polymorphism. ***Zh Mikrobiol Epidemiol Immunobiol*** 2004; (5): 39- 43.
15. Hall L, Doerr KA, Wohlfiel SL, Roberts GD. Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. ***J Clin Microbiol*** 2003; 41 (4): 1447- 53.
16. Telenti A, Honoré N, Bernasconi C, March J, Ortega A, Heym B, et al. Genotypic assessment of isoniazid and rifampin resistance in Mycobacterium tuberculosis: a blind study at reference laboratory level. ***J Clin Microbiol*** 1997; 35 (3): 719- 23.
17. Kiepiela P, Bishop KS, Smith AN, Roux L, York DF. Genomic mutations in the katG, inhA and aphC genes are useful for the prediction of isoniazid resistance in Mycobacterium tuberculosis isolates from Kwazulu Natal, South Africa. ***Tuber Lung Dis*** 2000; 80 (1): 47- 56.
18. Narvskaia OV, Mokrousov IV, Otten TF, Vishnevskiĭ BI. Genetic marking of polyresistant mycobacterium tuberculosis strains isolated in the north-west of Russia. ***Probl Tuberk*** 1999; (3): 39- 41.
19. Titov L.P., Problems of new and recently appeared infections of present time. 1997. International scientific conference, Minsk, Belarus, May, 26-27.4–10 (in Russian).
20. Sajduda A, Brzostek A, Poplawska M, Augustynowicz-Kopec E, Zwolska Z, Niemann S, et al. Molecular characterization of rifampin- and isoniazid-resistant Mycobacterium tuberculosis strains isolated in Poland. ***J Clin Microbiol*** 2004; 42 (6): 2425- 31.
21. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, et al. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. ***Lancet*** 1993; 341 (8846): 647- 50.
22. van Doorn HR, Claas EC, Templeton KE, van der Zanden AG, te Koppele Vije A, de Jong MD, et al. Detection of a point mutation associated with high-level isoniazid resistance in Mycobacterium tuberculosis by using real-time PCR technology with 3'-minor groove binder-DNA probes. ***J Clin Microbiol*** 2003; 41 (10): 4630- 5.
23. Silva MS, Senna SG, Ribeiro MO, Valim AR, Telles MA, Kritski A, et al. Mutations in katG, inhA, and ahpC genes

of Brazilian isoniazid-resistant isolates of Mycobacterium tuberculosis. ***J Clin Microbiol*** 2003; 41 (9): 4471- 4.

24. Bobadilla-del-Valle M, Ponce-de-Leon A, Arenas-Huertero C, Vargas-Alarcon G, Kato-Maeda M, Small PM. rpoB Gene mutations in rifampin-resistant Mycobacterium tuberculosis identified by polymerase chain reaction single-stranded conformational polymorphism. ***Emerg Infect Dis*** 2001; 7 (6): 1010- 3.
25. Kurabachew M, Enger Ø, Sandaa RA, Lemma E, Bjorvatn B. Amplified ribosomal DNA restriction analysis in the differentiation of related species of mycobacteria. ***J Microbiol Methods*** 2003; 55 (1): 83- 90.
26. Brennan PJ. Structure, function, and biogenesis of the cell wall of Mycobacterium tuberculosis. ***Tuberculosis (Edinb)*** 2003; 83 (1-3): 91- 7.
27. García M, Vargas JA, Castejón R, Navas E, Durantez A. Flow-cytometric assessment of lymphocyte cytokine production in tuberculosis. ***Tuberculosis (Edinb)*** 2002; 82 (1): 37- 41.
28. Sajduda A, Brzostek A, Poplawska M, Augustynowicz-Kopec E, Zwolska Z, Niemann S, et al. Molecular characterization of rifampin- and isoniazid-resistant Mycobacterium tuberculosis strains isolated in Poland. ***J Clin Microbiol*** 2004; 42 (6): 2425- 31.