ISONIAZID-MONORESISTANT TUBERCULOSIS IN BULGARIA

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ABSTRACT

Background. Isoniazid is a common drug in the treatment regimens for TB infection. Mono-isoniazid resistance reduces the probability of a successful treatment outcome and increases the risk of acquiring additional drug resistance.

Material and methods. For the period 2015-2016 a total of 36 TB cases were confirmed in NRL TB, Sofia, as isoniazid-monoresistant *Mycobacterium tuberculosis* complex. Minimum inhibitory concentration testing for isoniazid was conducted with BACTEC MGIT 960 System in the following concentrations of the drug: 0.1 μg/ml, 0.15μg/ml, 0.2μg/ml, 0.3μg/ml, 0.4μg/ml. Molecular testing was performed with GenoType® MTBDR*plus* in order to detect the most common mutations associated with resistance to isoniazid.

Results. Only 25% of the tested *M. tuberculosis* complex isolates with phenotypic isoniazid monoresistance had the S315T1 mutation in *katG*; all isolates were with MIC over 0.4 μg/ml. C15T in the promoter region of *inhA* was detected in 22.22% of cases and only 1 of them showed MIC below 0.4 μg/ml. No mutations were detected in nearly half of the cases (n=19, 52.78%) and most of these isolates were with lower MIC values (n=12).

Conclusion. The rapid testing with GenoType® MTBDR*plus* can be used as a screening procedure indicating whether further examination of isoniazid MIC is relevant in resistant *M. tuberculosis* and whether higher doses could be considered.

KEYWORDS:

tuberculosis, isoniazid, resistance

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INTRODUCTION

Isoniazid (isonicotinylhydrazide) is a common drug in the treatment regimens for active and latent tuberculosis (TB) infection. It is estimated that the highest burden of isoniazid resistance is in the Eastern European region of the World Health Organisation (WHO) – nearly half of the TB cases, while outside the region one in seven incident TB cases has resistance to isoniazid (1).

Isoniazid performs its activity only in metabolically active bacterial cells. It requires intracellular activation by the KatG peroxidase (encoded by the *katG* gene) in order to inhibit the mycolic acid synthesis via the NADH-dependent enoyl-acyl carrier protein reductase (ACP) encoded by *inhA* (2). The most common molecular mechanisms of isoniazid resistance are related to mutations in *katG*, *inhA* or its promoter. The most frequent mutation in *katG* is S315T1 (Ser → Thr) which results in insufficient activation of the drug and is associated with a high level of resistance (MIC> 1 μg/ml). Mutations in the *inhA* gene or its promoter region lead to InhA overexpression and are most commonly associated with low level of resistance (MIC <1 μg/ml). The most frequent mutation in the promoter is at position -15C/T (Cys→Thr) (2).

The level of phenotypic isoniazid resistance cannot be predicted only from results of testing with GenoType® MTBDR*plus* because mutations outside the tested regions can elevate minimum inhibitory concentration (MIC) values.

Mono-isoniazid resistance reduces the probability of a successful treatment outcome and increases the risk of acquiring additional drug resistance (1).

The aim of this retrospective study was to describe isoniazid-monoresistant clinical strains isolated

in Bulgaria in the period 2015-2016 and further tested in the National Reference Laboratory for Tuberculosis (NRL TB) at the National Centre of Infectious and Parasitic Diseases (NCIPD).

MATERIAL AND METHODS

Patients and clinical isolates: 36 TB cases were confirmed in NRL TB as isoniazid-monoresistant *Mycobacterium tuberculosis* complex. Polyresistant or multidrug-resistant strains were not in the scope of the study. All TB cases were mapped according to the official address registration and each patient was represented by a single strain.

Identification of the isolates as *M. tuberculosis* complex was carried out by BD MGIT™ TBc Identification Test.

Drug susceptibility testing was performed with BACTEC MGIT 960 System to first-line drugs as follows: rifampin (RMP) – 1.0 μg/ml, isoniazid (INH) – 0.1 μg/ml, ethambutol (EMB) – 5.0 μg/ml and streptomycin (STR) – 1.0 μg/ml. Minimum inhibitory concentration testing for isoniazid was conducted in the same conditions with the following concentrations: 0.1 μg/ml (the official critical concentration of the drug), 0.15μg/ml, 0.2μg/ml, 0.3μg/ml, 0.4μg/ml.

Molecular testing was performed with GenoType®MTBDR*plus* in order to detect the most common mutations associated with resistance to isoniazid.

RESULTS

As isoniazid-monoresistant were identified 36 clinical isolates of *M. tuberculosis*; 21 of them were isolated in 2015 and 15 in 2016. Most of the patients were male – 80.56% (n=29) and 19.44% (n=7) were female. In 1 of the cases there was a co-infection with HIV. Ten of the patients were previously treated for tuberculosis and the other 26 were new cases.

Figure 1. Distribution of 36 TB cases with phenotypic isoniazid monoresistance, Bulgaria 2015-2016.

The distribution of the isoniazid-monoresistant TB cases in the country showed higher occurrence in the boundary districts, mainly at the seaside – 38.9% (n=14) of the cases were from Dobrich, Varna and Burgas (Fig. 1). Two of the patients were prisoners – one in Varna, the other one in Sofia.

Table 1. GenoType® MTBDR*plus* results of 36 TB cases with phenotypic isoniazid monoresistance, Bulgaria, 2015-2016.

Mutation		Frequency	
katG	***inhA***	**n**	**%**
S315T1		9	25
S315T2		0	0
	C15T	8	22.22
	A16G	0	0
	T8C	0	0
	T8A	0	0
S315T1	C15T	0	0
WT1	WT 1-2	19	52.78

Among the 36 *M. tuberculosis* isolates with phenotypic isoniazid monoresistance 25% (n=9) had the S315T1 mutation in *katG*. C15T in the promoter region of *inhA* was found in 22.22% of the tested strains. In 52.78% (n=19) no mutations were detected with GenoType® MTBDR*plus*. Co-occurrence of S315T1 and C15T was not found among the tested strains (Table 1).

As expected, all strains harbouring the *katG* S315T1 mutation showed MIC over 0.4 µg/ml. Most of the strains with C15T mutation in the promoter of *inhA* had MIC over 0.4 µg/ml (n=7) and only 1 had MIC below 0.4 µg/ml (Table 2). The majority of strains with mutations in other genes (those without detected mutations using the molecular assay) showed lower MIC values (n=12) and one-third of them were with MIC>0.4 µg/ml.

Table 2. Isoniazid MIC values and detection of mutations with GenoType® MTBDR*plus* in 36 TB cases with phenotypic isoniazid monoresistance, Bulgaria, 2015-2016.

n	%	INH MIC	Detected mutation, n	
22	61.1	> 0.4µg/ml	S315T1	9
			C15T	7
			no mutation detected	6
2	5.6	0.2µg/ml	C15T	1
			no mutation detected	1
11	30.5	0.15µg/ml	no mutation detected	11
1	2.8	not valid	no mutation detected	1

DISCUSSION

This retrospective study on isoniazid-monoresistant clinical strains isolated in Bulgaria for the period 2015-2016 has some noteworthy findings. Out of 131 cases resistant to any anti-TB drug 27.48% (n=36) were confirmed in NRL TB as isoniazid-monoresistant (3, 4). Most of the patients were newly diagnosed. The distribution of cases was mainly in the boundary districts.

Although the mutation S315T1 in *katG* is well-known as the leading cause of isoniazid resistance in 42-95% of the cases globally, in our study it was detected in only 25% of the examined strains (5). When comparing its frequency in Bulgarian isolates with different level of resistance we found that S315T1 occurs in 50% of the XDR-TB and is insignificantly represented in MDR with fluoroquinolone resistance (Table 3).

The mutation C15T in the promoter region of *inhA* was detected in only 22.22% of the tested strains although it is very common among MDR-TB strains in Bulgaria (6, 7, 8, 9) (Table 3).

As expected, co-occurrence of S315T1 and C15T was not detected among the isoniazid-monoresistant strains. So far, we have found these mutations simultaniously only in multidrug-resistant Beijing strains (7, 8, 9).

The most unexpected result of the study was that in 52.77% of the tested isolates no mutations were detected using GenoType® MTBDR*plus*. The resistance can be explained with mutations in many other loci or genes (*furA-katG, fabG1-inhA, ahpC-oxyR* intergenic region, *efpA, fadE24, iniA, iniB, iniC, kasA, nat, ndh)* (5, 10).

Table 3. Frequency of S315T1 and C15T mutations in Bulgarian clinical isolates of *Mycobacterium tuberculosis* with different types of resistance.

TB resistance	S315T1 %	C15T %	Sensitive by GenoType® MTBDR*plus*	Clinical isolates, n
Mono-isoniazid resistance	25	22.22	52.78	36
MDR (7)	27.92	50.45	19.82	222
MDR with additional fluoroquinolone resistance (8)	11.54	80.77	0	26
XDR-TB (9)	50	55.56	5.55	18

The rapid testing with GenoType® MTBDR*plus* can exclude the possibility of a therapeutic effect of isoniazid in high doses in each TB case (if the presence of S315T1 in *katG* is detected). In all other cases isoniazid MIC testing in resistant *M. tuberculosis* strains is suitable and higher doses could be considered (16 - 20 mg/kg body weight per day) (11).

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