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STUDY OF DRUG-RESISTANT TUBERCULOSIS IN KHARKIV REGION, UKRAINE

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Summary. A total of 93 isolates from pulmonary tuberculosis (TB) patients in Kharkiv region were characterized by drug susceptibility testing and genotypic analyses (VNTR using five exact tandem repeat loci). Obtained data demonstrated that the Beijing genotype was the most common (in 64 cases) among studied strains. Twelve isolates had individual VNTR profile. Among all patients 41 (44%) were diagnosed as MDR TB and 52 (56%) — TB with extending resistance. The most common resistance of *Mycobacterium tuberculosis* was observed to streptomycin. The frequency of resistance to kanamycin, 4-aminosalicylic acid and ethionamide was revealed significantly higher for LAM strain in compare to Beijing strain ($p<0.05$).

Keywords: tuberculosis, *Mycobacterium tuberculosis*, strains, Kharkiv region, Ukraine, resistance, PCR

Introduction. Today tuberculosis (TB) is a major public health and social problem not only in Ukraine but also all over the world (WHO, 2014). Drug resistance of *Mycobacterium tuberculosis* is one of the main factors limiting the effectiveness of TB treatment.

On the background of gradual decrease of epidemic indicators at the present stage there is the risk of the multidrug-resistant tuberculosis (MDR TB) spreading in Ukraine (ed. Tolstanov, 2015).

MDR TB is the form of tuberculosis when isolated mycobacteria have resistance to at least isoniazid and rifampin or often to more anti-TB drugs from I and II groups, which could be confirmed by laboratory method to the drug sensitivity test (MHU, 2014).

According to the WHO data in Ukraine MDR TB was found in 16 % of initially diagnosed patients and 44% of previously treated TB patients. About 8 % of newly diagnosed patients interrupt treatment and the death rate is up to 12 % (ed. Tolstanov, 2015).

The number of MDR TB cases has increased almost in three times last 10 years due to the implement of modern methods of molecular diagnostics. From the total number of patients with MDR TB 13% of them were registered with extending resistance to anti-TB drugs (Feshchenko and Melnyk, 2013).

Development of molecular genetics gave opportunities to carry out genetic typing of *M. tuberculosis*, which allows distinguishing between strains of the pathogen and determining their role in the further clinical course. The genetic families or clades of *M. tuberculosis* complex have been identified in different geographic regions (Beijing, East African Indian (EAI), Central Asian (CAS), T, Haarlem I, X and Latin American Mediterranean (LAM) families) as well as unidentified strains and other widely distributed and maintained epidemiologic TB at a high level. In a number of observations it found that the in difficult cases and non-effective treatment often associated with strains from Beijing family (Liashenko, 2015). This family genotype is also common in the other countries of the former Soviet Union (Lillebaek et al., 2003).

In this article, we report the analysis of 93 isolates of *M. tuberculosis* from MDR-TB patients collected during 2014–2015.

The aim of the study was to estimate resistant of different *M. tuberculosis* strains that were isolated from MDR TB patients in Kharkiv region.

Materials and methods. In the period between September 2014 and September 2015 the 93 cases of TB were studied in patients who were treated in the hospitals in Kharkiv region in Ukraine. Patients were selected by blind method.

Mycobacterium identification and testing of the drug susceptibility of these strains to four first-line anti-TB drugs (isoniazid, rifampin, ethambutol, pyrazinamide and streptomycin) and two second-line anti-TB drugs (kanamycin, amikacin, capreomycin, ofloxacin, levofloxacin, moxifloxacin, prothionamide, 4-aminosalicylic acid, cycloserine and ethionamide) were performed as recommended by WHO (WHO, 2014). The samples of expectoration were used for the strain isolation on Lowenstein-Jensen medium. A strain was referred resistant to the specific drug when the growth rate exceeded 1% compared to the control.

All obtained strains were inactivated by heating and used for DNA extraction by commercial kit 'Diatom DNA Prep 200' (Ukraine) according to the manufacture instruction. PCR was performed using 'GenPak PCR Core' (Russian Federation). VNTR genotyping was done by using sets of primers for amplification of five exact tandem repeat ETR loci (A, B, C, D, E) as previously described (Frothingham and Meeker-O'Connell, 1998;

Liashenko, 2015). PCR was carried out in a total volume of 25 μL using 5 μL of template DNA. PCR protocol included an initial denaturation of 5 min at 95 °C that was followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 62 °C for 1 min and extension at 72 °C for 25 s. A final extension was at 72 °C for 5 min. Obtained amplicons were analyzed in 1.8% agarose gel after electrophoresis followed by staining with ethidium bromide.

Statistical analysis was done using Excel 2007 package soft (Microsoft, license № RW2FR-7DFDD-TCF8J-9K9BJ-MJ678).

Results and discussion. Among 93 patients 80% were men (n=74) and 20% women (n=19). Age of the patients was between 23 and 84 years. Average age was 49±1.2 years.

All patients were observed by clinical, radiological and laboratory examination in accordance with Decree № 620 of Ministry of Health of Ukraine (MHU, 2014). There was widespread pulmonary tuberculosis in all patients with massive recovery of mycobacteria, which was confirmed by culture and smear.

As the first stage of our work we provided VNTR analysis by 5 ETR loci. It was determined the variability of each locus by PCR from all 93 strains. The results of VNTR-genotyping were shown that the most common profile was 42 435 that belong to the Beijing genotype (69%). It was demonstrated genotype LAM (13%), Africanum (3%) and Harlem (2%) (Fig. 1). This data correlated with previously studies in Kharkiv region by Dymova et al. (2011). Twelve strains were not identified by reason of unique VNTR profiles.

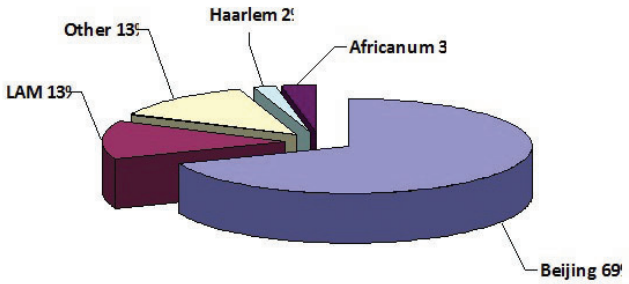

Figure 1. Prevalence of different genotypes among 93 *M. tuberculosis* isolates in Kharkiv region TB surveillance.

All isolates were tested for drug resistance using liquid and solid media. The results of resistant to the first-line anti-TB were shown in Table 1.

We observed the most common resistance to streptomycin (100%), followed by resistance to isoniazid (99%), rifampin (96%) and ethambutol (89%). There were no significant statistical differences in resistance frequency to the first-line anti-TB drugs among *M. tuberculosis* strains (p>0.05). Our findings showed that 26 patients with active TB could transmit MDR mycobacterium from Beijing clade that is stable to all first-line anti-TB drugs. All isolates were tested as well to the second-line anti-TB drugs (Table 2).

Table 1 – Resistant of *M. tuberculosis* isolates to the first-line anti-TB drugs

Resistance to	*M. tuberculosis* strains					
	Beijing (n=64)	LAM (n=12)	Haarlem (n=2)	Africanum (n=3)	Other (n=12)	Total (n=93)
Isoniazid (H)	63 (98%)	12 (100%)	2 (100%)	3 (100%)	12 (100%)	92 (99%)
Rifampicin (R)	63 (98%)	11 (91.5%)	1 (50%)	3 (100%)	11 (91.5%)	89 (96%)
Ethambutol (E)	58 (90.5%)	11 (91.5%)	0	3 (100%)	11 (91.5%)	83 (89%)
Pyrazinamide (Z)	26 (40.5%)	2 (16.5%)	1 (50%)	1 (33.3%)	6 (50%)	36 (39%)
Streptomycin (S)	64 (100%)	12 (100%)	2 (100%)	3 (100%)	12 (100%)	93 (100%)

Table 2 – Resistance of *M. tuberculosis* isolates to the second-line anti-TB drugs

Resistance to	*M. tuberculosis* strains					
	Beijing (n=64)	LAM (n=12)	Africanum (n=3)	Haarlem (n=2)	Other (n=12)	Total (n=93)
Kanamycin (Km)	42 (65.5%)	12 (100%)*	3 (100%)	0	7 (58%)	64 (69%)
Amikacin (Am)	8 (12.5%)	5 (41.5%)	1 (33.3%)	0	4 (33.3%)	18 (19%)
Capreomycin (Cm)	20 (31.2%)	5 (41.5%)	3 (100%)	0	4 (33.3%)	32 (34%)
Ofloxacin (Ofx)	37 (57.8%)	9 (75%)	3 (100%)	0	7 (58%)	56 (60%)
Levofloxacin (Lfx)	11 (17%)	3 (25%)	1 (33.3%)	0	1 (8.3%)	16 (17%)

Moxifloxacin (Mfx)	10 (15.5%)	4 (33.3%)	2 (66.6%)	0	2 (16.5)	18 (19%)
Prothionamide (Pt)	8 (12.5%)	3 (25%)	1 (33.3%)	0	3 (25%)	15 (16%)
4 Aminosalicylic acid (PAS)	13 (20.3%)	11 (91.5%)*	0	0	2 (16.5)	26 (28%)
Cycloserine (Cs)	2 (3%)	2 (16.5%)	2 (66.6%)	0	3 (25%)	9 (10%)
Ethionamide (Et)	21 (33%)	9 (75%)*	1 (33.3%)	0	4 (33.3%)	35 (38%)

* — $p<0.05$ vs. Beijing

Km and Ofx did not inhibit growth of *M. tuberculosis* isolates in 69% and 60% cases respectively. The most effective anti-TB drug was Cs (10%).

The frequency of resistance to Km, PAS and Et was revealed significantly higher for LAM strain in compare to Beijing strain ($p<0.05$). Among all 93 patients 41 (44%) were diagnosed as MDR TB and 52 (56%) — TB with extending resistance. It needs to prove preventive therapy with second-line anti-TB drugs for a long duration.

Conclusions. There is difficult situation for MDR TB in Kharkiv region. It was confirmed in 52 cases (56%) of TB from totally 93 cases. Using VNTR-genotyping it was found that 68.8% of MDR strains belong to Bejing family. Differences in the chemoresistance to the second-line anti-TB drugs depending on the *M. tuberculosis* strains have been not identified. Resistance test to the second-line anti-TB drugs showed tolerance to Km, PAS and Et. In the structure of chemoresistance to the second-line anti-TB drugs depending on the strains it was determined that the stability revealed significantly higher for LAM strain in compare to Beijing strain.

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