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THE IMPACT OF THE DELETION OF THE MMP-1 GENE ON THE EXPRESSION OF SYMPTOMS AND THE EFFECTIVENESS OF TREATMENT IN PATIENTS WITH PULMONARY TUBERCULOSIS

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The objective of the study is to examine the impact of the deletion of the MMP-1 gene on the development, expression, dynamics of clinical syndromes and the effectiveness of treatment in patients with pulmonary tuberculosis.

73 patients with pulmonary tuberculosis, receiving treatment in the hospital of the state health care facility "Regional TB Dispensary" in the city of Astrakhan. Depending on the genotype of MMP-1, three groups were formed: the first group — with G1/G1 — 15 people (20.5%), the second — with G2/G1 — 27 people (50.7%) and the third one — with G2/G2 — 21 people (28.8%).

During the treatment, the disappearance of symptoms of intoxication and bronchopulmonary disorders in patients of the 1st and 2nd groups occurred earlier than in the 3rd group ($\chi^2 = 11.5$, $p = 0.02$). Dissolving of infiltration in the lung tissue also started earlier in the first and second groups ($\chi^2 = 9.2$, $p < 0.05$). The discharge of *Mycobacterium tuberculosis* (MBT) stopped earlier in patients of the 1st group. After four months of treatment, MBT were not determined by any method in 94.6% of the patients in the 2nd group and in 90.4% in the 3rd group ($\chi^2 = 9.9$, $p = 0.07$). Closure of decay cavities after 2 months of treatment was observed in 70% of cases in patients of the 1st group, in 53.6% of cases — of the second and in 15.6% of cases — of the third. In 47.4% of patients with G2/G2, destructive changes persisted for more than 4 months of treatment ($\chi^2 = 10.3$, $p = 0.03$).

Patients with genotype G2/G2 have a tendency to a protracted tuberculosis. The study of the polymorphism of the MMP-1 gene in patients with pulmonary tuberculosis can be used as a prognostic criterion of the clinical course of tuberculosis, correction of specific therapy, and justification for prescribing drugs affecting collagen metabolism.

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IN VITRO ACTIVITY OF BEDAQUILINE AGAINST NON-TUBERCULOUS MYCOBACTERIA

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Infections caused by non-tuberculous mycobacteria (NTM) have become more frequent in the last years since they have emerged as important pathogens in immune-compromised patients. Unfortunately, their treatment is long, toxic and often with poor results. Bedaquiline (BDQ) (trade name Sirturo; Janssen Therapeutics, Inc.) has been approved for the treatment of multidrug-resistant tuberculosis and there is increasing interest in its potential use for treating NTM infections.

The aim of this study was to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of BDQ against clinical isolates of NTM obtained from different hospitals. We investigated the possible role of gene mutations on the activity of BDQ against NTM by sequencing the *atpE* gene.

The MIC was determined by the broth dilution method in 7H9 medium supplemented with OADC and glycerol using the resazurin microtiter assay (REMA). The MBC was determined by conventional 7H10 plate agar dilution method.

Range concentration of BDQ in REMA was assessed from 2 to 0.0035 µg/ml. Each experiment was performed in triplicate. The MIC of BDQ was found at ≤ 0.015 µg/ml. The MBC of all NTM tested was found to be higher than 4 times the MIC. No nonsynonymous mutations in the *atpE* gene that conferred BDQ resistance in all NTM tested were identified.

BDQ exhibited a strong inhibitory effect against all the NTM clinical isolates tested. These promising results indicate that BDQ could be potentially useful for the treatment of NTM. However, according to the MBC data it lacks bactericidal activity. Nevertheless, BDQ could still have excellent potential for use in patients with NTM infections and further investigation is needed.

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EMERGING OPPORTUNISTIC PATHOGEN MYCOBACTERIUM ABSCESSUS IN SLOVENIA: MOLECULAR ANALYSIS OF RESISTANCE GENES COMPARED TO MIC METHOD

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Mycobacterium abscessus complex (MABSC) is a group of three closely related subspecies: *M. abscessus* subsp. *abscessus* (MAA), *M. abscessus* subsp. *bolletii* (MAB) and *M. abscessus* subsp. *massiliense* (MAM). Correct species identification is important, especially for patients with cystic fibrosis (CF), due to distinct molecular resistance profiles among subspecies. Gene *erm(41)* with T/C polymorphism at nucleotide 28 is responsible for inducible macrolide resistance (only in MAA and MAB, in MAM gene is not functional), meanwhile mutation in *rml* gene causes high-level macrolide resistance. Aminoglycoside resistance occurs with mutation in *rrs* gene.

We aimed to differentiate Slovenian MABSC isolates to subspecies level and to assess their molecular resistance profile. Selected isolates were further tested with microdilution method to obtain full phenotypic resistance profile.

Molecular analysis was performed on 37 clinical isolates recovered from 37 patients in the period 2000–2017 from Slovenian National Mycobacterial Collection. GenoType NTM-DR (Hain Lifesciences, Nehren, Germany) was used to identify subspecies and resistance mutations. Antimicrobial susceptibility testing (AST) was performed on selected isolates with reference microdilution method using Sensititre RAPMYCO microplates (TREK Diagnostic Systems, Cleveland, Ohio, USA). Susceptibility and resistance were assessed according to CLSI guidelines.

GenoType NTM-DR showed the highest prevalence rate for MAA 72.9% (27/37), followed by MAB 16.2% (6/37) and MAM 10.8% (4/37). Resistance profile showed that T28 polymorphism in *erm(41)* gene is present in all MAB and MAM isolates and in 88.8% (24/27) MAA iso-

lates. Mutations in *rrl* and *rrs* genes were not detected. AST for 4 MAM isolates confirmed that inducible resistance is not present even with *erm(41)* T28 mutation. AST for 3 MAA with *erm(41)* C28 polymorphism showed MIC values below 2 mg/L which is interpreted by CLSI guidelines as sensitive strain. AST showed that MIC values for amikacin are between 8–16 mg/L interpreted as sensitive and concordant with molecular analysis.

In Slovenia, for macrolide and aminoglycoside resistance, phenotypic and genotypic results of *Mycobacterium abscessus* complex are concordant. Prevalent subspecies is MAA where high percentage of strains have inducible macrolide resistance. No other unknown genetic mutation was present in our isolates that can cause inducible macrolide resistance which is important for treatment patients with CF, where clarithromycin is first drug of choice.

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MOLECULAR FEATURES OF MYCOBACTERIUM TUBERCULOSIS ISOLATES FROM PATIENTS LIVING IN CLOSED CITY IN THE URAL REGION, RUSSIA

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Novouralsk is a closed town in the Sverdlovsk region, Middle Ural area in Russia, with a total population of 81 500 and travel and residency restrictions. We aimed to identify the molecular-epidemiological features of *M. tuberculosis* circulating in Novouralsk under these specific conditions.

A total of 87 *M. tuberculosis* clinical isolates obtained between 2013 and 2016 from TB patients living in Novouralsk town were analyzed. According to clinical data, 34 (39.1%) of TB patients were HIV-infected. 53 (60.9%) of TB cases were newly diagnosed. Using real-time PCR we divided *M. tuberculosis* clinical isolates into Beijing/non-Beijing genetic groups. Beijing genotype variant B0/W148 was detected by multiplex PCR assay. VNTR loci MIRU26 and QUB26 were used for subtyping Beijing strains. Spoligotyping was used for further subtyping non-Beijing isolates. Drug susceptibility testing for first and second line drugs was performed by absolute concentration method.

Genotyping identified the predominance of the Beijing genotype isolates (75.5%), among new TB cases, that is almost 20% higher than the average for the Ural region ($p < 0.05$). 52.8% isolates belonged to variant Beijing B0/W148. The majority of Beijing isolates — 35 (40.2%) had seven copies in MIRU26 and QUB-26 loci. Nine (10.3%) Beijing B0/W148 isolates had 2 copies in QUB26 locus that was unusual for this genetic cluster; six patients from this group had TB/HIV co-infection. Seven (8.0%) of non-Beijing isolates belonged to SIT35 spoligotype (Ural family). 20.7% of patient had prison history and 72.2% of them were infected with B0/W148 genotype. The MDR prevalence rate was higher than in Sverdlovsk region (66% vs 43.9%, $p < 0.05$) and MDR status was associated with the Beijing B0/W148 genotype (94% and 6% of its isolates were MDR and polyresistant, respectively).

Epidemiological situation with TB in Novouralsk is characterized by high level of TB/HIV co-infection, predominance of Beijing B0/W148 isolates, which is an underlying reason of high level of MDR-TB.

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PREVALENCE AND DIVERSITY OF NONTUBERCULOUS MYCOBACTERIA IN DIFFERENT REGIONS OF THE RUSSIAN FEDERATION

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Today, more attention is being paid worldwide to the nontuberculous mycobacteria (NTM) infections due to their increase in various regions of the world. The prevalence of different NTM species depends on the geographical location. The prevalence and distribution of the NTM species in Russian Federation have not been sufficiently studied to date.

The objective of this study was to demonstrate the diversity of NTM species isolated from patients in different regions of the Russian Federation. NTM were isolated from solid and liquid media from patients with suspected tuberculosis/mycobacteriosis in the period from July, 2013 to June, 2017 and identified using GenoType Mycobacterium CM/AS assay (Hain Lifescience, Germany) and real-time PCR assay co-developed with Syntol LLC (Moscow, Russia). Seventeen NTM species were identified in 1400 cultures from 876 patients. Exact species was identified for isolates from 840/876 (95.89%) patients. The prevalence of slowly growing NTM was 76.5% (643/840). The most common species was *M. avium* (223/846, 25.5%). Also the high incidence rate (in descending order) has been shown for *M. gordonae* (115/846, 13.12%), *M. lentiflavum* (113/846, 12.9%), *M. intracellulare* (80, 9.13%), *M. fortuitum* (83/846, 9.47%), *M. kansasii* (62/846, 7.1%), *M. abscessus* (52/846, 5.9%) and *M. xenopi* (31/846, 3.53%). 16 of 17 identified species were detected in Moscow region. This may be due to a large number of cases analyzed in this region in comparison with other regions. It was shown that NTM species distribution in Central Federal District, European part of Privolzhsky Federal District and Kaliningrad (Northwest Russia) was similar to European countries: MAC 33–39%, *M. gordonae* — 10–20% and *M. fortuitum* — 5–13%. The species distribution of NTM for neighboring Syktyvkar and Perm (North of European Russia) was similar and was characterized by high rates of *M. fortuitum* infection and low rates of *M. avium* infection. NTM, isolated from the border city of Khanty-Mansiysk were also characterized by low occurrence of *M. avium* and prevalence of *M. gordonae*.

To conclude, during the study period, 1400 samples collected from 876 patients in 6 federal districts were analyzed. The greatest species diversity — 16 NTM species — was shown for Moscow region. In most of the regions analyzed, slow-growing NTMs prevailed and the most abundant were species belonging to MAC complex. However, even on a relatively small number of observations, some regional features can be observed.

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MOLECULAR CHARACTERIZATION OF MYCOBACTERIUM BOVIS ISOLATES FROM CATTLE IN BULGARIA

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The observation of the population dynamics, diversity of genotypes and dissemination of *Mycobacterium bovis* strains in Bulgaria and neighboring countries play