All TB patients in Arkhangelsk region were tested with molecular-genetic tests before the treatment enabling quicker diagnostics and earlier treatment initiation. Early diagnosis ensures proper treatment regimen for patients with TB and NTM diseases. As a result management of TB patients is improved leading to better treatment outcomes and subsequently reduced TB transmission in the region.

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DETECTION OF EXTRACELLULAR *MYCOBACTERIUM TUBERCULOSIS* SMALL RNAs

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According to WHO, tuberculosis infection is one of the top ten deadly infections in the world, and about one-fourth of the human population is a carrier of latent tuberculosis infection (LTBI) without manifestation of disease symptoms. The LTBI associated with the conversion of *M. tuberculosis* bacilli to a dormant, metabolically inactive state; however, the molecular mechanisms of this change is not well studied. In many researches the small RNAs (sRNAs) was proposed as regulators of these processes. The aim of the study was detection of sRNA transcripts in cultural supernatant of *M. tuberculosis* strain H37Rv and into the blood serum of mice (C57Bl) infected with LTBI.

Mycobacterial cells were grown in Middlebrook 7H9 containing 10% ADC supplement at 37°C and harvested at different growth phase for use. The culture of *M. tuberculosis* was centrifuged at 6000g for 20 min at 4°C. The supernatant was filtered through 0.22 μm filters to remove the remaining bacteria. Bacterial total RNA was extracted from *M. tuberculosis* cultural supernatant by ExtractRNA reagent (Evrogen, Moscow, Russia), followed by digestion with Turbo DNase-free kit (Ambion, Austin, TX, USA) to remove contaminating DNA. cDNA was synthesized with 1.5 μg of total RNA by M-MLV (Evrogen, Moscow, Russia) transcriptase and random hexadeoxynucleotides according to the manufacture's instruction. The quantitative RT-PCR (qPCR) was carried out with the qPCRmix-HS SYBR (Evrogen, Moscow, Russia) and the CFX-96 real-time PCR detection system (BioRad, USA). All primers used in this study were designed using VectorNTI 11 (Invirogen, USA) and GeneRunner software (http://www.generunner.net) and synthesized at Evrogen (Moscow, Russia). LTBI C57Bl mouse model was designed as previously described (Shramko et al., 2010). The certain sRNA transcripts have been detected in *M. tuberculosis* culture supernatant at different growth phases (exponential phase, stationary phase and late-stationary phase) and into the blood serum of mice infected with LTBI. Obtained data allow us to propose *M. tuberculosis* sRNAs as new markers for LTBI diagnostic in the future.

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TB PORTALS PROGRAM: DATA-DRIVEN MULTINATIONAL CONSORTIUM AGAINST DRUG-RESISTANT TUBERCULOSIS

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TB Portals Program (TBPP) is an international initiative that is founded, developed and steered by doctors, researchers, IT specialists and radiologists to combat drug-resistant tuberculosis (DR-TB). The efforts of hospitals and biomedical research institutes from ten countries on three continents are organized and supported by TBPP to actively establish and grow an integrated open-access network of innovative tools and data from real patient cases of TB (tbportals.nid.nih.gov). The TB Portals collect, analyze, standardize, and present anonymized clinical, laboratory, and socioeconomic data, full bacterial genomes, and radiological data (CXR and CT).

The TBPP database currently has more than 1300 published (22 250 total cases), 75% of which are MDR or XDR-TB. Clinicians supply and validate all patient data. Once validated, the data become published with open access status in accordance with NIH FAIR principles. Importantly, both original and derived (expert-based and computational annotations) data remain patient-centric, i.e. linked to a unique patient identifier. This cornerstone principle enables users to define and analyze cohorts of patients, augmenting OMICS studies with diverse clinical information.

To date the database contains 730 published (1300 total) fully sequenced and annotated *Mycobacterium tuberculosis* genomes associated with the patient case record. We will highlight several TBPP projects studying 1) genomic signatures for TB relapse and reinfection, and 2) comparative analysis of *M. tuberculosis* strains isolated from sputum vs. surgically removed parts of lungs.

TBPP assists doctors and researchers in testing and refining their hypotheses with friendly and powerful tools. Starting from genomics and the molecular basis of drug resistance, we will demonstrate how our online tools enable anyone to simultaneously look at clinical, microbiological and radiological evidences in order to (1) search for genomic clues for inconsistencies in existing DR-TB diagnostics and to (2) study common and countries-specific evolutionary patterns of *M. tuberculosis*.

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SIMPLIFYING NGS APPROACHES TO OPTIMIZE TRACING OF TRANSBORDER SPREAD OF *MYCOBACTERIUM TUBERCULOSIS*

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Molecular epidemiology, and more recently genomic epidemiology, based on whole genome sequencing (WGS), improve our understanding about the transmission dynamics of *Mycobacterium tuberculosis* in a population. However, in many countries, including many of those with a high burden of TB, systematic genomic epidemiology cannot be implemented. Trying to find a solution to this situation, we propose an alternative line of progression, which tries to conciliate the discriminatory power of WGS with the speed, low cost and simplicity of PCR-based approaches. The cost of this shortcut is that it sacrifices the complete knowledge of all the transmission clusters in a population, because it needs to focus on surveying the strains that deserve special attention because they are more actively transmitted, or correspond to high-risk MDR or XDR strains. This short-cut strategy has proved to be efficient to survey actively transmitted strains, to fast track outbreak-strains, to update the presence of high-risk strains in a population or to give an urgent answer to public-health alerts, such as to rule out secondary cases due to the importation of XDR-TB cases. More recently, we are integrating this strategy to optimize the characteriza-

tion and tracking of trans-national transmission events. We have activated a decentralized multinational network of surveillance nodes. This network simultaneously analyze the cross-border distribution of relevant strains by means of sharing the same set of strain-specific PCRs. Once new cases infected by the surveyed strains are captured by the strain-specific PCRs the isolates are characterized by WGS. From these data we to define in detail the network of relationships between the involved cases. It allows us to differentiate transmissions after arrival of migrants to the host countries from independent importations from their countries of origin. Integrative multinational efforts supported on novel simplified strategies can transform the way in which we survey TB transmission in a new global scenario.

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GENETIC DIVERSITY AND DRUG RESISTANCE OF *MYCOBACTERIUM AVIUM* IN ITALY

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Mycobacterium avium complex is responsible for most of the human-associated nontuberculous mycobacteria infections. *M. avium* is classified into 4 subspecies, each endowed with specific pathogenetic and host range characteristics; among these, *M. avium* subsp. *hominissuis* (MAH), that is usually isolated from human and swine sources, is an important pathogen that causes infections in the respiratory tract, lymph node, and, occasionally, soft tissue of immunocompetent patients; moreover, it causes disseminated diseases in patients with human immunodeficiency virus infection. In Italy, as in many other countries worldwide, MAH is the most common cause of nontuberculous mycobacteria infection and the incidence of MAH infections is increasing. In the present study, we determined the VNTR-based genetic diversity of a collection of 71 MAH human strains isolated from 2010 to 2016 in order to estimate the genetic relationships among MAH isolates in our setting. Moreover, we performed the clarithromycin susceptibility test in order to investigate whether there was any association between the VNTR pattern and the minimal inhibitory concentration (MIC) of clarithromycin. The VNTR analysis revealed 24 distinct VNTR patterns; of these, 16 patterns were unique, while 8 patterns were shared by 2 or more isolates, thus yielding 8 lusters including a total of 55 isolates. Our results showed that most MAH isolates displayed a close genetic relationship, indicating that the MAH genotypes are quite homogeneous in our geographical area. Such genotypic stability of the MAH strain population circulating in our region supports the hypothesis of the presence of possible local sources of infection and transmission pathways at the local level.

Clarithromycin showed strong antimicrobial activity against MAH isolates, as indicated by the high proportion (94.4%) of susceptible strains. No significant association between VNTR genotype and MIC of clarithromycin was found; moreover, due to the small number of resistant isolates, it was not easy to evaluate the correlation between VNTR genotypes and clarithromycin susceptibility.

Further investigations on larger collections of MAH strains of human, animal and environmental origin, are needed both to define the correlation between genotypes and clinical features or drug resistance and to clarify the sources of infection and the specific transmission pathways of our region, in order to achieve a better control of MAH infection.

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GENOTYPES OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM DIFFERENT ORGANS OF PATIENTS WITH GENERALIZED TB AND HIV-COINFECTION

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The purpose of the study was to genotype *Mycobacterium tuberculosis* isolates from internal organs of patients from St. Petersburg, Russia, with generalized tuberculosis (TB) and HIV coinfection, to assess possible association between the *M. tuberculosis* genotype and localization of TB disease.

A total of 128 strains of *M. tuberculosis* were recovered from 55 patients with HIV infection of stages 4 or 5 and generalized TB with multiple lesions of internal organs (from 2 to 10), were studied. Most of the patients had affected lungs (50 cases), intrathoracic and intra-abdominal lymph nodes (46 and 34 cases respectively), spleen (40 cases), kidneys (32 cases), brain and meninges (32 cases). *M. tuberculosis* isolates were cultured from lungs (48), intrathoracic and intra-abdominal lymph nodes (40), spleen (20), kidneys (14), meninges and brain (7). *M. tuberculosis* were isolated from one affected organ in 11 patients, and in the remaining cases isolates from 2 or 3 affected organs (16 and 28 persons, respectively) were obtained. Genotyping was performed by spoligotyping (all strains) and IS6110-RFLP typing (Beijing genotype), the obtained profiles were compared with the international database SITVIT_WEB and a proprietary database (Narvskaya, 2003), respectively. The data were subjected to statistical analysis.

67.3% (37 of 55) patients were infected by *M. tuberculosis* Beijing genotype. Of the 37 patients infected with Beijing strains, strains of cluster A0 were isolated from 14 patients, B0 from 5 patients, and 10 other RFLP types were obtained in the remaining 18 patients. The non-Beijing genotypes were represented by LAM (4), Ural (5), T (5) and 4 others (1 strain of each genotype), all of which belong to the Euro-American lineage of *M. tuberculosis* (lineage 4). No difference was observed for isolates from different organs of the same patient. Of the 37 patients infected with Beijing strains, the lungs were affected in 33 patients, intrathoracic lymph nodes in 28, spleen in 28, intra-abdominal lymph nodes in 28, kidneys in 24, and brain and meninges in 24 patients. Of 18 patients infected with non-Beijing strains, lungs were affected in 17 patients, intrathoracic lymph nodes in 18, spleen in 12, intraabdominal lymph nodes in 11, kidneys in 8, brain and meninges in 10. Comparison of different organs for association with infection by Beijing and non-Beijing strains did not reveal statistically significant differences: lungs ($P = 0.890$); intrathoracic lymph nodes ($P = 0.503$); spleen ($P = 0.777$); intra-abdominal lymph nodes ($P = 0.970$); kidneys ($P = 0.448$); brain and meninges ($P = 0.886$).

Beijing genotype was predominant among *M. tuberculosis* isolates studied. Among Beijing family strains, cluster A0 (corresponding to the Central Asian Russian strain) prevailed. There were no statistically significant differences between Beijing and any of non-Beijing genotypes with regard to frequency of isolation from particular organs.