6.36 doi: 10.15789/2220-7619-2018-4-6.36

POLYMICROBIAL BIOFILM FORMATION AS A POSSIBLE CAUSE OF UNEXPECTED DEFAULTED TREATMENT OF PULMONARY TUBERCULOSIS

O. Ogarkov[1,2], A. Suzdalnitsky[3], V. Sinkov[1,4], N. Belkova[5], M. Koshcheev[3], S. Zhdanova[1]

[1]Scientific Center for Family Health and Human Reproduction Problems, Irkutsk, Russia; [2]Irkutsk State Medical Academy of Continuing Education, Irkutsk, Russia; [3]Irkutsk Regional Clinical Tuberculosis Hospital, Irkutsk, Russia; [4]Irkutsk Diagnostic Center, Irkutsk, Russia; [5]Limnological Institute Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia

Microbes rarely exist as single species planktonic forms as they have been commonly studied in the laboratory. Instead, the vast majority exists as part of complex polymicrobial biofilm communities attached to host and environmental surfaces. *Mycobacterial tuberculosis* (MBT) is no exception. A number of researchers have shown that in the experiment *in vivo* model, MBT can form biofilm-like structures in the lungs.

The aim of the study is to demonstrate the role of tuberculomas satellite microbiota as example of polymicrobial biofilm existent in a lung of TB patients.

Our study of clinical MBT strains shown less 5% of them were able to produce mature biofilms (pellicle) on a liquid medium. Although we might expect that pathogenic MBT could gain obvious advantage in case of growth in necrotic foci in lungs and it should keep this ability in the first passage *in vitro*. It was found feature of MBT strains produced pellicle on liquid medium to grow on Levinshtein–Jensen by specific R colonies. It looks as disk with a convex center, "UFO-colonies". It was shown on *in vitro* model that about of 50% clinical MBT strains can coexist together with *Bacillus licheniformis*, also isolated from sputum of TB patient. Moreover, after pellicle formation by bacilli in the first 3 days, the growth of MBT was continued for next 30 days under the bacillary pellicle. It is very important that investigated bacilli had a high tolerance to streptomycin, ethionamide, isoniazid and ethambutol, e.i. to four of the 12 basic anti-TB drugs.

The study on 16S rRNA metagenomic and massively parallel sequencing (NGS) DNA of several tuberculomas was conducted. It was shown that quantity of MBT genomes were less 3% in all cases. The vast majority species belonged to Gram-positive *Firmicutes* like *Staphylococcaceae* and also a small amount of Gram-negative taxons was found.

We can assume that anti-tuberculosis therapy is confronted with not only MBT, but with polymicrobial biofilm communities, which formed by the etiological agents of tuberculosis and also by a large number of other satellite microorganisms in lungs. It is very important that this microbial community in TB-patient lungs of should form a cumulative resistance to anti-tuberculosis therapy during long-term treatment. We can expect that the cumulative resistance of a polymicrobial biofilm in the TB-patient lungs may be significantly differing from the resistance of detected in the clinical laboratory TB strains.

6.37 doi: 10.15789/2220-7619-2018-4-6.37

BACTERIAL WGS AND HOST GENOME-WIDE SNP ANALYSIS OF TUBERCULOSIS PATIENTS IN THAILAND

P. Palittapongarnpim[1], S. Mahasirimongkol[2], K. Tokunaga[3]

[1]Department of Microbiology, Faculty of Science, Mahidol University and the National Science and Technology Development Agency, Bangkok, Thailand; [2]Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand; [3]University of Tokyo, Tokyo, Japan

Mycobacterium tuberculosis has been a human pathogen for a long time, providing ample opportunities for genomic interactions between the two organisms. Evidences of co-evolution has been reported. We have performed genomic studies in a cohort of tuberculosis patients in Chiangrai, northern Thailand. The genomes of *M. tuberculosis* isolated from 1170 patients during 2003–2010 were sequenced. The genomes of the same patients were also evaluated using high-density SNP arrays. The bacteria were genetically heterogeneous, with majority belonging to various sublineages of lineages 1 and 2. Refinement of classification of lineage 1 were proposed and a few novel sublineages of the others were identified especially in remote populations. The patients mostly belonged to three genetic groups, identified by principal component analysis, and three self-identified ethnicity groups. The profiles of patients infected by sublineages varied especially among sublineages of lineage 2. There were strong correlations between the bacterial genotypes and human ethnicity. GWAS identified a few genes associated with particular genotypes of the bacteria. Together with historical records, this study indicated that both the founder effects and co-evolution may explain the associations. This study provided some insights to the bacterial host interactions and useful information for the development of vaccines and other control measures for tuberculosis and is being replicated in a cohort of 600 patients in 2016–2018 with some patients studied by WGS.

6.38 doi: 10.15789/2220-7619-2018-4-6.38

POPULATION STRUCTURE OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM TB-HIV COINFECTED PATIENTS IN OMSK REGION, WEST SIBERIA, RUSSIA

O.A. Pasechnik[1], A.A. Vyazovaya[2], I.V. Mokrousov[2]

[1]Omsk State Medical University, Omsk, Russia; [2]St. Petersburg Pasteur Institute, St. Petersburg, Russia

A clear trend of the increasing incidence of tuberculosis (TB) associated with HIV infection is observed in the Omsk region in West Siberia. The TB-HIV incidence increased from 0.3 in 2006 to 15.2 in 2017 per 100 000 population. The aim of this study was to analyze the population structure of *Mycobacterium tuberculosis* isolated from TB-HIV coinfected patients.

A total of 150 *M. tuberculosis* isolates were recovered from 150 patients with pulmonary tuberculosis in 2013–2017 were included in this study. They included 110 men (74.8%) and 40 women (25.2%), the average age was 35.2 years (from 22 to 58 years). *M. tuberculosis* culture and drug susceptibility testing were performed according to standard protocols. DNA was extracted from *M. tuberculosis* isolates using the recommended method. Beijing genotype was detected by PCR analysis of the *dnaA-dnaN*::IS*6110* insertion. Beijing B0/W148 cluster was identified by PCR analysis of the *Rv2664-Rv2665*::IS*6110* insertion. Spoligotyping was performed according to standard protocol (Kamerbeek et al., 1997) and the profiles were compared to SITVIT_WEB (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE) for family assignment which was corrected by expert assessment. A chi-square test was used to detect any significant difference between the two groups.

Almost 3/4 of the studied *M. tuberculosis* isolates belonged to the Beijing genotype (109/150, 72.6%). Beijing B0/W148-cluster (Russian MDR Beijing clone) included 29 isolates (26.6% of Beijing population). Majority of the Beijing isolates (62/109; 56.8%) belonged to the Beijing 94-32-cluster (Central Asian/Russian strain).

Forty-three non-Beijing isolates were subdivided into 17 spoligotypes shared by 1 to 5 isolates. They represented the following genetic families: LAM (n = 19), T (n = 10), Ural (n = 6), Haarlem (n = 3), X (n = 1); for two isolates the family status was "unknown".

Population structure of *M. tuberculosis* isolates from TB-HIV coinfected patients in Omsk region is dominated by the Beijing genotype (72.6%) while the other, non-Beijing families belong to the Euro-American superlineage. Beijing genotype is dominated by the isolates of the epidemiologically important Beijing 94–32 cluster (56.8%).

Funding: this study was supported by Russian Foundation for Basic Research (grant 17-04-00367).

6.39 doi: 10.15789/2220-7619-2018-4-6.39

LOOKING INSIDE THE FOREST: FROM CLASSICAL GENOTYPING OF *MYCOBACTERIUM TUBERCULOSIS* TO WHOLE GENOME SEQUENCING IN HIGH MULTIDRUG RESISTANCE SETTINGS

J. Perdigão

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Molecular typing of *Mycobacterium tuberculosis* is an increasingly important public health tool that can provide a framework to investigate the dissemination and emergence of specific strains. Classical typing methods have relied upon the genetic analysis of repetitive loci, whose presence, number and layout on the *M. tuberculosis* genome have enabled the distinction between clinical isolates of different genotypes.

Over the last decade, the massive development of Next Generation Sequencing and ability to carry out Whole Genome Sequencing (WGS), which provides the ultimate resolution power, has revolutionized bacterial typing by enabling one to infer on the directionality of tuberculosis (TB) transmission. Herein, the importance of seeing deeper in the genome of *M. tuberculosis* will be analysed in two distinct epidemiological scenarios: the emergence of strains associated with drug resistance due to migratory movements and, the discrimination and study of the transmission dynamics of endemic multidrug and extensively drug resistant strains.

Regarding the emergence of drug resistant strains, WGS does provide sufficient evidence to delineate and discriminate within cross-border clusters that were otherwise impossible to discriminate. In Portugal, this has been of special relevance for multidrug resistant (MDR) superclusters of the Beijing family in Europe (such as the 94-32 and 100-32 types) that are spreading through vast geographical areas. This can be of great importance to inform concerted efforts aimed at screening migrant populations arriving from high-incidence settings and new epidemiological links can be uncovered even within the country. The same inability to discriminate using classical typing methods can be generated by outbreak strains whose circulation is occurring for decades. In such a scenario multiple transmission sub-clusters are usually present and WGS can effectively resolve these transmission networks. Good examples are the KZN, Lisboa or Q1 strains, all of which associated with extensively drug resistant (XDR) TB. Furthermore, recent evidence obtained by WGS shows that MDR-TB and XDR-TB within Lisboa and Q1 clades has emerged multiple times instead of more conservative predictions based on classical typing. Some roadblocks still lie ahead, but, the latter also highlights the advantage of genome-wide based phylogenetic analysis of *M. tuberculosis* clinical isolates in TB surveillance and, the need for a switch from classical typing to WGS-based typing.

6.40 doi: 10.15789/2220-7619-2018-4-6.40

ADVANCES IN THE STUDY OF MOLECULAR BASIS OF RESISTANCE TO NEW ANTI-TB DRUGS

I.V. Peretokina[1], L.Yu. Krylova[1], O.V. Antonova[2], M.S. Kholina[2], D.A. Gryadunov[2], E.Yu. Nosova[1], D.V. Zimenkov[2]

[1]Moscow Research and Clinical Center for Tuberculosis Control, Moscow, Russia; [2]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

Bedaquiline is an effective drug for the treatment of MDR and XDR tuberculosis allowing up to 85% cure rate in complex therapy. Unsuccessful treatment is accompanied with elevation of bedaquiline MIC and acquisition of mutations in *mmpR* and *atpE* genes. However, the clinical significance of mutations detection is still obscure due to an insufficient number of clinical isolates, characterized by phenotypic and molecular methods.

Bedaquiline MIC of clinical MTB isolates from patients, who obtain complex therapy including bedaquiline, were tested using both the agar proportion method on 7H11 plates and Bactec MGIT system. Genes *mmpR* and *atpE*, associated with an elevated MIC of bedaquiline, were sequenced.

191 clinical isolates were divided into several groups based on the genetic analysis: strains with wild-type sequences of all analyzed genes; heteroresistant strains, where both wild-type and mutant sequences could be identified; isolates where only mutant, or mix of different mutant sequences was found; and a group of isolates with the mutated *atpE* sequence. Most of the strains, isolated prior the bedaquiline treatment, had wild-type sequences and liquid media MICs ranged from 0.06 to 0.50 mkg/ml with the mode at 0.12 mkg/ml. Isolates with mutated *mmpR* gene possessed MIC range of 0.12–4.00 mkg/ml with mode at 0.25 mkg/ml. Heteroresistant isolates had an intermediate MICs from 0.12 to 2.00 mkg/ml. Four isolates with AtpE substitutions (D28N, A63P, A63V) had bedaquline MICs of 4.00 and 8.00 mkg/ml. The MICs distributions of wild-type and mutated isolates on 7H11 media had the distinct border between 0.06 mkg/ml and 0.12 mkg/ml: most of the strains with a MIC of ≥ 0.12 mkg/ml bore mutations.

During the treatment with bedaquiline, intermediate resistance emerged by selection of *mmpR* mutations, and high-level resistance caused by substitutions in AtpE. Our results also raise the question of reliability of currently used critical bedaquiline concentrations for 7H11 agar (0.25 mkg/ml) and Bactec MGIT 960 (1 mkg/ml) tests.

This work was financially supported by the Russian Science Foundation (grant no. 14-50-00060).

6.41 doi: 10.15789/2220-7619-2018-4-6.41

THE IMPLEMENTATION OF NEXT-GENERATION SEQUENCING FOR EPIDEMIOLOGICAL STUDIES AND DRUG RESISTANCE INVESTIGATIONS IN MICRO-EPIDEMICS INVOLVING PEDIATRIC TUBERCULOSIS PATIENTS

I. Pole[1,2], I. Ozere[2,3], I. Norvaisa[2], R. Ranka[1,3]

[1]Latvian Biomedical Research and Study Centre, Riga, Latvia; [2]Riga East University Hospital, Centre of Tuberculosis and Lung Diseases, Riga, Latvia; [3]Riga Stradiņš University, Riga, Latvia

In Latvia, the childhood TB epidemiology trends very clearly reflected the increase of TB transmission from the year 1992 and the decrease of transmission rate since 2001. There was also a small increase of TB notification rate in children in 2011 which clearly predicted an increas-