POLYMICROBIAL BIOFILM FORMATION AS A POSSIBLE CAUSE OF UNEXPECTED DEFAULTED TREATMENT OF PULMONARY TUBERCULOSIS
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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 (MTB) is no exception. A number of researchers have shown that in the experiment in vivo model, MTB can form biofilm-like structures in the lungs.

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

Our study of clinical MTB strains shown less 5% of them were able to produce mature biofilms (pellicle) on a liquid medium. Although we might expect that pathogenic MTB 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 MTB strains produced pellicle on liquid medium to grow on Levinstein—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 MTB 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 MTB 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.t. to four of the 12 basic anti-TB drugs.

The study on 16S rRNA metagenomic and massively parallel sequencing (NGS) DNA of several tuberculosomas was conducted. It was shown that quantity of MTB genomes were less 5% in all cases. The vast majority species belonged to Gram-positive Firmicutes like Staphylococcaceae and also a small amount of Gram-negative taxa was found.

We can assume that anti-tuberculosis therapy is confronted with not only MTB, 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 will be significantly differing from the resistance of detected in the clinical laboratory TB strains.

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

POPULATION STRUCTURE OF MYCOBACTERIUM TUBERCULOSIS ISOLATES FROM TB-HIV CONINFECTED PATIENTS IN OMSK REGION, WEST SIBERIA, RUSSIA
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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 dnaN-dnaA fragment. Beijing B0/W148 cluster was identified by PCR analysis of the Rv2664-Rv2665:IS6110 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).
Fourty-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%).

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

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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.