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GENOMIC EPIDEMIOLOGY OF TUBERCULOSIS: FROM WITHIN HOST EVOLUTION TO GLOBAL MIGRATION PATTERNS

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The availability of thousands of *Mycobacterium tuberculosis* genomes allows not only to infer how the pathogen emerged and spread but also to identify specific loci associated. I will present our work using evolutionary analyses to generate a high-resolution picture of the emergence, global spread and local transmission of the pathogen as well as genomic determinants associated. I will show how non-selective processes like genetic drift contribute to the genomic diversity of the pathogen with downstream consequence at the transcription and methylation levels. In the second part of the talk I will also discuss different approaches to identify and track new drug resistance determinants using a combination of functional genomics and genomic diversity analyses from different countries and from different patients through time and space. Overall our analyses reveal new bacterial factors associated to virulence and drug resistance. I will show however how the frequency of genetic variants associated to different traits, even if advantageous, depend on the conditions of local TB control more than on the high fitness of the bacterial genotype.

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EMERGENCE OF BEDAQUILINE RESISTANCE AFTER COMPLETION OF BEDAQUILINE-BASED DRUG-RESISTANT TB TREATMENT: A CASE STUDY FROM SOUTH AFRICA

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Treatment outcomes for drug-resistant tuberculosis (DR-TB) are poor with only 52% of MDR-TB and 24% of XDR-TB patients successfully treated. To address the global DR-TB epidemic WHO has released guidelines for the use of bedaquiline (BDQ) for the treatment of rifampicin-resistant or MDR-TB for specific indications. However, standardised methods to perform drug susceptibility testing (DST) have not been defined and BDQ resistance mechanisms remain poorly characterised.

Illumina NextSeq whole genome sequencing (WGS) was used to characterise serial *Mycobacterium tuberculosis* (Mtb) isolates from a patient receiving BDQ in Khayelitsha, South Africa. Phenotypic drug susceptibility testing (DST) for BDQ was performed in MGIT-960 media (concentration 1 μg/ml).

WGS showed an initial infection with a strain resistant to 7 drugs (rifampicin, isoniazid (low-level), ethambutol, ethionamide, fluoroquinolones, pyrazinamide and streptomycin). Following initial treatment failure with a standardised MDR-TB regimen, the patient was placed on a regimen containing 6 effective drugs (including BDQ, based on WGS). Isolates taken prior to BDQ initiation were BDQ-susceptible (phenotypically). WGS of subsequent serial isolates revealed the acquisition of a variant in *Rv0678* (conferring BDQ-resistance) one month after stopping BDQ treatment. Subsequent isolates showed the loss and gain of several other *Rv0678* variants, with only one variant (138 G insertion) fixed in the last available isolate. All isolates with *Rv0678* variants were BDQ-resistant.

The systematic gain and loss of *Rv0678* variants in isolates taken after completion of BDQ-based treatment illustrates the complex ongoing evolution patterns of *M. tuberculosis* as the concentration of BDQ decreases in the patient (long half-life). An alternative explanation is the emergence of existing BDQ-resistant Mtb from lesions which rupture following continuation of treatment without BDQ and after stopping all TB treatment. The emergence of BDQ resistant *M. tuberculosis* following stopping of treatment poses a risk of transmission of BDQ resistant clones to close contacts. Monitoring of pre-existing and emerging BDQ resistance should be a priority for all routine use and should continue post BDQ cessation.

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WHOLE GENOME SEQUENCING SHEDS LIGHT ON THE TRANSMISSION DYNAMICS OF A MULTI-DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* OUTBREAK OVER 23 YEARS IN A HIGH INCIDENCE SETTING

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Whole genome sequencing (WGS) has shown that *Mycobacterium tuberculosis* strains are more genetically diverse than previously assumed and that traditional genotyping methods cannot discriminate strain heterogeneity with high resolution, which may mask their ability to accurately define the directionality of an outbreak, particularly in high tuberculosis (TB) incidence settings.

The objective of this study was to examine the evolution of a single *M. tuberculosis* cluster defined by a particular IS*6110* RFLP pattern to understand transmission and strain diversity over time.

Clinical *M. tuberculosis* isolates (n = 97) with identical IS*6110* RFLP fingerprint patterns were selected from a longitudinal sample bank of *M. tuberculosis* isolates collected from a high TB incidence suburb in the Western Cape, South Africa from 1993–2015. DNA was extracted from *M. tuberculosis* cultures for WGS and subsequent analysis. Available WGS of *M. tuberculosis* isolates from surrounding suburbs were screened and additional isolates

from the same time period with the same genotype were included in the phylogenomic analysis. The genomic variants identified by WGS were used for phylogenomic inference, drug resistance prediction and to determine genomic distances between isolates.

WGS analysis revealed unexpected genomic diversity within the seemingly homogenous IS*6110* cluster of *M. tuberculosis* isolates. Despite the IS*6110* RFLP based uniformity, at least six non-time dependent sub-clusters and several orphan-isolates were evident from the WGS-based phylogeny and genomic comparisons. Sub-clusters gained drug resistance conferring mutations (beyond MDR) on multiple occasions and *M. tuberculosis* isolates from surrounding suburbs were observed throughout the phylogeny.

IS*6110* RFLP typing underestimated the complexity of this 23-year outbreak. This study suggests that there is continuous circulation and reintroduction of this *M. tuberculosis* cluster in the community setting. Even with the advent of the WGS-era, confirming direct epidemiological links or outbreak directionality remains a challenge in high TB burden, low-income settings.

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A 15-YEAR SPATIOTEMPORAL ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* LINEAGES 1 AND 2 IN CHIANG RAI, THAILAND

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Chiang Rai is the northernmost province of Thailand with high burden of tuberculosis (TB) and high TB death rate. Chiang Rai consists of various ethnic groups in three different geographic areas including (1) the bordering area in the northern and northeastern, (2) the central area, and (3) the outlying districts in the southeastern, southern and southwestern.

We aimed to assess the spatial and temporal distribution of *Mycobacterium tuberculosis* (MTB) lineages in the three different areas over 15 years.

Whole genome sequence (WGS) data was used to classify the genotypes of 1497 MTB using lineage-specific single nucleotide polymorphisms (SNPs). The spatiotemporal distribution of MTB lineage was analyzed in the three different areas during early 2000s (2002–2006), late 2000s (2007–2011) and 2010s (2014–2018). Stoddart and Taylor's index (G) was calculated to determine genotypic diversity of MTB lineage in the different settings.

In 2000s, lineage 2 (East Asian) was a highly predominant genotype (45%) in Chiang Rai followed by lineage 1 (Indo-oceanic) (41%). In 2010s, lineage 1 became the most dominant genotype (51%) replacing lineage 2 (35%). The overall change in predominant lineage from lineage 2 to lineage 1 was caused by a dramatic increase proportion of lineage 1 in the central area (from 44 to 53%) and in the outlying districts (from 39 to 59%). In the bordering area, a combined impact of increasing distribution of lineage 1 (from 32 to 40%) and other lineages (from 18 to 24%) was an additional cause of changing predominant lineage. The Stoddart and Tayloy's analysis of genotypic diversity showed that the central area had the highest diversity of MTB lineage ($G = 4.14 \pm 0.46$) followed by the bordering area ($G = 3.67 \pm 0.76$) and the outlying districts ($G = 3.63 \pm 0.62$).

Our study combining genotypic and space-time analysis has revealed a dynamic population changes in MTB lineage over 15-year period in Chiang Rai. Further studies on social determinants and patient's demographic data in the different geographic areas have the potential to provide effective TB control in the different setting.

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MOLECULAR-GENETIC METHODS OF DETECTION OF TUBERCULOSIS AND ITS DRUG RESISTANCE IN ARKHANGELSK REGION IN 2017

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Burden of tuberculosis (TB) is decreasing in Arkhangelsk region in northwestern Russia with incidence declining from 45.9/100 000 in 2012 to 21.6/100 000 in 2017 in civil society (excluding penitentiary society). Introduction of molecular-genetic tests of detection of TB and its drug resistance (DR), including multidrug-resistant (MDR) and extensively drug resistant (XDR) TB, is one of the key components of regional TB program. It plays an important role in improvement of diagnostics and management of TB patients in the region.

The objective was to evaluate performance of molecular-genetic tests used for detection of TB and its DR among patients with TB registered in civil society in Arkhangelsk region in 2017.

Line probe assay (LPA) (Hain Lifescience, Germany) and GeneXpert MTB/RIF (Cepheid, USA) were used for detection of *M. tuberculosis* (MTB) and its DR alongside conventional sputum smear microscopy (SM) and culture. All patients were initially tested with SM followed by LPA (Genotype MTBDR*plus*) if result of SM was positive or GeneXpert if result was negative using the same sample. In case of DR to isoniazid and/or rifampicin, Genotype MTBDR*sl* was performed to detect additional DR to fluoroquinolones and injectables, including XDR. In cases suggestive of nontuberculous mycobacteria (NTM), SM or culture positive with negative results of LPA or GeneXpert for MBT, identification was performed using Genotype Mycobacterium CM/AS.

Total of 214 "new cases" and 28 "relapses" of TB were registered in Arkhangelsk region in 2017. MTB was detected in 160 (74.8%) out of 214 "new cases" and all of them were tested for DR using molecular-genetic tests. 53 patients (31.1%) had MDR-TB, among them 3 patients had additional DR to injectables and 2 patients had XDR-TB. Among 28 "relapses" MTB was detected in 24 (85.7%) patients. 13 patients (54.2%) had MDR-TB, among them in 1 patient additional DR to injectables and in 2 patients to fluoroquinolones was detected. NTM associated disease was diagnosed in 4 patients (2 — *M. avium*, 1 — *M. gordonae*, 1 — *M. interjectum*).