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PHYSIOLOGICAL IMPACT OF THE EVOLUTION OF THE *rpoB* MUTATION

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Bacilli within an infected lung cavitary lesion spontaneously evolve mutations that confer resistance and are subsequently selected following antibiotic treatment. During this evolutionary process both drug susceptible and drug resistant bacilli may be present. This mix state of susceptive and resistant bacilli captured at a distinct point in time may change during the course of infection and drug selection. The complexity of the population structure in each sputum sample may thus define the outcome of molecular and phenotypic drug resistance testing which in turn may determine how the patient will be treated. We hypothesize that the *rpoB* mutation will influence the transcriptome of the rifampicin mono-resistant isolate compared to the progenitor rifampicin susceptible isolate.

A sputum sample from an individual patient containing a heterogeneous population of both a rifampicin mono-resistant Beijing Ser531Leu clone and its susceptible progenitor was selected. DNA was extracted and sequenced using the Illumina HiSeq platform and analyzed using an in-house bioinformatic pipeline. RNA was extracted and sequenced using the Illumina platform and analyzed using Chipster, an open source bioinformatic platform.

The small number of variants between the two isolates suggests that the resistant isolate evolved from the susceptible progenitor. Our comparative transcriptomic analysis showed that microevolutionary events within the *rpoB* gene had a considerable influence on transcription. Consequently, the expression of bacilli's stress response, sigma factors, and regulatory genes were down regulated. This in turn led to a down-regulation of expression of a large number of genes, suggesting that the rifampicin resistant mutant has an altered physiology.

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MYCOBACTERIUM TUBERCULOSIS DRUG RESISTANCE MUTATIONS AND UNDERSTANDING OF PK/PD: TREATMENT AND CARE IMPLICATIONS

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Russian Federation has the third-highest burden of multidrug-resistant tuberculosis (MDR-TB) in the world and complicated by high rates of human immunodeficiency virus (HIV) co-infection which leads to mortality and risk for acquired *Mycobacterium tuberculosis* drug resistance. Treatment outcomes may be a consequence of pharmacokinetic/pharmacodynamics (PK/PD) variability.

In Irkutsk, we aimed to describe pharmacokinetic variability, minimum inhibitory concentrations (MICs) for key anti-TB drugs and their molecular correlates of resistance, and to determine if PK/PD variability associates with treatment response.

Consecutive people living with HIV initiating TB treatment at Irkutsk Regional TB Referral Hospital were recruited. After 2 weeks of treatment, medications were directly administered and plasma samples collected at 2 and 6 hours after administration. Drug concentrations were measured using validated liquid chromatography-mass spectrometry assays for peak concentration (C_{max}), the highest value in the dosing interval, and area under the concentration curve from time 0 to 6 hours (AUC_{0-6}). *M. tuberculosis* MIC testing was performed using the MYCOTB Sensititre plate. A drug was classified as active when C_{max} was greater than MIC. PK/PD variability as a predictor of treatment outcome was determined by classification and regression tree (CART).

69 patients with HIV had PK/PD testing. Mean age was 34 years (SD±6.2), 45 (65.2%) were male. Mean CD4 count was 180 (±202) cells/mL. Thirty-six (52.2%) had drug susceptible TB, 10 (14.5%) MDR-TB, 17 (24.6%) pre-extensively drug-resistant (XDR)-TB and 6 (8.7%) with XDR-TB. Based on PK/PD testing, patients were treated with a lower number of active drugs (3.25±1.40) compared to the number presumed to be active when initially prescribed (4.81±0.94), $p \leq 0.001$. Fifty patients had treatment outcomes and 16 (32.0%) had treatment failure. In CART analysis, regardless of molecular mutation for drug resistance, having less than 4.5 active drugs as redefined by PK/PD testing, correctly identified 15 of 16 (93%) of patients with treatment failure.

In Irkutsk, PK/PD testing predicted treatment outcome for patients with HIV/TB. Screening for mutations in *M. tuberculosis* resistance determining regions is an important method for constructing initial regimens, but should be followed by PK/PD testing to attain the highest likelihood of drug activity.

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GENOMICS AND LOCAL ADAPTATION OF *MYCOBACTERIUM AVIUM*

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Mycobacterium avium subsp. *hominissuis* (MAH) is a human pathogen that causes *M. avium* complex (MAC) lung disease, which is difficult to cure by current antibiotics treatment. It has been suggested that MAH circulates between the human body and the environment. Despite its clinical significance, the genetic mechanisms underlying local adaptation of this pathogen are unknown due to a lack of population-wide genomic data. To overcome this issue, we evaluated the genetic population structure of MAH using genome-scale data from 36 global strains (including 12 Japanese strains sequenced in this study), and then sought to identify alleles unique to Asian populations by comparative genomic analysis. The population structure analysis was extended to include 652 global strains using the multiple-locus variable-number tandem repeats data set, which revealed that two genetic population groups dominated the Asian isolates.

By analyzing mutual homologous recombination and gene content, we revealed that MAH reproduces sexually and has an unlimited gene repertoire. The results of these analyses predict the presence of a chromosome