Multi-Drug Resistance in *Mycobacterium tuberculosis* Strains Collected from Patients in the Ukraine

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Background: In the Ukraine, there are an estimated 700,000 people infected with *Mycobacterium tuberculosis* (MTB) and the country ranks among the top twenty with the highest multi-drug resistant tuberculosis (MDR-TB) burden in the world. Next-generation sequencing (NGS) has become an important molecular tool for confirming, characterizing, and discovering genetic mutations in MTB genes known to confer antibiotic resistance. There is little molecular data characterizing the types resistance mutations from clinical isolates obtained from the Ukraine.

Aim: We used whole-genome sequencing (WGS) to characterize the *rpoB, inhA, and katG* genes from a random sampling of 73 MTB clinical isolates collected from four regions in the Ukraine.

Methods: Clinical isolates grown in Löwenstein–Jensen medium were inactivated in PrimeStore Molecular Transport Medium® (PS-MTM) and shipped from Kiev, Ukraine to San Antonio, Texas, USA at ambient temperature. MTB in PS-MTM was extracted and subsequently subjected to WGS using the Illumina MiSeq. Bioinformatics analysis of drug resistance genes was performed using LaserGene Version 12.3 (DNAStar).

Results: Of 73 total strains, 66 (90%) contained one of several known resistance-conferring mutations in the 81-bp *rpoB* determining region. The S-531-L mutation was most prevalent, but mutations at positions 511, 516, 526, 533 and 535 were also noted with varying amino acid substitutions including four isolates containing a mixed-strain amino acid. Analysis of the *katG* gene revealed the S-315-T isoniazid conferring resistance mutation in 64 (89%) of 73 strains. There were a total of 59 (81%) clinical isolates that contained an R-463-L, a mutation with no antibiotic effect. Analysis of the *inhA* gene revealed that all clinical isolates (100%) were 'wild-type' compared to the H37Rv strain. There were 3 strains that were *rpoB* mono-resistant and one strain that was mono-resistant for *katG* but wild-type in the *rpoB* gene.

Discussion: This study is one of the first to use WGS to characterize MDR genes from TB patients in the Ukraine. Rapid analysis of genes associated with drug resistance is a major challenge for successful treatment of TB in the Ukraine. In this study several unique substitution mutations were deduced that may have been overlooked by Hain LPA or other molecular methods. Detection of rifampin or isoniazid-mono-resistance indicates that *rpoB* or *katG* analysis alone cannot be used to determine MDR cases. WGS is a powerful tool for direct detection of MDR/XDR as well as discovery of novel resistance-conferring amino acids outside of known resistance sites. A thorough genome analysis is underway to evaluate pre-XDR and XDR resistance genes from these samples.