TB Genome sequencing in the Kyrgyz Republic

The Kyrgyz Republic has one of the highest TB drug-resistance (DR-TB) rates in the world, with nearly one-third of new and 60% of retreatment cases estimated to have DR-TB. Current laboratory methods, however, for testing of drug-resistance can be challenging to implement and have not kept up with developments of new drugs. Genome sequencing allows both for the identification of *Mtb* and if there are any mutations indicative of drug-resistance. Next-generation sequencing technology was first introduced in TB settings in the Kyrgyz Republic in 2019 under the *USAID Defeat TB project* implemented by Abt Associates. As part of the project, the Illumina MySeq system was procured along with consumables; the overall cost was about $300,000. The equipment has been installed at the National Reference Lab (NRL) at the National TB Program in Bishkek and has been fully functional since then. The main objectives of the project were to introduce genotyping in a high burden setting and develop national capacities for use of genotyping for both surveillance and in routinized clinical and program operations.

From 2019 to date, 679 whole genome sequencing tests were performed at the National Reference Lab. Initial inclusion criteria covered MDR TB patients from the Bishkek, Kemin, and Issyk Kul areas. Following the sequencing of DNA, the results were uploaded to the global database and evaluated by Borstel Supranational Lab (SNRL) in Germany, a partner organization in the Defeat TB Project. The project successfully analyzed the existing resistance patterns in the Kyrgyz Republic and results revealed that 72% of analyzed strains belonged to the Beijing lineage.

As a result of this initiative, both whole-genome and targeted sequencing were included in the updated diagnostic algorithm in 2019, which is part of the MOH approved MDR TB guidelines adopted in 2020. The Kyrgyz Republic was the first country in the region to incorporate genotyping in its diagnostic and treatment algorithms.

Also, the NRL has successfully passed an external quality assurance assessment performed by Borstel SNRL in 2019 as part of the lab-to-lab comparison for next-generation sequencing of MTB, with 100% accuracy on 28 genome sequencing compared. The next external quality assurance is taking place in 2021.

With the *Defeat TB project* ending in 2019, next-generation sequencing has not stopped at the NRL and was further supported through the *SeqMDRTBNet project* started in 2019 and funded by the German government. Under this global project also implemented in Moldova, Namibia, and Mozambique, next-generation sequencing was supported in select patient groups focusing on M/XDR TB. Apart from consumables, the project also covered several trainings.

The *SeqMDRTBNet project* has recently introduced targeted sequencing as well. 78 tests were performed to date and the target is tentatively set to increase to 500 tests in 2021.

While NGS in both *Defeat TB and SeqMDRTBNet projects* was primarily implemented for research purposes, starting from 2020, the sequencing results are being released to clinicians as part of clinical case management. In 2021, with the introduction of an electronic TB register, the register has designated fields for NGS results as well. The plans for 2022 also include trainings for clinicians to ensure that the NGS results are being integrated in routine clinical management to ensure more precise and appropriate treatment regimens based on drug resistance patterns and reducing probabilities for the development of further amplified resistance.

To build on these successful developments, the current USAID Cure TB project, implemented by JSI in the Kyrgyz Republic, is planning the procurement of additional 700 kits for targeted sequencing and 300 kits for whole-genome sequencing to introduce universal NGS testing for DR-TB patients in 2022.

In addition, the platform will be used to integrate COVID-19 sequencing for COVID-19 PCR positive patients and help improve the public health response to the COVID-19 epidemic. The results from this study will: a) determine virus strains prevalent in Kyrgyz Republic and their effect on the epidemiological situation; b) conduct regular monitoring of the virus evolution/mutations and c) study the influence of specific strains on the clinical manifestations of COVID-19 disease. While the total number of tests is yet to be determined, tentatively 300 tests will be performed in 2021.

This is another example of how TB platforms and approaches can benefit the COVID-19 response.