Validation of Whole Genome Sequencing of *Mycobacterium tuberculosis* Antibiotic Resistance Functional Mutations at the Health and Environmental Testing Laboratory

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BACKGROUND:

By sequencing the entire genome of clinical *Mycobacterium tuberculosis* isolates and comparing high quality single nucleotide polymorphisms and other mutations to a curated database, it is possible to identify all known functional resistance mutations and build a repository of potential antibiotic resistant mutations. Bioinformatic analysis of whole genome sequenced *Mycobacterium tuberculosis* can influence the clinical management of tuberculosis weeks before traditional drug susceptibility results are finalized by increasing successful antibiotic treatment and reducing the likelihood of antibiotic resistance developing.

METHODS:

Nucleic acid was extracted and purified from historical *Mycobacterium tuberculosis* isolates grown on Lowenstein-Jensen medium using a combination of heat, bead beating, and solid phase anion exchange matrix. Mycobacterial genomes were sequenced on the Illumina MiSeq using Illumina NexteraXT protocols. CLC-BIO (Qiagen) bioinformatics software was used to align sequenced isolates to the *Mycobacterium tuberculosis* H37Rv NC_018143 reference strain. *In silico* analysis of antibiotic resistance functional mutations was performed using an in-house database modeled after TBDReamDB, Tuberculist, PhyResSE, TB profiler, Resfinder, and TGS-TB databases.

RESULTS:

For this validation, 31 samples in total were analyzed; 24 *in silico* only samples and 7 samples grown from culture, sequenced, and then analyzed. All samples were analyzed by an in-house workflow created in CLC-BIO (Qiagen) and results compared to samples analyzed by Mykrobe and ResFinder. After accounting for differences in individual databases, our in-house analyses were 94.28% sensitive, 100% specific, and 99.26% accurate. *In silico* variability studies and interpretations between technicians were 100% sensitive, 100% specific, and 100% accurate.

CONCLUSION:

HETL will now routinely perform whole genome sequencing on all *Mycobacterium tuberculosis* isolates. Despite this technology, whole genome sequencing still cannot supplant traditional methods as the laboratory and clinical guidelines still mandate that all results must be interpreted in the context of other molecular or growth-based drug susceptibility test results and clinical history. While there is enough laboratory evidence that there are so called ‘high-quality’ and ‘low quality’ functional mutations, there is also a growing consensus that there is a synergistic relationship between functional mutation phenotypes, where a combination of inefficient functional resistance mutations can create a high level resistant bacterium whereas individually they cannot. Not until the laboratory and medical community agree that ‘high-quality’ mutations always result in a resistant phenotype will this technology finally supersede pyrosequencing, PCR, and susceptibility testing.