• In the list of the mutations in the table presented regarding confidence grading- what do the bold mutations represent?

The table includes all the mutations graded according to the proposed standardized approach for providing confidence levels to their association with phenotypic drug resistance. Standard type represents associations based on nominal p-values (putative); bold type represents associations based on corrected p-values.

• In your presentation only LR is used or described, how are OR with LR used together?

In the reference PMID: 29284687 supplementary tables report also OR; since LR and OR are basically similar ways to present the same thing, for easiness during the webinar I used LR only. Details on what LR and OR are representing in the grading system can be found in slides 16-17-18 of the webinar.

• What do we know about the newer drugs? Is there data on BDQ, DLM, and repurposed LZD, CFZ?

At this stage there is little evidence available, thus it was not possible to provide any grading score for mutations associated with resistance to such drugs. The Cryptic Project (http://www.crypticproject.org/), and other initiatives will likely contribute to build such missing evidence.

• In TB are there any known mutations that increase a strain’s susceptibility to a drug? I am thinking about HIV, where an M184V mutation confers resistance to lamivudine and emtricitabine but increases susceptibility- to tenofovir.

There are no many mutations known, but at least a combination of mutations in the gyrA gene has been associated with hyper-susceptibility to fluoroquinolones (PMID: 16377674). Genome-wide association studies will allow to identify any additional mutation causing increased susceptibility to drugs.

• Is this constructed using samples from only pulmonary Tb patients?

• The grading system (PMID: 29284687) was based on any sequencing and related phenotypic data available, irrespective of isolation source, but most of the isolates were from pulmonary TB. Similarly, the ReSeqTB platform is considering any WGS data, but most are from PTB isolates.

• There are many factors such as strain, resistance which are directly known for a change in nucleotide, how is it the SNP that is for resistance can be selected.

SNPs, insertions and deletions associated with resistance were compiled from several different published sources, you can find one example listed here: https://www.nejm.org/doi/full/10.1056/NEJMoa1800474

Presentation 3: “Standardized clinical reporting of sequencing data for DR-TB diagnosis”, Angela Starks, CDC

• How long does TB sequencing take from sample to results? TAT total in lab.

Whole genome NGS: sample collection to culture growth 7-15 days + sequencing and reporting 5-6 days
Target-based NGS: from sample collection, sequencing to reporting – 5-6 days

• Who would be considered “experts” for consultation?

This is a very good question. This should be determined by each country. It should be an individual with vast amount of experience with DRTB treatment and a strong knowledge base regarding mutations that correlate with resistance. This will need further consideration as to how one will define and “expert” related to the interpretation and use of sequencing data for clinical case management.