MARCH 2025 LabCoP ECHO SESSION

Phenotypic drug susceptibility testing – general concepts
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Understanding drug resistance

1. Is MIC also for other drugs varying by lineage?
   This has not been systematically studied for all anti-TB drugs, yet MIC distributions seem to be normal for wild type strains for other drugs than pretomanid and pyrazynamid. Indeed, for lineage 1, the MIC is increased also for pyrazinamide (https://doi.org/10.1128/aac.01916-20 and https://doi.org/10.1128/aac.02617-20). In addition, few exceptions are known as depicted in the table below.

	Pyrazinamid	Cycloserine	Isoniazid	Pretonamid	Fluoroquinolones	Marcolides
M. bovis	Resistant *pncA*_H57D mutation					
M. bovis (BCG)	Resistant *pncA*_H57D mutation		Increased MIC *mmaA3 mutations*			
M. bovis (BCG) RD2 deleted						Susceptible
M. canettii	Resistant unknown	Resistant *cycA*_G122S mutation		Resistant unknown		
*M. tuberculosis gyrA*_T80A/ A90G mutants					*Hyper susceptible*	
M. tuberculosis lineage 1				Increased MIC		

2. Could you explain a bit more about the risk of false resistant results in settings with low resistance prevalence and the need to do MIC in such a setting? What do you consider low prevalence?
   See Chapter 3.2 "Pretest probability and test accuracy considerations" in the WHO operational handbook on tuberculosis: rapid diagnostics for tuberculosis detection, third edition. The predictive values of a test vary depending on the prevalence of the disease or drug resistance in the population being tested. Low prevalence is usually defined when it is lower than a certain prevalence threshold (2-5%). If pretest probability is low and given that specificity for the diagnostic test is high but not 100%, the absolute number of FP is higher because there are more TN in the population (TP are rare). MIC testing provides a value for the level of resistance (nearby or distantly from the critical concentration, while binary pDST only provides an R/S classification which may be erroneous.

3. There are strains of Mtb carrying rpoB I491F mutations, which are known to be missed by traditional MGIT culture prediction due to the concentration of RIF used in these methods. In these instances, how can DST be done to detect such strains?
   Solid medium for pDST allow growth for up to 6 weeks, overall detects the I491F mutation. Also, the alternative (not yet WHO recommended) thin-layer agar method with direct DST allowed for detection of most I491F mutants in Eswatini (doi.org/10.1128/aac.02263-20).

4. How do we interpret resistance to fluoroquinolone and susceptibility to RIF and INH. Is this scenario even possible?
   In theory it is possible, particularly in areas and settings where FQs are used frequently in the community to treat common diseases. In case of undetected coinfection with MTB, these TB bacilli could acquire FQ resistance. Recent molecular studies identified 6.2% (20/324) FQ-resistance among rifampicin-susceptible TB in Taiwan (doi.org/10.3389/fpubh.2022.990894), and 4.0% (27/676) among rifampicin-susceptible TB in the Zhejiang Province in China (doi.org/10.3389/fmicb.2024.1413618). Also in Pakistan, high levels of FQ-resistance have been observed (https://doi.org/10.1016/j.ijmyco.2014.10.046). In conclusion, even though it is not expected to be very common, the prevalence is context specific. Knowing your epidemiology is very important. Of course, also your QC and EQA data will determine the reliability of your observed FQ-resistant results.

5. On heteroresistance, do I understand well that when comparing Xpert vs pDST (as reference) results, you would expect false sensitive Xpert results but not false resistant results?
   Correct, in case of sufficiently high bacterial load (high, medium and low) we don't expect false-resistant rifampicin results. For both Xpert-Ultra and Xpert-XDR, heteroresistance for rifampicin, isoniazid and fluoroquinolones remains undetected if the frequency of the minority variant is too low. The detectable % varies across mutation types: 20-40% for Xpert-Ultra RMP-heteroresistance detection (doi.org/10.1128/mbio.00812-17), 20% to 50% for Xpert-XDR FQ-heteroresistance detection (oi.org/10.1128/jcm.02314-20), and ~20% for Xpert-XDR INH-heteroresistance detection. Another cause of false resistance may be the presence of silent mutations (nucleotide change in the DNA but not leading to amino acid change in the protein), regardless of the bacillary load. This phenomenon is rare though, particularly in essential genes such as the *rpoB* gene.

6. How has Whole genome sequencing advanced our understanding in MDR and XDR MTB?
   WGS data provides the most comprehensive genetic data when it comes to (potentially) drug-resistance associated mutations. Particularly, in combination with MIC data (and even binary phenotypic DST data) they provide insight in the molecular mechanism behind the observed phenotypic resistance. This data helps us to associate genotypic and phenotypic resistance. For research purposes, WGS data allows to have a comprehensive overview of all potentially associated mutations and hence is informative for developing rapid molecular assays (in case the number of genes and variants involved is limited). For patient management, WGS data allows us to have more comprehensive molecular information compared to rapid tests and targeted next-generation sequencing. This is only relevant if the TB bacilli have mutations in (regions of) genes (sequences) that are not targeted in the rapid molecular or tNGS assays. For the newer drugs, not all resistance mechanisms/genetic variants are known. Hence, WGS data in combination with MIC (pDST) data is still needed to gain more insight.

7. I want to know if we must consider pDST results instead of genotypic ones in case of drug resistance. Also, where to find bedaquiline drug for pDST.
   This may be drug dependent. For rifampicin for instance, where we know that MGIT-DST or other rapid phenotypic tests may miss detection of borderline mutations, genotypic testing is the preferred method, particularly if the entire rpoB gene is considered. This can be done by sequencing, while WHO recommended rapid assays only target the rifampicin-resistance-determining region (RRDR). In some geographical regions, RR-TB strains with a mutation outside the RRDR may be very common (e.g. the I491F mutation in Eswatini).

For many of the new drugs (bedaquiline, delamanid, pretomanid), our knowledge about the association of observed mutations in the various genes involved in the drug (resistance) mechanisms, is incomplete. Therefore, phenotypic testing is still the preferred method for patient management guidance for these drugs. For research purposes, there is a need to have more combined pheno- and genotypic data for isolates from different lineages and geographical regions. The Global Drug Facility (GDF) recently announced to have larger quantity vials of bedaquiline available in their catalogue (https://www.stoptb.org/news/stop-tbs-global-drug-facility-announces-38-price-decrease-bpalm-regimen-and-newly-available).

8. Could you elaborate a bit on resistance to PZA? I understand it is the only drug active against population C. How common is resistance (compared to rifampicin and isoniazid)?
   PZA is active in an acidic environment such as the macrophages, hence targeting the intracellularly residing MTB bacilli.
   PZA resistance occurs mostly among rifampicin-resistant TB, with a global prevalence of ~50-60% in this target population (doi.org/10.1371/journal.pone.0133869; http://dx.doi.org/10.1016/ S1473-3099(16)30190-6).

9. Sometimes we found BDQ mono Res, in pDST, how you interpret this? If you found phenotypically BDQ res but no mutation in NGS (t), what can be done in this case?
   Epidemiologically and historically, we wouldn't expect BDQ-mono resistance, as BDQ has been introduced relatively recently, and meant for patients with TB bacilli already resistant to at least rifampicin (and isoniazid).
   However, BDQ resistance-associated mutations leading to phenotypic BDQ resistance, has been documented occasionally in BDQ-naïve patients, i.e. in TB bacilli of patients who have never been treated with BDQ (https://doi.org/10.1186/s12866-022-02475-4 ; https://doi.org/10.1128/aac.00322-22; https://doi.org/10.1101/2020.05.27.120451)
   However, all these isolates had evidence of genetic variants.
   In case, no genetic variants are observed along with phenotypic BDQ-mono resistance, false phenotypic resistance is suspected. It is important to carefully check the results of the QC strain of that batch of medium (H37Rv = susceptible), to check the expire date of the medium used, and particularly of the stock solution used (maximum storage of 3 months at -20°C for BDQ). Also, BDQ phenotypic testing (and preparation of all drug solutions) should only be done in glass or polystyrene. Do not use polypropylene, as the BDQ will stick to it, and hence not be available in your solution/medium.

Procedures for pDST

1. Are there any procedures or SOPs for performing DST using pretomanid drug?
   The BACTEC MGIT 960 PZA kit (cat No. 245128BD, Cat No. GDF 106033) is used for PZA MGIT testing. More details on Susceptibility testing for anti-TB agents using liquid media in the WHO Annex available at this link: https://iris.who.int/handle/10665/376286

   Instructions for testing pretomanid and cycloserine can be found in the WHO Annex B available at this link: https://iris.who.int/handle/10665/376285

2. Has pretomanid reconstitution been updated in WHO manual?
   You will find more details here: https://iris.who.int/handle/10665/376286 and https://iris.who.int/handle/10665/376285 and more in the upcoming GLI training material on pDST.