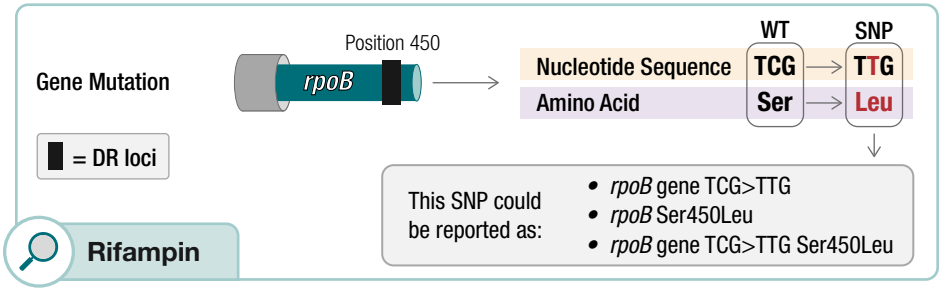
TB: Next Generation Sequencing and Molecular Drug Susceptibility Testing

As laboratories expand their next generation sequencing (NGS) capabilities, it is increasingly possible to use molecular drug susceptibility testing (mDST) to predict *Mycobacterium tuberculosis* (MTB) drug resistance (DR) by identifying key mutations in the MTB genome known to be associated with DR.

This document focuses on resistance associated with changes at the genetic level (i.e., mutations), though other factors can also result in observed resistance, such as intrinsic resistance and expression of efflux pumps.

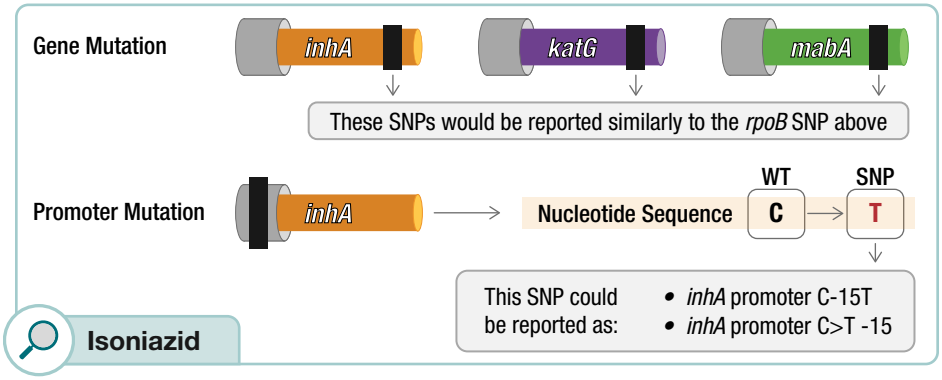
Mutations that confer DR can be simple...

Sometimes resistance to a drug is associated with a single gene or area of a gene.



...or complicated.

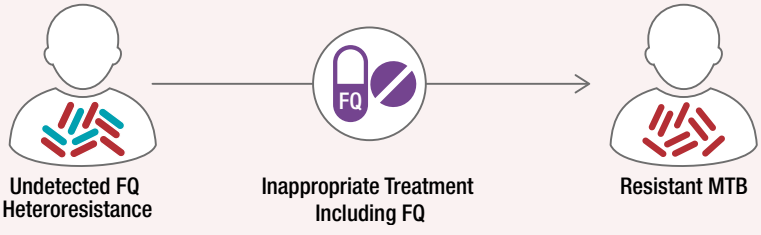
Resistance may stem from a mutation within one of several genes or their promoters, making identification more challenging.



Heteroresistance is important to detect.

If heteroresistance is undetected in a patient infected with a mixed population of fluoroquinolone (FQ) susceptible and FQ resistant MTB, FQ treatment may not effectively clear the FQ-resistant subpopulation.

NGS-based methods can be used to detect heteroresistance in MTB.



Drug Resistance Terminology

DST: Drug susceptibility test; there are two categories of tests:

- **Phenotypic DST (pDST):** Growth-based antimicrobial susceptibility (e.g., MGIT, agar proportion)
- **Molecular DST (mDST):** Detection of genetic mutations associated with DR

Discordance: Lack of agreement between laboratory results (e.g., when two pDSTs or a pDST and mDST produce discrepant results)

Heteroresistance: Coexistence of organisms susceptible and resistant to the same MTB drug in a patient

Intrinsic Resistance: Innate ability of a particular species to resist a certain antibiotic or family of antibiotics

Low-level Resistance: Organisms resistant to low-level drug concentrations

Genetic Terminology

Amino Acid: Building blocks of protein

Codon: Three consecutive nucleotides that code for an amino acid

Gene: DNA sequence that encodes specific traits

Locus/Loci: Fixed position on chromosome where a particular gene, genetic marker or mutation is located

Numbering System: Specific location in TB gene or loci where a mutation is located

Promoter: Region within the genome that initiates the expression of a gene; promoters do not code for protein

Indel: Insertion/deletion

Silent Mutation: Change to nucleotide secuence that does not result in a change to the amino acid

SNP: Single nucleotide polymorphism, a variation at a single nucleotide position; the most common type of mutation causing DR in MTB

Variant or Mutation: Alteration in the nucleotide sequence of an organism that may or may not have a phenotypic effect

WT: Wild Type; standard genetic sequence to which mutations are compared (e.g., H37Rv is a commonly used WT reference strain for MTB)

Overview of Drug Resistance Mutations in MTB

Decades of research have helped define many genetic mutations associated with DR. Understanding the most common of these mutations is essential for effectively leveraging the power of mDST.

Notable DR-associated mutations for each drug/gene are noted as:

Mutation(s)

Rifamycins (RIF) & *rpoB* Gene

- Rifamycins include: rifampin (rifampicin), rifabutin and rifapentine.
- ~95% of DR mutations are found in the 81-basepair RIF Resistance Determining Region (RRDR).
- Although less common, mutations outside of the RRDR can confer DR.
- A subset of mutations confer low-level resistance and may not be detected by pDST.

***rpoB* Ser450Leu, His445Tyr, His445Asp, Asp435Val**

Isoniazid (INH) & *katG* Gene

- *katG* mutations are responsible for ~85% of observed INH DR.
- Most frequent *katG* mutation is Ser315Thr, though other *katG* variants also cause resistance.
- Mutations in the promoter and genes for *inhA* and *fabG1(mabA)* may also be associated with INH DR.
- Silent *fabG1(mabA)* mutation Leu203Leu is also know to be associated with INH DR.

***katG* Ser315Thr**

Ethionamide (ETH) & *inhA* and *ethA* Genes

- INH and ETH are structural analogs and cross-resistance is common.
- Mutations in *ethA* are known to be associated with ETH DR.
- *inhA* promoter mutation C-15T is often associated with low-level INH and ETH resistance.
- Mutations in *fabG1(mabA)* are also associated with ETH DR.

***inhA* C-15T**

Pyrazinamide (PZA) & *pncA* Gene/Promoter

~85% of *pncA* genetic variants are associated with phenotypic PZA DR.

All *M. bovis* (including BCG strain) have a *pncA* His57Asp mutation and are resistant to PZA

Ethambutol (EMB) & *embB* Gene

- SNPs in *embA*, *embC* and the embC-embA promoter region have also been associated with EMB DR.
- Not all EMB DR associated mutations are known and discordance between methods can be observed.

***embB* Met306Val**

Fluoroquinolone (FQ) & *gyrA* Gene

- FQs used for MTB treatment include moxifloxacin, levofloxacin and ofloxacin.
- Mutations within the quinolone-resistance-determining region (QRDR) of DNA gyrase subunit *gyrA* are most frequently linked to DR; mutations in the QRDR of *gyrB* can also be linked to DR.
- Heteroresistance is common and can cause discordant results.

***gyrA* Asp94Gly, Ala90Val**

Second Line Injectable Drugs & *rrs, eis* and *tlyA* Genes

Second line injectable drugs include amikacin, kanamycin and capreomycin.

***rrs* A1401G**

New TB Drugs

The following drugs and genes have known DR associations:

- **Bedaquiline** & **Rv0678** and ***atpE***
- **Linezolid** & ***atpE, rplC*** and ***rrl***
- **Delamanid** and **pretomanid** & ***fgd1, ddn, fbiA, fbiB, fbiC*** and ***fbiD***
- **Clofazimine** & ***pepQ, Rv0678, mmpL5*** and ***mmp***

Note that newer drugs have less robust DR data, so continued tracking is essential.

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