Resistance Testing and Interpretation of Discordant Results

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…can you help me with these DST results?

Case #1

- 23 y/o patient gave birth 8 days ago to a healthy male child that she has been breastfeeding. She visits the ED for cough and a CXR shows bilateral upper lobe cavities
- Sputum is AFB smear positive, GeneXpert MTB/RIF was positive for the presence of *M.tb* and for **rifampin resistance**. MDDR was ordered. Her newborn was evaluated and ruled out for active disease, then started on levofloxacin and high dose INH
- The MDDR returned with mutations in *inhA*/*katG* (not fabG1), *rpoB*. Mutations in *embB* and *pncA* of unknown effect. No mutations in *gyrA* or *gyrB*
- Q: The baby is on window prophylaxis due to 8 days of exposure. What can this tell you about likely fluoroquinolone resistance in the organism?

MDDR Results

- Assuming high-level INH-R from *inhA*/*katG* results
- Heteroresistance often observed for fluoroquinolones (FQs) so cannot rule out resistance
  - Typically, would need ~20% of population to have mutant allele for detection by Sanger sequencing
  - Next generation sequencing will help to lower limit of detection (~10%)
- Growth-based DST should follow sequencing
  - Would detect FQ-R by growth-based methods (possible discordance with molecular results)
  - If growth on FQ (i.e., resistant), could sequence growth to determine specific mutation
  - If no growth on FQ, would consider susceptible

Case #2

- 46 y/o woman presents with dysfunctional uterine bleeding
- An endometrial biopsy specimen showed granulomas on pathology and cultures grew out *M.tb*. Susceptibilities returned with low level INH and PZA resistance
- Q: How reliable is PZA DST and would checking for *pncA* mutations be more reliable?

Pyrazinamide Testing

- Reports of issue with false resistance and susceptibility but primarily false resistance
- Testing for PZA requires acidified media but pH range is narrow
- PZA DST sensitive to inoculum size
  - Reduced or modified inoculum can mitigate occurrence of false resistance
- PZA is prodrug and must be converted to pyrazinoic acid for activity
  - Pyrazinamidase (PZase) for this activity encoded by *pncA*
  - Mutations in *pncA* that inhibit PZase activity= PZA resistant
- Mutations in *pncA* primarily responsible for PZA-R (70–95%)
- *M. bovis* inherently resistant to PZA (His57Asp in *pncA*)

https://jcm.asm.org/content/55/12/3552

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5698819/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3536208/

Sequencing *pncA* may be more reliable than current phenotypic testing

- In United States, some laboratories already performing pncA sequencing instead of or in addition to growth-based methods
  - Wild type might be considered susceptible
  - Nonsynonymous mutations might be considered resistant unless otherwise proven not to be associated
- No hot spot region like other genetic loci
  - Mutations can be spread throughout the open reading frame
- Still understanding contribution of some mutations
  - Not all nonsynonymous mutations are associated with PZA-R
- Other potential mechanisms?

https://www.researchgate.net/publication/267737255_Mycobacterium_tuberculosis_Pyrazinamide_Resistance_Determinants_a_Multicenter_Study

https://pubmed.ncbi.nlm.nih.gov/26292310/

Case #3

- 40 y/o woman with history of adherence difficulties x1-year for pan-sensitive PTB, now returns with molecular evidence by **Xpert, PSQ and MDDR for RIF resistance (*rpoB*)**
- PSQ and MDDR report Asp435Tyr mutation as a **"disputed mutation"** – can you help me understand what this means for me as a clinician making decisions?
- Phenotypic DST showed sensitive for RIF – **molecular vs. phenotypic discordance** – now what?

Case contribution: S. Katrak CDPH

Low level RIF-R mutations

- Asp435Tyr (Asp516Tyr) is located within the rifampin resistance determining region (RRDR)
  - Referred to as discordant, disputed, or low-level resistance conferring mutation
- Mutation results in elevated MIC near the current critical concentration used for testing
  - Isolates may test as susceptible or have variable results

Low level RIF-R mutations (2)

- Recent WHO technical report recommended change to rifampin critical concentration
  - Resolve discordance between molecular and growth-based results
- WHO Technical Expert Group recommended that mutation would indicate need for treatment using MDR-TB regimen

file:///C:/Users/eog0/Downloads/9789240017283-eng.pdf

Case #4

- Patient treated for TB in India 2011, slow response so treatment extended
- Recurrent respiratory infections 2013-2017 with multiple rounds of levofloxacin and linezolid
- In 2019, the patient has relapsed TB; Xpert positive for *M.tb* and rifampin resistance
  - MDDR suggests INH, rifampin, EMB, PZA resistance
  - *gyrA*, no mutations
  - *gyrB*, Asn538Asp mutation
- Questions:
  - What additional evaluation can the lab do to address possible FQ resistance, especially to levofloxacin and moxifloxacin?
  - What is the significance of not finding a *gyrA* mutation?
  - What is the significance of the *gyrB* mutation?
  - Would WGS add any information

Next steps…

- Mutations associated with FQ-R primarily in quinolone resistance determining region (QRDR) of *gyrA*
- Mutations in *gyrB* QRDR are infrequent and less well characterized
- Asn538Asp mutation (also reported as Asn499Asp with revised numbering) associated with resistance to MFX and LFX
  - Functional genetic studies confirmed role in resistance
- Growth-based DST should be performed to assess cross-resistance among FQs
  - Level and pattern of resistance might vary with different *gyrB* mutations
- Lack of *gyrA* mutation could be due to heteroresistance with variant population below limit of detection for molecular assay
  - In MDDR service, presence of both *gyrA* and *gyrB* QRDR mutations in same isolate uncommon

https://pubmed.ncbi.nlm.nih.gov/22761889/

Case #5

Patient with an initial episode of TB

- MDDR:
  - *rpoB* mutation (Ile572Phe) – probably clinically relevant low-level resistance
    - Is additional evaluation available to determine degree of resistance?
    - Is MIC testing for rifampin and rifabutin available? If not, are there plans for it in the future?
      - Rifabutin MIC at another lab ≤0.12; if real has clinical implications for treatment of patient and her 11 y/o daughter
  - *gyrA* mutation (Ser91Pro)
    - Does this mutation give additional information on how resistant the organism is to the FQs?
    - How do specific mutations give suggestion on degree of resistance?
    - What additional testing can be done?
      - MIC for moxifloxacin = 4.0 (resistant)

Low level RIF-R mutations

- Ile572Phe also referred to as Ile491Phe when using *M. tuberculosis* numbering system is located outside the rifampin resistance determining region (RRDR)
  - Referred to as discordant, disputed, or low-level resistance conferring mutation
  - Experimentally, determined to elevate MICs in *M. tuberculosis*
- In United States, 8 isolates with this mutation (2018–2020) compared to 256 isolates with the most common Ser531Leu mutation

Low level RIF-R mutations (2)

- MIC testing might be performed
  - Proposed breakpoint for RIF (≤ 1 µg/ml) for commercial broth microdilution assay (CLSI document M62)
  - MIC testing not widely available and no FDA cleared assay for this purpose
  - CDC TB Laboratory implementing MIC testing in 2021 with reporting for limited drugs
    - Additional drugs to be added to clinical report in future

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Fluoroquinolone resistance

- Resistance primarily associated with nonsynonymous mutations in the QRDR of *gyrA*
  - Mutations in *gyrB* also less frequently reported
- Ser91Pro considered a high-confidence resistance marker
- Substitutions occur in *gyrA* at codons 88, 90, 91, and 94
  - Gly88Cys, Asp94Gly, Asp94His, and Asp94Asn—high MFX MICs
  - Asp89Asn, Ser91Pro, and Asp94Tyr—moderate MFX MICs
  - Ala90Val and Asp94Ala—low MXF MICs
- Level of resistance to FQs dependent on specific mutation
- Heteroresistance often observed in FQ-R isolates
- Additional testing: growth-based drug susceptibility testing and perhaps MIC testing

https://jcm.asm.org/content/jcm/54/3/727.full.pdf
https://www.who.int/tb/publications/2018/WHO_technical_report_concentrations_TB_drug_susceptibility/en/