### Journey to the Center of the MTB Complex

#### (Making Sense of Laboratory Test Results in TB Management)

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**Why is the laboratory so confusing?**

Why don’t all laboratories do it the same way?

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<table>
<thead>
<tr>
<th>Drug Susceptibility Testing</th>
<th>Genotypic</th>
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<tbody>
<tr>
<td>Agar Proportion</td>
<td>Molecular</td>
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<tr>
<td>BACTEC</td>
<td>PCR</td>
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<td>MGIT</td>
<td>Beacons</td>
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<td>TRENK</td>
<td>Sequencing</td>
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<td>MIC</td>
<td>Sanger</td>
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<td>Critical Concentration</td>
<td>Pyro</td>
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<tr>
<td>Equivalent Critical Concentration</td>
<td>MDDR</td>
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<td>Culture-based</td>
<td>Silent mutation</td>
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<tr>
<td>Conventional</td>
<td>rpoB</td>
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<tr>
<td>Phenotypic</td>
<td>inhA</td>
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<td></td>
<td>katG</td>
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<td>gyrA</td>
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<td>etc., etc., etc.</td>
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Learning Objectives:
Laboratory cases will be used to:

- Explain how molecular tests are used for rapid detection of drug resistance;
- Discuss considerations for interpretation of molecular tests; and
- Identify benefits and limitations of these tests and conventional drug susceptibility testing.

“Rules of the Lab”

- No lab test is perfect
- Do not order a lab test if you are not ready to deal with the result
- Treat the patient, not the lab test
- If you do not know what something means, call the lab
- For TB—There is a lot we still need to learn about DST and molecular detection of drug resistance
  - Discordance
Benefits of Molecular Detection of Drug Resistance

- Rapid results within days as compared to weeks for conventional testing
- Expedite further conventional testing (e.g., second-line drug susceptibility testing)
- Development of technologies requiring limited biosafety infrastructure; does not require BSL-3 once DNA is extracted
- Information provided by some platforms may be used to enhance accuracy of conventional DST

Limitations and Considerations

- Still filling in gaps in knowledge about drug resistance (phenotypic and genotypic testing)
  - Not all mechanisms of resistance are known and the lack of a mutation ≠ susceptibility
  - Not all mutations are associated with phenotypic resistance
  - Silent (synonymous) mutations—no change in protein
  - Neutral polymorphisms (e.g., gyrA codon 95 may be Ser or Thr)
  - Gold-standard DST may not be perfect
  - Mutations resulting in elevated MICs but S at critical concentration (e.g., Leu511Pro in rpoB)

- Limited genes and sites are targeted
  - inhA and katG only 85-90% sensitive for detecting INH resistance

- Emerging resistance (mixed populations) may not be detected; limit of detection of assay

Limitations and Considerations (2)

- Clinical utility: Do results impact patient care? Will clinicians and programs "trust" these results or "wait for the conventional DST result?"
  - Output from the assay depends on the platform
  - Need to understand platform to understand limitations

- Expertise of staff (laboratory, clinical, and program)
  - Output from the assay depends on the platform
  - Need to understand platform to understand limitations

- Educational partnerships (laboratory, program, and clinicians) need to be developed
How to report results?

Weighing Genotypic versus Phenotypic Results

- The term ‘Gold Standard’ can be misleading.
- New, previously uncharacterized or poorly characterized mutations
  - Reported as clinical significance unknown
  - Anecdotal information may be reported
- Functional genetic analysis is necessary to definitely determine effect of mutation on resistance
- Need to develop standardized reporting language

Why don’t all laboratory results agree?

Understanding Discordance

- Can have discordant results
  - Between different phenotypic DST results (e.g., MGIT 960 and agar proportion)
  - Between phenotypic and genotypic (molecular) results
  - Between different genotypic results (e.g., GeneXpert and Sanger sequencing)
- Can occur within a lab, between labs, between different methods, and within the same method
- Which is correct?
  - Both, only one, neither
What causes discordant DST results?
- "Human error/lab error"
- Transcription, labeling errors
- Cross contamination / specimen mix-up
- Different "inoculum" / bacterial population
  - e.g., isolates from different specimens; sampling from same specimen; original isolate vs. subculture
  - Size of inoculum/clumps
  - Different growth kinetics
- Different method or media
  - "equivalent" critical concentrations
  - "calling" result too soon
- The "bug" - MIC is close to the critical concentration
  - Evaluations performed with "highly resistant" bugs

What causes discordance between molecular and phenotypic DST results?
- "Human error/lab error"
- Not all mechanisms of resistance are known
  - The lack of a mutation ≠ susceptibility
- Limited genes and sites are targeted
- Emerging resistance (mixed populations) may not be detected
- Not all mutations are associated with phenotypic resistance
  - Silent (synonymous) mutations
  - Neutral polymorphisms
  - Output is "platform dependent"
- "Gold-standard" DST may not be perfect

What causes discordance between different molecular platform results?
- "Human error/lab error"
- Not necessarily looking at the same segment of DNA
  - e.g., looking for a particular single nucleotide polymorphism (SNP) in one codon versus looking at 30 codons
- Limited genes and sites within genes are targeted
  - katG only versus katG+inhA
- Emerging resistance (mixed populations) may not be detected; limit of detection for platform's different
Cases

Case # 1—Is it RMP-R?  
(RMP Discordance between molecular and conventional results)

☐ Smear (+) pulmonary TB; prisoner
☐ At hospital
  ▪ Xpert (X2) — RMP Resistance Detected
  ▪ DST (MGIT) — INH-R and RMP-S
☐ AP DST pending at State lab

☐ At CDC, rpoB DNA sequence – Phe514Phe
  • Silent (synonymous) mutation – no amino acid change and not clinically significant
  • Xpert detects wild type sequence; even though the mutation is not significant, called resistant

Case # 2 - Is it RMP R?  
(RMP discordance between broth and AP)

☑ State PHL DST results:
  • Bactec 460—R to INH; S to RMP (2 µg/ml)
  • AP (7H10)—100% R to INH; 80% R to RMP (1 µg/ml)

☑ At CDC:
  • rpoB—Asp516Tyr; RMP resistant
  • inhA—C(-15)T; INH resistant
  • 40% R to RMP by AP
Case # 3—Is it RMP-R?  
(RMP Discordance between molecular and conventional results)

- Pulmonary TB; Burma (Nepal camp)
- State Lab DST (MGIT) — INH-R and RMP-S
- rpoB DNA sequence — Asp516Tyr; RMP resistant
- CDC AP — RMP-S

rpoB mutations associated with highly discordant DST results  

- "Low-level" or "borderline" resistance
- Probably clinically relevant resistance
- Resistance often missed by standard, growth-based systems, especially automated broth systems
  - Critical concentration may be too high to cover all clinically relevant resistance.
  - Maybe the methods need modification (e.g., prolonged incubation, larger inoculum size) to detect resistance
- Frequency of these strains unknown
- Mutations: Asp516Tyr, Leu511Pro, Leu533Pro, His526Leu, His526Ser, Ile572Phe

Clinical failures associated with rpoB mutations in phenotypically occult MDR TB  

- Significant association between the presence of rpoB mutations that are not detected in DST and treatment failure
  - 4 had rpoB mutations (GeneXpert)
    - RRDR sequenced—Leu511Pro/Met515Ile, His526Asn/Ala532Val, Asp516Tyr, His526Leu
  - 3 of 4 were treatment failures; other was unknown
Asp516Tyr (CDC MDDR) (cases #2 and 3)

<table>
<thead>
<tr>
<th></th>
<th>RMP-R by DST # with mutation/Total</th>
<th>RMP-S by DST # with mutation/Total</th>
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<tbody>
<tr>
<td>Original (retrospective) validation</td>
<td>2/152</td>
<td>0/102</td>
</tr>
<tr>
<td>Prospective validation</td>
<td>0/17</td>
<td>1/63</td>
</tr>
<tr>
<td>Total</td>
<td>4/253</td>
<td>4/308</td>
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</tbody>
</table>

Case #4—Persistently smear (+) patient with drug susceptible MTBC

- **MDDR**
  - *rpoB, inhA, katG* all wildtype (**no mutations**)
  - *pncA* – mutation with unknown significance
- **AP DST**
  - R to INH, S to RMP
- **MGIT PZA—S**

- 10-15% of INH-R MTBC do not have a mutation in either *inhA* or *katG*

Case #5—Discordant results for INH and RMP between 2 laboratories

- **Lab A:** INH-R and RMP-R (MGIT)>>MDR
- **Lab B:** INH-S and RMP-S (MGIT and AP)
- **MDDR**
  - *rpoB, inhA, katG* all wildtype (**no mutations**)
- **AP DST**
  - S to INH, S to RMP
Question

What are the possible reasons for the discordant results?
1. Specimen mix-up
2. Technical error in lab A
3. Lab B and CDC did not test same bug as lab A
4. Lab A isolate mixed (e.g., with MAC)
5. All of the above

How have molecular tests for the detection of drug resistance changed clinical practice in United States?

Rapid Drug Susceptibility Testing with a Molecular Beacon Assay is Associated with Earlier Diagnosis and Treatment of Multidrug-Resistant Tuberculosis in California

- Retrospective analysis of 127 MDR TB patients
  - 27 had specimens tested by MB with confirmatory phenotypic DST (all with pulmonary disease)
  - 100 had phenotypic DST alone (80% with pulmonary disease and 20% with either extrapulmonary or both pulmonary and extrapulmonary disease)
- MB assay was associated with more timely detection and treatment of MDT TB

How important is it to rule out drug resistance?

Recent MDDR Case
- 28 yo male; born in US; traveled to India to get married; fell ill on return to US
- Community hospital referred isolate of MTBC from specimen to State PHL; contaminated with *M. fortuitum*; cannot get pure isolate for DST
- Patient on 8 drugs and not doing well
- Over a month later, mixed culture received at CDC – no mutations associated with resistance detected

What is more desirable?
An assay with the problem of false-R or an assay with the problem of false-S?

There are known knowns; there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also unknown unknowns – there are things we do not know we don’t know.

—Former United States Secretary of Defense Donald Rumsfeld
Conclusions

- Paradigm shift in laboratory diagnosis of TB and detection of drug resistance in MTBC
  - Molecular tests for diagnosis do not replace culture
  - Molecular tests do not replace conventional DST
  - Need to develop cost-effective algorithms for incorporating new technology; timely referral
- Results from genotypic and phenotypic tests for drug resistance need to be used in conjunction with one another (may depend on drug and genetic locus)
  - Molecular (genotypic) tests may
    - Elucidate “truth” in certain cases
    - Add to confusion in certain cases
    - Help us “fine-tune” conventional DST
- Communication is essential
- There is still a lot to learn!

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