New Tools in the Diagnosis of TB: Principles, Indications and Limitations (Understanding TB Laboratory Results and Challenges of Reporting Results)

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Division of TB Elimination

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Objectives

- Briefly review mycobacteriology testing practices in US with emphasis on potential “problems”
- Describe the new molecular tests available for detecting drug resistance in MTBC
- Use case-based scenarios to explain the use of molecular test results and the benefits and limitations of these tests

“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 don’t know what something means, please call the lab
- For TB—We are still learning about DST and molecular detection of drug resistance
  - Discordance will happen
  - Things are not always black and white

TB testing/mycobacteriology in U.S.

- Types of laboratories (not mutually exclusive):
  - Hospital/medical center laboratories
  - Public health laboratories (e.g., State, county, city)
  - Commercial laboratories (e.g., LabCorp, Quest, ARUP)
  - Reference Laboratories (Nat. Jewish, CDC, Mayo)
- Mycobacteriology laboratory services are often dispersed
  - Work is often piecemeal – specimens or isolates referred from one lab to another
  - Difficult cases may have drug susceptibility results from several laboratories (discordance not uncommon)
  - Communication between labs may be a problem
- Communication with care-giver/TB program a problem especially when testing becomes further removed from originating lab

AFB Microscopy

- Not very sensitive
  - 50-70% for pulmonary TB
- Not specific for MTBC
- Value for TB
  - Inexpensive and rapid; 1st bacteriologic evidence of TB
  - Infectiousness; follow therapy
  - Determine need for additional testing (e.g., NAAT)
- Primary method for TB diagnosis in developing countries
Microscopy vs. Culture

- 5,000 to 10,000 AFB/mL for smear
- 10 to 100 AFB/mL for culture

Significance of culture

- Confirm TB/mycobacteriosis; obtain isolate for DST, genotyping; evaluate therapy
- Only 85-90% cases of pulmonary TB are culture-positive (culture-negative TB; clinical diagnosis)

Identification of Mycobacteria from Growth in Culture

- Conventional biochemical tests
- HPLC of cell wall mycolic acids
- DNA probes (AccuProbe®; Gen-Probe, Inc.)
  - Does not require Nucleic Acid Amplification
- “in-house” or laboratory developed tests (LDT) such as PCR/RE analysis/genetic sequencing

Direct Detection of MTBC in Clinical Specimens; Nucleic Acid Amplification Tests (NAAT)

- Objective is to detect/identify MTBC directly from clinical specimens and avoid the weeks required for culture
  - Rapid turnaround time of 24 to 48 hours after specimen receipt
- Positive result demonstrates the presence of MTBC
  - Does not distinguish live and dead bacilli
- Negative result does not necessarily mean the absence of MTBC
  - Inhibition of amplification
  - Target below the limit of detection

NAAT for Direct Detection of MTBC

- FDA-approved for use with respiratory specimens
  - Amplified MTD® (Mycobacterium tuberculosis Direct) Test: Gen-Probe, Inc.
- FDA market authorization for use with raw sputum or sputum sediments
  - GeneXpert® MTB/RIF: Cepheid
- Non-FDA approved tests (RUO; Research Use Only)
  - Hain Lifescience Genotype® MTBDRplus and MTBDRsl
  - Laboratory developed tests or LDT (e.g., DNA sequencing, and real-time PCR assays)

Limitations and Considerations

- Sensitivity
  - Reduced for smear negative specimens and some specimen types?
  - Do you want to “rule in” or “rule out”? Platform dependent
- Specificity
  - Platform dependent
- Does not replace need for culture
  - Culture still needed for conventional DST, genotyping
- Amplicon cross contamination in open systems
- Cost and sustainability
  - Expense can limit utilization

Drug Susceptibility Testing (DST) of MTBC

Current recommendations (Clinical and Laboratory Standards Institute [CLSI] M24-A2)

- Initial isolate should be tested against primary or first-line drugs (FLD)
  - INH, RMP, PZA, EMB
- For RMP-resistant isolates, or resistance to any 2 FLD, test second-line drugs (SLD)
  - To include FQ, AMK, KAN, CAP
**Possible Definitions of Drug Resistance**
- MIC that is beyond or at the extremes of the MIC distribution of presumed wild-type isolates
- Comparison of the MIC to a PK parameter (e.g., peak/MIC or AUC/MIC)
- MIC that is associated with an increased risk of failure/relapse (clinical validation)
- Change in MIC from baseline to time of treatment failure
- Change in genotype (i.e., wild type to mutation) from baseline to time of treatment failure

*Bill Burman, June 2011*

**Current Practice for DST**
- For FLD, FDA-cleared broth-based methods are routine and widely available
  - Results generally available within 28 days of specimen receipt in laboratory
- Molecular assays (RMP, INH) are available in some jurisdictions – Cepheid GeneXpert® MTB/RIF, LDT or RUO tests
  - Performed directly on clinical specimens or on culture isolates and results available within 1–2 days
- For SLD, testing often performed in piecemeal fashion through referrals; few laboratories with technical expertise / capacity
  - Slow turn-around-time; Indirect agar proportion takes ~28 days after isolation from culture
  - Some laboratories have validated methods for broth-based testing
  - Some laboratories offer molecular assays (LDT or RUO tests)

**Agar Proportion Method for MTBC DST**
- The method of proportion using Middlebrook 7H10 agar has been considered the “gold standard” method in the U.S. for several decades – used at CDC
- Plate bacteria onto media containing
  - no drugs (growth control)
  - critical concentrations of a drug
- Incubate for 3 weeks
- Count colonies
- Isolate is resistant if the number of colonies on drug-containing media is >1% of the colonies on drug-free media

**Critical Concentration**
- DST of MTBC typically involves testing the susceptibility of the organism against the critical concentration of a drug
- Critical concentrations were adopted by international convention
  - Ideally the critical concentration is the lowest concentration of a drug that discriminates between susceptible and resistant strains of MTBC
  - That means, it inhibits growth of all susceptible strains AND allows growth of all resistant strains
- It is difficult to find a drug concentration that precisely meets this definition, we settle for the concentration that BEST DISCRIMINATES between susceptibility and resistance

**Equivalent Critical Concentrations**
- Critical concentrations were originally determined in Lowenstein-Jensen medium
- Equivalent concentrations of drugs later established in Middlebrook 7H10 and 7H11 for agar proportion method and in media used in commercial DST systems

<table>
<thead>
<tr>
<th>Drug</th>
<th>7H10</th>
<th>7H11</th>
<th>Bactec</th>
<th>MGIT</th>
<th>Versa</th>
<th>TREK</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>RMP</td>
<td>40</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>EMB</td>
<td>2.0</td>
<td>5.0</td>
<td>7.5</td>
<td>2.5</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*No longer manufactured*

**What about MIC testing?**
- Sensititre (ThermoScientific/Trek Diagnostic Systems)
  - Dry Microdilution Plate for Mycobacterium spp.
**Critical Concentration Differs from Minimum Inhibitory Concentration**
- The minimum inhibitory concentration (MIC) is defined as the lowest concentration of a series of drug dilutions (usually serial two-fold dilutions) that prevents visible / detectable growth of MTBC
  - Can perform in agar or broth system
  - Interpretive criteria needed to define categorical result of resistant or susceptible
- MIC testing differs from testing using critical concentrations which uses single drug concentrations and provides a categorical result of resistant or susceptible

**Limitations of MIC Testing for MTBC**
- Procedures are not standardized
- Assays not FDA-cleared
- No universally established breakpoints or interpretive criteria
- Few studies on how MIC correlates with clinical presentation or patient outcome
- MIC results may not correlate with results obtained by critical concentration methods
- Additional research is needed to understand how DST results using different methods correlate with treatment efficacy

**What is the Gold Standard for DST?**
- Traditionally believed to be the result obtained by growth-based testing
  - BUT, discordance between methods is not rare
  - Equivalent critical concentrations (different media)
- Lack of clinical validation of drug resistance (especially 2nd and 3rd line drugs)
- Introduction of genotypic tests –
  - Significant mutation detected but S by growth-based test
- Multidrug therapy (challenge to get clinical validation)

**Molecular Detection of Drug Resistance (Molecular DST)**
- Examining DNA of specific genes for mutations known to be associated with phenotypic resistance
  - Mutations in what genes are associated with resistance?
  - Where are the mutations within the gene?
  - Some areas are “hot spots”—resistance determining regions

**Molecular-based Tests for Detecting Mutations Associated with Drug Resistance**

<table>
<thead>
<tr>
<th>Company</th>
<th>Test Name</th>
<th>Market Authorization</th>
<th>FDA approval</th>
<th>Expected turn-around time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cepheid</td>
<td>GeneXpert® MTB/RIF</td>
<td>N/A</td>
<td>No</td>
<td>1-2 working days</td>
</tr>
<tr>
<td>HAIN Lifescience</td>
<td>HAIN Genotype® MTBDRplus</td>
<td>N/A</td>
<td>N/A</td>
<td>1-2 working days</td>
</tr>
<tr>
<td>Not Applicable</td>
<td>DNA sequencing</td>
<td>Laboratory developed</td>
<td>Laboratory developed</td>
<td>1-2 working days</td>
</tr>
<tr>
<td>N/A</td>
<td></td>
<td></td>
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<td>1-2 working days</td>
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Output from the assay depends on the platform (e.g., DNA sequencing, molecular beacons, line probe assay)
Important to understand platform to understand limitations

**Cepheid Xpert MTB/RIF Assay**
- Semi-automated real-time PCR
- Market authorization: No
- Turn-around time: 1-2 working days
Cepheid Xpert MTB/RIF Assay
- Automated commercial system for identification of *M. tuberculosis* complex and mutations in *rpoB*
- Uses real-time PCR with molecular beacons
  - 5 probes for wild-type RRDR in *rpoB* and 1 probe for amplification control (*B. globigii*)
- Decontamination, digestion, DNA extraction, amplification, and detection in same cartridge; Limited biosafety requirements
- Results in ~2 hours
- Minimal hands on manipulation- technically simple
- Platform is random access

Genotype MTBDRplus
- NAA and hybridization-based test use immobilized DNA probes on nitrocellulose membranes (line probe assay [LPA])
  - Colorimetric change indicates hybridization
  - “Read” the bands to determine MTBC or not and to detect resistance-associated mutations for RMP and INH

Conventional DNA Sequencing (Sanger sequencing)
- PCR Amplification of target regions
- DNA Sequencing
- Sequence Analysis

Pyrosequencing
- Direct DNA sequencing of PCR products
- Unique chemistry
  - Biotin labeled PCR product (1 strand)
  - Biotinylated DNA strand captured on beads
  - Beads hybridized with sequencing primer
  - Instrument carries out DNA sequencing reaction and analysis <2 hrs

Most commonly observed *rpoB* mutation:
\[ T \rightarrow G \] Ser531Leu

Actual DNA sequence detected
“Differences” in Testing Platforms (simplified version)
- Sanger sequencing
  - Identifies actual mutations
  - Can examine long sequences
- Pyrosequencing
  - Identifies actual mutations
  - Short sequence reads
  - Minor or mixed populations are harder to detect
- GeneXpert MTB/RIF
  - Detects wild type sequence and thus may miss mixed populations
- Hain
  - Interpretation of banding pattern may give indication of actual mutation

Advanced Molecular Diagnostics (AMD)
- Next Generation Sequencing
- High-throughput sequencing
- ‘Whole Genome’ sequencing
- High throughput, multi-use platforms – need to batch
- DTBE using next generation sequencing tools and bioinformatics to identify outbreaks so TB control programs will be able to focus their investigations, conserve scarce resources, and identify and respond more quickly to outbreaks to interrupt transmission.

“Real World” Considerations
- Silent mutations
  - Base changes; amino acid does not change
  - No alteration in protein sequence
- Missense mutations
  - Base changes; amino acid changes
  - Protein sequence changes
  - Not all result in phenotypic resistance
    - Neutral polymorphisms (possible lineage markers, not associated with resistance)
    - "Border-line" or low-level resistance
- “New” mutations
  - Not previously observed, not reported in the literature, not yet shown to be associated with resistance
- Mixed wild type / mutant populations
  - Emerging resistance
  - >1 strain of TB in patient
  - Limit of detection
  - Lack of a mutation does not confirm susceptibility
  - Our “gold standard” DST is not perfect

MDDR Service at CDC: Rationale (2008-2009)
- Clinical/Program
  - Make rapid confirmation of MDR TB available
  - Make laboratory testing data available to clinicians about SLD resistance in cases of RMP-R or MDR TB
  - New technologies may fill the role in the future but demand exists now
- Development
  - Continuous correlation of molecular (genotypic) results and DST (phenotypic) results
  - Addition of new drugs and alleles
- Research
  - Determination of mechanisms of resistance
  - “Fine tune” DST

MDDR Service History
- Implemented in September 2009 (CLIA compliant)*
  - DNA sequencing, ABI 3130xl
  - MTBC isolates
  - Anticipated workload - conservative estimate, 1-2 isolates/week
  - Loci examined for INH, RMP, FQ, and injectables
  - Loci for EMB and PZA incorporated October 2010
- MDDR V 2.0 implemented in June 2012
  - Incorporation of pyrosequencing screen (INH and RMP only)
  - MTBC isolates and NAAT(+) sediments (not raw specimens)

Criteria for MDDR Testing Version 2.0* (Expanded MDDR)
- Isolate or NAAT (+) sediment (not raw specimen)
- High-risk patients (RMP-R, MDR TB)
  - From population with high rates of drug resistance
  - Exposed to DR case
  - Failing therapy
- Cases of public health importance
  - Impact on public health measures & public health response
- Known RMP Resistance
  - Conventional or molecular test by submitter
- Mixed or non-viable cultures
- Other Reasons


*June 2012
MDDR Service:
Sanger Sequencing
Drugs and Genes for Panel
- Rifampin
- Isoniazid
- Ethambutol
- Pyrazinamide
- Fluoroquinolones
- Amikacin, Kanamycin, Capreomycin
- Kanamycin
- Capreomycin
- rpoB (81bp region)
- inhA (-15)
- katG (Ser315)
- embB (Met306, Gly406)
- pncA (promoter and coding regions)
- gyrA (coding region)
- rrs (nt1401/1402,1484)
- eis (promoter region)
- tlyA (coding region)

How should we report the mutation detected?

rpoB TGG>TGT; Ser531Leu

761155CT = Ser450Leu = S450L
Ser531Leu (E. coli) = S531L (E. coli) = S31TTG

What about tests which do not determine actual genetic mutation / provide actual genetic mutation as output?

Hain Test Report (lab 1)

Hain Test GenoType MTBDRplus
rpoB point mutation detected
katG point mutation detected
No inhA point mutation detected

As with any DNA-based assay, this test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, it is possible that mutations that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild type probes. The GenoType MTBDRplus test only indicates those resistances of the M. tuberculosis complex that have their origins in the rpoB, katG, and inhA regions examined here. Resistances originating from mutations of other genes or gene regions as well as other rifampin and isoniazid resistance mechanisms will not be detected by the test. Theoretically, a resistance can exist in spite of a wild type pattern. If, at investigation, the sample contains a strain that has developed only a partial resistance that is not covered by the mutation probes, the wild type pattern will appear. If the sample contains more than one M. tuberculosis strain (due to mixed culture or contamination) and one of these harbors a mutation that is not covered by the mutation probes, the wild type pattern will appear.
Hain Test Report (lab 2)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>RIFAMPIN GENO</th>
<th>S.</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rpoB MUTATION DETECTED</td>
<td>RMP</td>
<td>RESISTANT TO RIFAMPIN</td>
</tr>
<tr>
<td></td>
<td>NO katG MUTATION DETECTED AND NO inhA MUTATION DETECTED</td>
<td>S.</td>
<td>SUSCEPTIBLE TO S.</td>
</tr>
</tbody>
</table>

NOTE: The line probe assay only indicates those resistances of MTB complex that have their origins in the rpoB, katG, and inhA regions examined here. Resistances originating from mutations of other genes or gene regions will not be detected by this test. This test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, it is possible that mutations that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild-type probes.

Interpretative Comments Considerations

- Will differ with platform
- Examples
- “Unknown” or “novel” mutation
  - Defer to DST?
- Discordance between molecular and DST results
  - Report both; no comment?
  - Defer to DST?
  - Defer to molecular result?
  - Report both; clarifying comment?

Discordant Results – CDC Example

- **rpoB Leu533Pro**
  - the Leu533Pro mutation detected in rpoB is associated with low-level, but probably clinically-relevant, RMP resistance. Isolates with this mutation often test as susceptible by conventional techniques.
  - RMP S by AP DST
    - RMP reported as “see comments”
    - EXCEPTION: rpoB —Leu533Pro mutation; RMP DST—RMP S. Low-level but probably clinically relevant rifampin resistance has been linked to the Leu533Pro mutation detected in the rpoB locus; isolates with this mutation may test as susceptible by conventional techniques.

Discordant Results – CDC Example

- No mutations detected in inhA and katG
  - Cannot rule out INH resistance. (86% of INH-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at one or both of these loci.)
- INH R by AP DST
  - INH reported as R
  - EXCEPTION: inhA and katG—no mutations; AP DST—INH-R. The absence of a mutation in inhA and katG does not rule out resistance to INH; ~14% of INH-R isolates do not have a mutation at either of these loci.

Considerations for Standardization of Reporting Tuberculosis Molecular Diagnostic Results

- What is considered the “gold standard” for interpretation?
- What interpretive comments are provided for novel mutations?
- How are silent mutations described?
- How much information should be provided to aid in understanding discordant results?
- What is the threshold when the complexity of information provided challenges data accuracy for surveillance reporting?

Must keep this complexity in mind with the ultimate goal of improving patient outcomes and public health
# CASES

**Illustrate rapid turn-around time**

<table>
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<th>Gene</th>
<th>Mutation</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>rpoB (RDRR)</td>
<td>No mutation</td>
<td>Probably Rifampin susceptible. (97% of RMP-R isolates have a mutation at this locus.)</td>
<td></td>
</tr>
<tr>
<td>inhA (promotor)</td>
<td>No mutation</td>
<td>Cannot rule out INH resistance. (85% of INH-R isolates have a mutation at one or both of these loci.)</td>
<td></td>
</tr>
<tr>
<td>katG (Ser315 codon)</td>
<td>No mutation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MGIT broth
Previous TB Treatment
From a country with a high rate of drug resistance (China)
University Student

Collection Date: 1/17/2014 (Friday)
CDC contacted: 2/6/2014 (Thursday)
Date sent to CDC: 2/6/2014 (Thursday)
Date received at CDC: 2/7/2014 (Friday)

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<td>Rifampin resistant</td>
<td></td>
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<tr>
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<td>No mutation</td>
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<td></td>
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<td>Mutation: AGC&gt;ACC; Ser315Thr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>embB (Met306, Gly406, other)</td>
<td>Mutation: ATG&gt;ATC; Met306Ile</td>
<td>Ethambutol resistant</td>
<td></td>
</tr>
<tr>
<td>pncA (promotor, coding region)</td>
<td>Mutation: GGC&gt;GAG; Ala146Glu</td>
<td>Likely PZA resistant</td>
<td></td>
</tr>
<tr>
<td>gyrA (QRDR)</td>
<td>Mutation: GAC&gt;GGC; Asp94Glu</td>
<td>Ofloxacin resistant</td>
<td></td>
</tr>
<tr>
<td>rrs (1400 region)</td>
<td>Mutations: A1401G and C1402T</td>
<td>Amikacin and Kanamycin resistant; Possibly Capreomycin resistant</td>
<td></td>
</tr>
<tr>
<td>eis (promotor)</td>
<td>Mutation: C-14T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blyK (entire ORF)</td>
<td>Frameshift mutation</td>
<td></td>
<td></td>
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**Pyrosequencing Report issued 2/10/2014**

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</tr>
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<td>rrs (1400 region)</td>
<td>Mutations: A1401G and C1402T</td>
<td>Amikacin and Kanamycin resistant; Possibly Capreomycin resistant</td>
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</tr>
<tr>
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<td>Mutation: C-14T</td>
<td></td>
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<tr>
<td>blyK (entire ORF)</td>
<td>Frameshift mutation</td>
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</tbody>
</table>

**NAAT+ sputum sediment**
Previous TB Treatment
From a country with a high rate of drug resistance (PERU)

Collection Date: 12/29/2013 (Sunday)
CDC contacted: 12/30/2013 (Monday)
Date sent to CDC: 1/2/2014 (Thursday)
Date received at CDC: 1/3/2014 (Friday)

<table>
<thead>
<tr>
<th>Location and Region examined</th>
<th>Gene</th>
<th>Mutation</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>rpoB (RDRR)</td>
<td>Mutation: TCG&gt;TG; Ser531Leu</td>
<td>Rifampin resistant</td>
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</tr>
<tr>
<td>inhA (promotor)</td>
<td>No mutation</td>
<td>Isoniazid resistant</td>
<td></td>
</tr>
<tr>
<td>katG (Ser315 codon)</td>
<td>Mutation: AGC&gt;ACC; Ser315Thr</td>
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<td></td>
</tr>
<tr>
<td>embB (Met306, Gly406, other)</td>
<td>Mutation: ATG&gt;ATC; Met306Ile</td>
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**CDC Sanger Sequencing Report issued 1/7/2014**

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Rapidly identified MDR / XDR TB
CASES
(Illustrate discordance)

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 — TTC>T; Phe514Phe

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)
- MDDR:
  - 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 or “disputed” mutations
- 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, or
  - 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


Rifampin
CDC MPEP Strain H (6/2008) and Strain T (5/2010)*

<table>
<thead>
<tr>
<th>Method</th>
<th>No. RMP-R/No. results</th>
<th>%</th>
<th>No. RMP-R/No. results</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>LJ Proportion</td>
<td>7/7</td>
<td>100</td>
<td>n/a</td>
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<tr>
<td>Agar Proportion</td>
<td>10/27</td>
<td>70</td>
<td>15/23</td>
<td>65</td>
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<tr>
<td>BACTEC 460</td>
<td>15/36</td>
<td>41</td>
<td>7/19</td>
<td>37</td>
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<tr>
<td>MGIT</td>
<td>13/69</td>
<td>18</td>
<td>9/61</td>
<td>15</td>
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<tr>
<td>VersaTREK</td>
<td>0/9</td>
<td>0</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>54/239</td>
<td>38</td>
<td>33/108</td>
<td>29</td>
</tr>
</tbody>
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*His526Leu mutation in rpoB
**Case # 4—Is it RMP-R?**

- Isolate submitted for MDDR
  - HIV+, prison, Mexico, intermittent therapy, “funky” RMP on Bactec 460
- CDC rpoB — wildtype; probably RMP-S
- CDC AP — contaminated
- Resubmit isolate (A) and a newer isolate (B)
  - AP (A)—RMP-R (5%)
  - AP (B)—RMP-R (12%)
- rpoB on colonies—His526Tyr (100% of isolates with this mutation are RMP-R)

**Delayed Test Results**

**Man-Made and Unexpected Delays**

- Recent submissions to CDC
  - 4 month TAT
    - Collection date 9/26/2013
    - CDC receives and approves request 1/22/2014 (Wednesday); “delay on sensitivity; patient already on treatment over 2 months”
    - Isolate received 1/24/2014 (Friday)
    - MDDR results 1/27/2014 (Monday)
  - 9 month TAT
    - Collection date 4/30/2013
    - CDC receives and approves request 1/23/2014 (Thursday); “Somalia”
    - Isolate received 1/27/2014 (Monday)
    - MDDR results 1/31/2014 (Friday)

**Does it make a difference?**

- Survey of TB Programs:
  - “We had two specimens R to rifampin by GX...with the "silent" rpoB mutation, ... After medical consultation to make recommendations for the providers... both cases were treated as MDR-TB.”
- MDDR request:
  - MDDR results: katG mutation (Ser315Thr) with interpretation as INH resistant; no mutation in rpoB
  - “while the katG mutation does suggest high level resistance, I hesitate to recommend stopping the INH until there are susceptibility results to confirm this.”

**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). The use of both may involve “trade-offs.”
  - Molecular (genotypic) tests may
    - Elucidate “truth” in certain cases
    - Add to confusion in certain cases
    - Help us “fine-tune” conventional DST
- Communication is essential

**Acknowledgements**

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Jeff Driscoll, PhD

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(404) 639-1285

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.