Molecular Diagnostics for TB: Testing for Drug Resistance Mutations

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Learning Objective

• Determine who should have rapid molecular testing for mutations associated with drug resistance to optimize early detection of drug-resistant tuberculosis

Outline: Molecular Tests for Drug Susceptibility

• What are the types of tests?
• What are the benefits?
• On whom and when should you use them?
• How to interpret the results?
• Areas for caution...
Terminology

- Growth-based susceptibility tests = culture-based susceptibility tests = phenotypic susceptibility tests = DSTs
- Molecular tests for drug resistance
  - “Molecular susceptibility tests”
  - “Genotypic susceptibility tests”

Molecular Tests for Drug Resistance

- Non-sequencing (reports presence of a mutation)
  - Molecular beacons: Cepheid Xpert MTB/Rif – FDA authorized
  - Line probes: Hain MTBDRplus and MTBDRs, Innogenetics INNO-LIPA RifTB
  - Laboratory developed tests
- Sequencing (reports specific mutation)
  - Pyrosequencing (PSQ)
    - e.g., California, New York public health labs
  - CDC’s Molecular Detection of Drug Resistance (MDDR)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Gene/locus</th>
<th>Sensitivity (Sequencing)</th>
<th>Specificity (Sequencing)</th>
<th>Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (INH)</td>
<td>katG</td>
<td>86.0</td>
<td>99.1</td>
<td>Hain, PSQ, MDDR</td>
</tr>
<tr>
<td>INH and Ethionamide</td>
<td>inhA promoter</td>
<td>4.5</td>
<td>100</td>
<td>PSQ</td>
</tr>
<tr>
<td>INH</td>
<td>ahpC promoter</td>
<td>97.1</td>
<td>97.4</td>
<td>Xpert, MDDR, PSQ, MODR</td>
</tr>
<tr>
<td>Rifampin (Rif)</td>
<td>rpoB</td>
<td>78.8</td>
<td>94.3</td>
<td>Hain, MDDR</td>
</tr>
<tr>
<td>Ethambutol (EMB)</td>
<td>embB</td>
<td>79.0</td>
<td>99.6</td>
<td>Hain, MDDR</td>
</tr>
<tr>
<td>Pyrazinamide (PZA)</td>
<td>pncA</td>
<td>86.0</td>
<td>95.9</td>
<td>MDDR</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>gyrA</td>
<td>79.0</td>
<td>99.6</td>
<td>Hain, MDDR</td>
</tr>
<tr>
<td>Amikacin (AMK)</td>
<td>rrs</td>
<td>90.9</td>
<td>98.4</td>
<td>Hain, MDDR</td>
</tr>
<tr>
<td>Capreomycin (CAP)</td>
<td>dlyA</td>
<td>55.2</td>
<td>91.0</td>
<td>MDDR</td>
</tr>
</tbody>
</table>

Lin, et al., J Clin Micro. 2014;52:479
Types of Mutations

<table>
<thead>
<tr>
<th>Silent</th>
<th>Missense</th>
</tr>
</thead>
<tbody>
<tr>
<td>• No amino acid change</td>
<td>• Amino acid change</td>
</tr>
<tr>
<td>• Not associated with drug resistance generally</td>
<td>• Some are associated with resistance</td>
</tr>
<tr>
<td>– 514 TTT mutation in rpoB is the most common silent mutation</td>
<td></td>
</tr>
</tbody>
</table>

Molecular Testing for Rifampin (rpoB)

• Rifampin cornerstone of TB treatment
  – Resistance requires a longer duration of therapy
  – Rif resistance without INH resistance rare

Xpert Probes: Coverage of rpoB

- Most common silent mutation (514 TTT)
- Most common resistance mutation (531 TTG)
Molecular Diagnostics for TB: January 21, 2015
What are NAATS and How Do You Use Them?

Xpert Performance
Rifampin Resistance

• Pooled median sensitivity:
  – 94% (95% CrI: 87, 97)
• Pooled median specificity:
  – 98% (95% CrI: 97, 99)


Benefits of Molecular Tests for Drug Resistance

• Reduced time to detection of resistance
  – Time from empiric treatment to MDR treatment
    40 days less (median)
  – Less transmission
  – Less acquired resistance while DSTs pending
  – Less ineffective LTBI treatment given to contacts

Banerjee et al, J Clin Micro, 2010
Risks of Molecular Tests for Drug Resistance

- Cost
- Confusion
- Possible unneeded exposure to second-line drugs

Number and Proportion MDR TB by Country/Region of Origin, CA 2009–2013

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>No.</th>
<th>%</th>
<th>PPV (99% spec)</th>
<th>PPV (98% spec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Former Soviet Republics</td>
<td>8</td>
<td>14.5</td>
<td>94%</td>
<td>89%</td>
</tr>
<tr>
<td>Laos</td>
<td>5</td>
<td>4.3</td>
<td>81%</td>
<td>68%</td>
</tr>
<tr>
<td>Korea, North and South</td>
<td>11</td>
<td>4.1</td>
<td>80%</td>
<td>67%</td>
</tr>
<tr>
<td>Burma</td>
<td>2</td>
<td>3.4</td>
<td>77%</td>
<td>63%</td>
</tr>
<tr>
<td>India</td>
<td>10</td>
<td>2.6</td>
<td>72%</td>
<td>56%</td>
</tr>
<tr>
<td>Central America</td>
<td>9</td>
<td>2.2</td>
<td>68%</td>
<td>52%</td>
</tr>
<tr>
<td>Peru</td>
<td>1</td>
<td>2.0</td>
<td>66%</td>
<td>50%</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1</td>
<td>1.9</td>
<td>65%</td>
<td>48%</td>
</tr>
<tr>
<td>Vietnam</td>
<td>17</td>
<td>1.9</td>
<td>65%</td>
<td>48%</td>
</tr>
<tr>
<td>Philippines</td>
<td>25</td>
<td>1.6</td>
<td>61%</td>
<td>44%</td>
</tr>
<tr>
<td>Kampuchea</td>
<td>2</td>
<td>1.4</td>
<td>57%</td>
<td>41%</td>
</tr>
<tr>
<td>China (incl Taiwan)</td>
<td>7</td>
<td>1.2</td>
<td>54%</td>
<td>37%</td>
</tr>
<tr>
<td>Mexico</td>
<td>16</td>
<td>0.8</td>
<td>44%</td>
<td>28%</td>
</tr>
<tr>
<td>United States</td>
<td>8</td>
<td>0.5</td>
<td>33%</td>
<td>20%</td>
</tr>
</tbody>
</table>

* Countries with >50 cases tested for MDR
**MDR TB Cases by Country/Region of Origin and Years in the US, CA 2009-2013**

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Total MDR TB cases</th>
<th>&lt;=2 years in US No. (%)</th>
<th>&gt;2 years in US No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Countries (excl US)*</td>
<td>123</td>
<td>29 (3.3)</td>
<td>93 (1.5)</td>
</tr>
<tr>
<td>Former Soviet Republics</td>
<td>8</td>
<td>3 (22.2)</td>
<td>5 (10.9)</td>
</tr>
<tr>
<td>Vietnam*</td>
<td>17</td>
<td>6 (7.1)</td>
<td>8 (1.1)</td>
</tr>
<tr>
<td>China (incl Taiwan)*</td>
<td>7</td>
<td>4 (6.4)</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Philippines*</td>
<td>25</td>
<td>7 (3.6)</td>
<td>18 (1.4)</td>
</tr>
<tr>
<td>Central America</td>
<td>9</td>
<td>2 (3.5)</td>
<td>7 (2.1)</td>
</tr>
<tr>
<td>India</td>
<td>10</td>
<td>2 (2.2)</td>
<td>8 (2.9)</td>
</tr>
<tr>
<td>All Other Countries</td>
<td>20</td>
<td>3 (1.7)</td>
<td>17 (1.9)</td>
</tr>
<tr>
<td>Mexico</td>
<td>16</td>
<td>0 (0.0)</td>
<td>16 (0.9)</td>
</tr>
<tr>
<td>Korea, North and South</td>
<td>11</td>
<td>0 (0.0)</td>
<td>11 (4.5)</td>
</tr>
</tbody>
</table>

* Difference is statistically significant.

**Increased Risk for MDR-TB?**

- Foreign-born patients from countries or groups with high prevalence of MDR
  - In California:
    - Hmong refugees
    - Tibetan ancestry
    - Immigrants from former USSR, Laos, Burma, Korea, Peru, Central America, India
    - Recent immigrants (e.g., within 2 years) especially from China, Vietnam, Philippines

**Increased Risk for MDR-TB**

- History of previous TB treatment, particularly if recent
- Poor response to standard 4-drug treatment
  - Culture remains (+) after 2 months treatment
- Known exposure to MDR-TB case
- HIV (+)
  - Higher incidence of Rifampin mono resistance
Other Indications for Molecular Resistance Testing

<table>
<thead>
<tr>
<th>Increased stakes of drug resistance</th>
<th>Laboratory issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Case with large numbers of contacts (e.g., corrections, healthcare facilities, schools)</td>
<td>1. Cultures mixed with other bacteria</td>
</tr>
<tr>
<td>2. Patients in whom unidentified drug resistance has increased risk, e.g., age &lt;5 years, immunocompromised</td>
<td>2. Smear-positive but culture negative</td>
</tr>
<tr>
<td>3. Contacts with risks for rapid progression to active TB disease in whom effective preventive “window” treatment is needed (e.g., aged &lt;5 years, or immunocompromised persons)</td>
<td></td>
</tr>
</tbody>
</table>

How to Interpret Results of Molecular Tests for Resistance

Case 1
- 70 yo asymptomatic man from India with abnormal preimmigration CXR, no TB history
- Domestic CXR with multifocal infiltrates
- Sputum smear positive x 3
- Xpert positive: rifampin resistant

What do you do next?
- Start MDR treatment
- Order pyrosequencing or MDDR
- Start RIPE
- Repeat Xpert on another specimen
- Start treatment for monoRif resistance
Case 1 (cont.)

- Treatment held; PSQ available within 2 days and clinically stable
- Pyrosequencing:
  - \( katG \) mutation: INH R
  - \( rpoB \): 531TTG mutation: RIF R
  - gyrA (FQ): no mutations
  - \( rrs \) (amikacin): no mutations

What do you do next?
- Start MDR treatment
- Order MDDR
- Start RIPE
- Repeat Xpert on another specimen
- Order second line DSTs
- Cancel DSTs (already have molecular results)

Case 2

- 70 yo man from Mexico in US x 25 years with 4 weeks of cough, no TB history
- CXR with multifocal infiltrates
- Sputum smear positive x 3
- Xpert positive, rifampin resistant

What do you do next?
- Start MDR treatment
- Order pyrosequencing or MDDR
- Start RIPE
- Repeat Xpert on another specimen
- Start treatment for monoRif resistance

Case 2 (cont.)

- RIPE started
- PSQ:
  - \( katG/\text{inhA} \): no mutation
  - INH Sens
  - \( rpoB \): 514TTT silent mutation: RIF Sens
Case 3

- US born high school student coughing x 6 months
- Sputum smear positive x 3
- CXR: bilateral cavities
- Xpert positive: rifampin sensitive

What do you do next?
- Start MDR treatment
- Order pyrosequencing or MDDR
- Start RIPE
- Repeat Xpert on another specimen

Case 3 (cont.)

- RIPE started
- Large contact investigation planned
- 12 dose INH/Rifapentine regimen planned for infected contacts
- PSQ ordered to look for INH resistance

How to Interpret Molecular Test for Resistance

- Put into clinical and epidemiologic context!
- Confirm non-sequencing tests (e.g., Xpert) with sequencing test
- Consider Rif resistance on Xpert to be MDR (not just rifampin monoresistant)
- Can usually treat based on sequencing test results; follow the growth based DST results
- If resistance result is unexpected, investigate other explanations
Areas for Caution

- Molecular tests vs. DST discordance
  - "Disputed" mutations
  - Undescribed mutations outside of loci in current molecular tests → resistance

Case 4

- 23 year old male from Mexico diagnosed with smear-positive, pulmonary TB
- PSQ results
  - $katG$ mutation: “Associated with INH resistance”
  - $rpoB$ mutation 526AAC: “Not associated with RIF or RFB resistance.”
- Growth-based DST results: INH resistance only
- Treated with RIF, EMB, PZA for 9 months
- End of treatment sputum: smear and culture-positive

Studies of Discordant Rifampin Results

Rifampin Resistance Identified in Automated Liquid Culture System for Mycobacterium tuberculosis Isolates with Lipopolysaccharide Mutations

Studies of Discordant Rifampin Results

- Diagnostic implications of discordant results associated with the Xpert MTB/RIF assay in detection of Mycobacterium tuberculosis strains with acquired lipopolysaccharide mutations
- Analysis shows that discordant results are associated with specific mutations in the $rpoB$ gene.
Summary of Literature on Discordant *rpoB* Mutations

- Discordant mutations are associated with high rate of treatment failure
- Many were also INH resistant
- Conclusions
  - Perhaps phenotypic DST should not be gold standard
  - Discordant mutations have clinical significance
- Studies from high incidence settings; mostly from cases being retreated

California Discordant Rifampin Investigation

- 17 cases identified; almost all with INH resistance
- Preliminary results show potential poor outcomes with certain mutations
- Consult your MDR experts; consider MDR treatment

Summary

- Drug resistance can be identified faster with molecular tests
- Confirm nonsequencing results (e.g., Xpert) with sequencing
  - Beware the silent mutation!
- Keep clinical picture in mind → investigate further if result unexpected
- Consult experts for unusual mutations
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