Issues in Tuberculosis Drug Susceptibility Testing: TB Subcommittee White Papers

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Drugs being Addressed

- Pyrazinamide
- Rifamycins
  - Rifampin
  - Rifabutin
- Ethambutol
- Fluoroquinolones
  - Moxifloxacin
  - Levofloxacin
  - Ofloxacin
What’s a “White Paper”

“A white paper is an authoritative report or guide informing in a concise manner about a complex issue and presenting the issuing body's philosophy on the matter. It is meant to help readers understand an issue, solve a problem, or make a decision.”

Courtesy of Wikipedia
Organization of the White Papers

- Background
  - Role in treatment regimen
  - Mechanism of action
  - Mechanisms of resistance
  - Drug side-effects
- Practical laboratory issues
  - Culture-based DST
  - Molecular methods
  - Other tests for resistance detection
  - Proficiency Testing
Organization of the White Papers

- Impact on clinical outcomes
  - Prolonged therapy
  - Use of more toxic drugs
  - Creation of MDR-TB
  - Treatment failure

- Areas of ongoing research
  - Defining role of new mutations in resistance
  - New resistance determinants

- Testing guidance

- References
PZA White Paper
PZA

- Critical component of first-line drug combination therapy
- Shortens chemotherapy regimen to 6 months (WHO Treatment Guidelines 4th Ed)
- Significant effect against non-replicating “persister” organisms or slowly replicating bacilli at acid pH (5.5)
  - Kills bacilli not eliminated by other TB drugs
PZA

- Inactive against organisms in the growth phase during culture conditions at neutral pH
  - Use pH 6.8 for culture-based DST
Mechanism of Action

- Prodrug that requires conversion to active form, pyrazinoic acid (POA)
- Converted by pyrizinamidase (Pzase) encoded by pncA gene
- POA expelled by putative efflux pump
- Outside of cell POA protonated and re-enters
- H+ acidification of cytoplasm
Mechanism of Action

- POA also targets ribosomal protein S1 (RpsA)
  - Inhibits protein synthesis (Shi et al, Science 2011)

http://www.biologyreference.com/images/biol_o4_img0400.jpg
Mechanism of Resistance

- Primary Mechanism—Loss of PZase activity
  - Due to mutations in *pncA* gene
  - Affect catalytic sites and Fe$^{2+}$ binding site
    - PZA not converted to POA
- Mutations found in 72-97% of PZA resistant isolates
  - Widely distributed throughout the gene
Mechanism of Resistance

- All *pncA* mutations do not result in resistance
  - Newly recognized mutations require evidence to determine if they truly cause phenotypic resistance.
  - Some PZA R isolates don’t have any *pncA* mutations
- Other mechanisms of resistance
  - Efflux of POA
  - Altered PZA uptake
  - Impaired POA binding to the drug target
  - Lack of *pncA* expression
Practical Laboratory Issues
Susceptibility Testing of M.tb

- Agar Proportion Method
- Bactec 460 Radiometric Method
  - Discontinued in 2011
- MGIT
- Versa Trek
- Trek Microbroth dilution MIC
  - Not FDA approved
  - PZA not included
Other Non-FDA Approved Methods

- Resazurin microtitre assay (REMA)
  - Detect growth in microtiter well format using redox reactions
- Colorometric nitrate reductase assay
- Dimethylthiazol diphenyl tetrazolium bromide (MTT) redox reaction
- Nitrate reductase assay
PZA Deaminase Test

- Detects deaminization of PZA to POA and ammonia
  - pink color in the medium due to pH change
- Negative test correlates well with resistance
- Cannot interpret a positive test as susceptible
  - May be resistant by other mechanisms
Reproducibility Issues

- pH effects
  - PZA active only in acid environment
    - Commercial systems use pH 6.8
    - A large inoculum $10^7$ to $10^8$ increases pH leading to false resistance
    - $10^6$/ml leads to a small increase in pH
  - Too low inoculum or old cultures may lead to false susceptibility
## False Resistant PZA DST Results

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Bactec 460</th>
<th>MGIT</th>
<th>VersaTREK</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0/17 (0)</td>
<td>1/64 (2)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>B</td>
<td>0/17 (0)</td>
<td>7/62 (11)</td>
<td>0/3 (0)</td>
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<tr>
<td>C</td>
<td>0/17 (0)</td>
<td>20/62 (32)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>D</td>
<td>0/17 (0)</td>
<td>21/63 (33)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>E</td>
<td>0/17 (0)</td>
<td>0/64 (0)</td>
<td>(0)</td>
</tr>
</tbody>
</table>

*A and E are same strain; C and D are same strain.*

Bactec 460 system no longer commercially available

- Data indicate potential false PZA resistance in some automated liquid systems

Data from 2010 CDC Model Performance and Evaluation Program for MTBC DST
Proficiency Programs

- CAP is the only CLIA-approved PT program for DST
  - Cannot ship resistant strains
  - Use the same strain every challenge
- WHO proficiency test panels
  - Supranational Reference Laboratories
  - PZA not included
Impact on Clinical Outcomes
Clinical Outcome

- PZA is a critical component of first-line drug combination therapy
  - For both pan-susceptible and MDR TB
  - Used in the first 2 months of therapy
- If PZA is reported as resistant the length of therapy is increased by three months
  - False-resistance may result in prolonged, unnecessary treatment
Areas of Ongoing Research

Mutations

- *pncA* gene mutations spread along entire gene as well as the upstream regulatory region for approx 700 bp
  - Large number of mutations published, but no predominant mutations
  - Some always associated with resistance, but ongoing studies needed to determine phenotypes of other mutations
Other Areas of Research

- Critical concentration revisions
  - Research on development of new testing breakpoints is ongoing
  - MGIT currently 100 ug/ml
  - Trek 300 ug/ml
  - Likely not to change for FDA-approved tests
- Role of other gene targets in resistance?
  - rpsA gene—Altered POA binding to ribosomal protein S1
  - panD—involved in co-enzyme A synthesis
Guidance
Laboratory Considerations to Optimize Results

- Use fresh cultures for preparation of inocula for culture-based DST
- Ensure standard inoculum
- Consider using a lower inoculum
  - MGIT---use Day 3-5 seed vial
  - Vortex well and allow to settle for 20 min
  - Take aliquot from top and dilute 1:5
- Repeat DST if initially resistant
  - Perform pncA sequencing
Laboratory Considerations to Optimize Results

- If using Pzase, a negative result correlates well with resistance, but not all positive isolates can be considered susceptible.
- All mono-resistant PZA isolates should be identified to determine if *M. bovis* or *M. bovis* BCG
  - Intrinsically R to PZA
Thank You