MIC = Many Inherent Challenges

Sensititre MIC for Antimicrobial Susceptibility Testing of *Mycobacterium tuberculosis* complex

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Objectives

• Discuss antimicrobial susceptibility testing for *Mycobacterium tuberculosis* complex (MTBC)
• Review Sensititre Minimal Inhibitory Concentration (MIC) method for MTBC
• Describe challenges with the Sensititre MIC method and provide solutions
Common Challenges with AST in the Mycobacteriology Laboratory

What is the biggest challenge that you face in the antimicrobial susceptibility testing (AST) for mycobacteria?

A. Test method selection/availability
B. Quality assurance
C. Discordance
D. Biosafety
E. All of the above
### CDC National TB Program Objectives and Performance Targets for 2020
#### Laboratory Targets*

<table>
<thead>
<tr>
<th>Objectives on Laboratory Reporting</th>
<th>Targets</th>
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<tbody>
<tr>
<td><strong>Turnaround Time - Culture</strong></td>
<td><strong>78%</strong></td>
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</table>
| For TB patients with cultures of respiratory specimens identified with *M. tuberculosis* complex (MTBC), increase the proportion reported by the laboratory within **25 days from the date the specimen was collected**.  
**NOTE:** 25 days includes 21 days for culture to grow and 4 days for specimen collection and delivery to laboratory. | |
| **Turnaround Time - NAA**         | **92%** |
| For TB patients with respiratory specimens positive for MTBC by nucleic acid amplification (NAA), increase the proportion reported by the laboratory within **6 days from the date the specimen was collected**.  
**NOTE:** 6 days includes 2 days for detection and 4 days for specimen collection and delivery to laboratory. | |
| **Drug-Susceptibility Result**    | **100%** |
| For TB patients with positive culture results, increase the proportion who have **initial drug-susceptibility results reported**. | |
| **Universal Genotyping**          | **100%** |
| For TB patients with a positive culture result, increase the proportion who have a **MTBC genotyping result reported**. | |

*Targets are based on a statistical model that uses data to find trends from 2000-2013 (or the latest year with data available). Specifically, the targets are the 90th percentile values in the latest year estimated from a quantile regression on 2000-2013 data, excluding jurisdictions with fewer than 150 cases from 2011–2013.
Antimicrobial Susceptibility Testing for MTBC

- **Direct Molecular methods:**
  - “MDR Screen” (Multi Drug Resistance Screen) - focus on the detection of Rifampin (RIF) resistance
  - Limited availability of tests that are FDA-approved or cleared, alternative is the use of laboratory developed tests (LDTs)
  - Further (downstream, reflex) testing is still required to confirm or rule-out resistance and guide patient treatment
    - e.g. rule out a silent mutations, confirm resistance mutations, detect additional resistance...
Molecular “MDR Screen”

Hain GenoType MTBDRplus

Cepheid Xpert MTB/RIF
“Laboratories should use or have prompt access to the most rapid methods available:

1) fluorescent microscopy and concentration for acid fast bacilli (AFB) smears

2) rapid nucleic acid amplification (NAA) testing for direct detection of M. tuberculosis in patient specimens

3) solid and rapid broth culture methods for isolation

4) nucleic acid probes or high pressure liquid chromatography for species identification

5) rapid broth culture methods for drug susceptibility testing...

Settings that cannot provide the full range of...testing...should contract with their referral laboratories to ensure rapid results...”

Antimicrobial Susceptibility Testing for MTBC

• Growth-based methods
  – Growth or culture-based detection of resistance to a range of drugs
  – Several methods are available but some may require in-house verification. The gold standard remains the agar proportion method
  – Algorithm may be based upon initial testing of ‘first-line’ drugs; testing of additional drugs is reflexed when resistance to first-line drugs is detected
  – Further testing may still be required
    e.g. additional drugs, other method to resolve discordance
Which method are you using for growth-based antimicrobial susceptibility testing for MTBC?

A. Agar Proportion Method
B. MGIT
C. VersaTREK
D. TREK Sensititre MIC
E. None, we refer out
Growth-based Antimicrobial Susceptibility Testing (AST)

• Test selection/availability
  – What test would best serve our patient population and TB clinicians?
  – What drugs can be tested?
  – How does the method fit into our algorithm?
  – What are the test method considerations?
    e.g. biosafety, skilled laboratorian, instrumentation, cost
  – Are there any specific (insurmountable?!?) challenges presented with implementing one method over another?
**Options for Growth-based AST**

1. **FDA-cleared or FDA-approved methods**
   - MGIT or VersaTREK
     - Critical concentration – categorical result (minimal inhibitory concentration may also be an option)
     - FDA-approved for RIF, INH, EMB and PZA

2. **Laboratory Developed Test/Research Use Only**
   - Sensititre MIC
     - Minimal inhibitory concentration
     - Range of drugs, customizable
   - Indirect Agar Proportion method
Why Sensititre MIC for AST?

- Treatment for TB is multi-drug which presents a challenge when comparing AST data with clinical outcomes. MIC provide meaningful data for treatment - more information than a categorical result.

  Critical Concentration: the lowest concentration of drug that inhibits 95% of wild-type strains that have never been exposed to anti-TB drugs, and does not inhibit patient strains that are considered resistant.

  Minimal Inhibitory Concentration (MIC): the lowest concentration of an antimicrobial, in a series of dilutions of a drug that will inhibit the visible growth of a microorganism.

- In 2012 Florida Bureau of Public Health Laboratories (FLBPHL) needed a replacement for the BACTEC 460TB method that was discontinued!
Growth-based Antimicrobial Susceptibility Testing (AST)

- **Test selection/availability**
  - What test would best serve our patient population and TB clinicians? What drugs can be tested? FL clinicians wanted AST for a range of drugs and flexibility to choose which ones, particularly for complex cases
  - How does the method fit into our algorithm? What are the test method considerations? FLBPHL performs AST on all TB cases, about 600/year. MIC is cost-effective, relatively easy to perform
  - Are there any specific (insurmountable?!) challenges presented with implementing one method over another? We had run a validation of this method to determine the feasibility of implementation in 2011. But what about PZA? (we’ll come to that later)
Overview of the Sensititre MIC Method

• Method:
  – Equipment:* Nephelometer, Sensititre AIM™ – Automated Inoculation Delivery System, Sensititre Vizion System® (PC, Software, Plate Reader)
  – Supplies: MYCOTB 96-well plates, Dosing Heads, Media, Plate Seal, ATCC Control Strain


*none of these are required as the test can be performed manually*
Overview of the Sensititre MIC Method

• Set-up/Workflow:
  – Batch test: FLBPHL performs once a week
  – Ideal results when performed on solid cultures
Overview of the Sensititre MIC Method

• Set-up/Workflow:
  – We have 3 staff members who are trained in performing this assay

  [Images showing steps of the workflow: Place MYCOTB Plate in Autoinoculator, Inoculate plate (100µl/well), Seal plate, place in bag and incubate at 37°C, Read in Vizion Plate Reader, Read at 14 and 21 days]
# Sensititre MIC – MDR Plate

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<th>MXF</th>
<th>RIF</th>
<th>AMI</th>
<th>STR</th>
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</table>

MDR - TB

Levofloxacin and Capreomycin
Challenges with Growth-based AST

• General barriers to performing AST for MTBC:
  – Availability of methods for the clinical laboratory
  – Biosafety considerations
  – Expertise in performing test/quality assurance
  – Low volume - maintain proficiency, cost

• Challenges with growth-based AST:
  – “Gold standard” requires considerable expertise
  – Performing non FDA-approved methods or off-label usage of FDA-approved method (e.g. second line drugs)
  – Discordance between methods
  – Result interpretation and reporting
  – Long turnaround time
Challenges with the Sensititre MIC method

• Performing non FDA-approved/-cleared method
  – Verification to determine test performance compared to an FDA-approved method (not available for all drugs)
    → FLBPHL performed a comparative study with the BACTEC 460TB on 230 MTBC isolates. Drugs for which there was no comparison are reported as MIC without an interpretation

• Discordance between genotypic and phenotypic methods, between two different phenotypic methods
  – Genotypic vs. phenotypic: “low-level rifampin resistance” associated with certain rpoB mutations but phenotypically susceptible (rifampin MIC of 0.12)
  – Phenotypic vs. phenotypic: Agar Proportion method vs. MIC
    → FLBPHL reports all results and is available for consultation with clinicians to discuss discordance
Challenges with the Sensititre MIC method

- Result interpretation and reporting
  - Delayed turnaround time
  - MIC versus Critical Concentration
    → FLBPHL actively performed education and is available for consultation regarding reporting language and interpretation of results.
    → FL TB Control Program and clinicians are knowledgeable and available to discuss results with healthcare providers. FL DOH utilizes a phone service for health care providers to call, 1-800-4TB-INFO (listed on all TB laboratory reports).
    → FLBPHL sets-up MIC plates with solid culture inoculum (not liquid) and reads plates at 10, 14 and 21 days. The average TAT is 15.68 days from set-up to final read (out of 1763 isolates tested between 2014 and 2016 with final read at 14 or 21 days)
### AST – Result Reporting

**FLBPHL – Example Report**

<table>
<thead>
<tr>
<th>Test Code</th>
<th>Description</th>
<th>MIC</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>3145</td>
<td>HAIN Test GenoType MTBDRplus</td>
<td>rpoB point mutation detected</td>
<td>1 µg/mL</td>
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<tr>
<td></td>
<td></td>
<td>No katG and No inhA point mutation detected</td>
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</tr>
<tr>
<td>3315</td>
<td>Streptomycin MIC</td>
<td>0.06 µg/mL</td>
<td>Susceptible: ≤0.25, Intermediate: 0.25-1.0, Resistant: ≥2</td>
</tr>
<tr>
<td></td>
<td>Streptomycin Interpretation</td>
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<tr>
<td></td>
<td>Isoniazid MIC</td>
<td>16 µg/mL</td>
<td>Susceptible: ≤1, Resistant: ≥2</td>
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<td>Isoniazid Interpretation</td>
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<tr>
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<td>Rifampin MIC</td>
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<tr>
<td></td>
<td>Rifampin Interpretation</td>
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04/20/2012
• PZA cannot be tested by MIC because it requires acidic conditions that cannot be accommodated by the Sensititre® system
  → FLBPHL performs Sanger DNA sequencing of the pncA gene on all TB-positive isolates
  – Non-synonymous mutations, other than His57Asp, are reported and isolates are forwarded to a reference laboratory for phenotypic testing
  – Isolates with the His57Asp mutation are intrinsically resistant and are characteristic for M. bovis/M. bovis BCG and are reported as such
Pyrazinamide (PZA) Resistance Testing

- Non synonymous and gene promoter pncA mutations identified by FLBPHL over 4-year period
  - n=2086 MTBC isolates tested
    - 139 MTBC strains with pncA mutations
    - 55 His57Asp (intrinsic R, M. bovis/M. bovis BCG)
    - 58% strains with non-synonymous mutations phenotypically confirmed (or intrinsically) resistant
Pyrazinamide Resistance Testing

• Challenges with *pncA* sequencing:
  – Sanger DNA Sequencing:
    • Requires a culture
    • Sequence 720bp of the *pncA* gene (no smaller target region like RRDR 81bp region of *rpoB* gene)
  – Resistance possible by mechanisms other than mutations in the *pncA* gene
  – A range of mutations are seen, some are novel and their effect on *in vivo* resistance is unknown
  – Non-synonymous mutations should be confirmed with phenotypic testing. Although frameshift, deletions/additions will likely result in resistance, there are non-synonymous mutations that do not confer resistance
Detection of MDR-TB Case

- FLBPHL MDR Screen (Hain): *rpoB* wild type bands 1&2 missing, no *rpoB* mutant bands present. *inhA* point mutation detected
- CDC MDDR program: no amplification of any genes
  - At this point, molecular results suggest patient has low level isoniazid resistance – but could it be MDR?
- Additional sample received and sent again to CDC MDDR program:
  - novel mutation in the *rpoB* is detected (amino acids 508-510 deleted), whether this confers *in vivo* resistance to RIF unknown
  - *inhA* point mutation detected
  - *embB* silent mutation detected
  - CDC Agar proportion: INH resistant (1 µg/mL), RIF susceptible, ETH susceptible
  - INH mono-resistant?
Detection of MDR-TB Case

- FLBPHL DST results by Sensititre MIC method:
  - RIF resistant (4 μg/mL), INH resistant (1 μg/mL), ETH resistant (>40 μg/mL)
  → MIC results determined the isolate was resistant to RIF and ETH as well as INH
- Despite discordance between methods, the MIC results were helpful for clinicians in deciding to treat this patient as an MDR-TB case with a successful treatment outcome
In Summary....
MIC = Maybe I Can!

• No matter the method, AST for TB is challenging
• Any algorithm for AST should include direct molecular testing, in addition to growth-based AST
• In evaluating AST methods, MIC has advantages in FL:
  – Easily implemented, cost effective, simple procedure
  – All results at once on a customizable plate
  – MIC results provide meaningful data to informed clinicians especially on drug dosage, critical for complex patients or drug resistant TB
• \textit{pncA} sequencing is a valuable addition to the algorithm
Thank You!

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