Laboratory’s Role in the Battle Against Drug Resistant Tuberculosis

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Understanding Drug Resistant Tuberculosis

Drug Resistant (DR)-TB

Multidrug Resistant (MDR) TB

Extensively Drug Resistant (XDR) TB

- Resistant to Isoniazid + Rifampin
- MDRTB + resistant to fluoroquinolone & second-line injectable
### Global Burden of Tuberculosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Estimated number of cases</th>
<th>Estimated number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All forms of TB</strong></td>
<td>8.6 million</td>
<td>1.3 million*</td>
</tr>
<tr>
<td></td>
<td>- 0.5 m in children</td>
<td>- 74,000 in children</td>
</tr>
<tr>
<td></td>
<td>- 2.9 m in women</td>
<td>- 410,000 in women</td>
</tr>
<tr>
<td><strong>HIV-associated TB</strong></td>
<td>1.1 million (13%)</td>
<td>320,000</td>
</tr>
<tr>
<td><strong>Multidrug-resistant TB</strong></td>
<td>450,000</td>
<td>170,000</td>
</tr>
</tbody>
</table>

*Including deaths attributed to HIV/TB*

Source: WHO Global Tuberculosis Report 2013
Capacity to Detect Drug-resistant TB

Global capacity for drug-susceptibility testing (DST), 2014

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.


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http://gamapserver.who.int/mapLibrary/Files/Maps/Global_TB_dst_2014.png
 Drug Susceptibility Testing (DST) of *Mycobacterium tuberculosis* (MTB)

- Globally in 2014, only 12% of new reported TB cases and 58% of retreatment cases were tested for drug resistance
- Global End TB strategy endorsed by WHO with focus placed on universal DST
- Growth-based DST long considered gold standard but paradigm is shifting

2015 WHO Global Tuberculosis Report
Reported TB Cases
United States, 1982–2014*

*Updated as of June 5, 2015.
Primary MDR TB, United States, 1993 – 2014*

*Updated as of June 5, 2015.
Note: Based on initial isolates from persons with no prior history of TB. MDR TB defined as resistance to at least isoniazid and rifampin.

**XDR TB Case Count Defined on Initial DST* by Year, 1993 – 2014**

*Drug susceptibility test.
**Updated as of June 5, 2015.
Note: Extensively drug-resistant TB (XDR TB) is defined as resistance to isoniazid and rifampin, plus resistance to any fluoroquinolone and at least one of three injectable second-line anti-TB drugs.

Preventing and Controlling MDR and XDR TB in the United States
Supporting the Battle Against Drug Resistant TB

- Strengthening laboratories through funding, training, and technical consultation
- Ensuring access to high-quality laboratory services
- Focusing on research to advance understanding and drive innovation
- Encouraging use of state-of-the-art technologies
- Partnering to provide technical assistance in use of new tools
Drug Susceptibility Testing for MTB in United States

- PHL serve as a primary provider of DST

- 580 (100%) labs perform AFB smear microscopy
- 474 (82%) perform culture
- 215 (37%) perform MTBC identification
- 94 (16%) perform first-line DST

- 60/94 (63%) are PHL
- 26/94 (28%) are clinical labs

APHL, National TB Services Survey Report, 2012
Test Volume Varies for DST

- Range of 1–685 patients/year for whom DST performed by PHL supported in part by DTBECoAg
- 24 PHL performed testing for <50 patients/year which is the recommended volume for maintaining technical proficiency*
  - <1 isolate each week

**APHL/CDC DST Reference Center for Mtb**

- Center established March 2015 at California Microbial Diseases Laboratory
  - Provides first-line growth-based drug susceptibility testing (DST) for *low volume* PHLs (i.e., <50 DSTs/year)
  - Access to second-line DST and rapid molecular detection of drug resistance

- Services complementary to those performed by CDC Reference Laboratory and offered free-of-charge
  - Shipping charges responsibility of submitting laboratory

- Participation is voluntary
**CDC's Molecular Detection of Drug Resistance (MDDR) Service**

- **Isolate or NAAT(+) Sediment Received for MDDR**
- **Molecular Analysis** (PSQ; PSQ then Sanger; Sanger)*
  - 2-3 day TAT
- **Conventional DST**
  - ~35 day TAT
- **Molecular Results (Interim Report[s])**
- **Molecular + Conventional DST Results (Final Report)**

*based on information supplied on request form

Public Health Laboratory Submissions to CDC for MTB Drug Susceptibility Testing

Number of submissions

- DST only
- MDDR only (DNA)
- DST and MDDR
- PZA only
- Total

Year:
- 2009
- 2010
- 2011
- 2012
- 2013
- 2014
- 2015
Expanding Targets Examined by MDDR

- Why expand targets?
  - Improve detection of drug resistance
  - Sometimes MDDR are only DST result
    - Mixed culture
    - No growth in culture
    - Fixed tissue sample

- Prioritized expanded capacity for RIF, INH, and FQs
  - Focus on MDR and XDR TB
MDDR Version 3
2015
(Isolates, NAAT+ Sediments, NAAT+ extractions by IDPB from fixed tissue)

- Rifampin
- Isoniazid
- Isoniazid
- Isoniazid
- Isoniazid
- Ethambutol
- Pyrazinamide
- Fluoroquinolones
- Amikacin, Kanamycin, Capreomycin
- Kanamycin
- Capreomycin

- rpoB (81bp region) + Val176
- inhA (-15, -8)
- katG (Ser315)
- fabG1 (mabA) Leu203
- ahpC (promoter)
- embB (Met306, Gly406)
- pncA
- gyrA + gyrB QRDR
- rrs (nt 1401/1402, 1484)
- eis (promoter region)
- tlyA (coding region)
Case – “Are we doing enough?”

- Isolate submitted for DST only; MDDR not requested
  - "preliminary results indicate R to STR, INH, and PZA"
- CDC DST
  - R to INH, Rif, STR, and PZA
- Communication with Submitting Laboratory
  - Them: What is status of DST? We’re concerned about PZA.
  - Us: Funny you should ask. We got Rif-R and are in the process of confirming.
  - Them: Rif MGIT (X2) was S
Further Communication

- Program: MDR not on our radar. Any chance of a mixed culture? Patient is responding clinically.
- Us: Pure culture.

- “in-house” MDDR to confirm DST result
  - *katG* mutation (Ser315Thr) – INH resistant
  - *rpoB* mutation (Leu533Pro)
    - RIF resistant. (Low level, but probably clinically relevant; RMP-R has been associated with the Leu533Pro mutation in the literature; isolates with this mutation may test as susceptible by conventional techniques.)
  - *pncA* mutation (frameshift; insertion) – PZA resistant
**NOTE**: Fortunate this isolate showed *RIF-R* on growth-based DST; otherwise would have missed since MDDR not ordered.

In process of changing our algorithm to include molecular testing for all submissions.
Areas of Applied Research for Drug Resistance

- **Improve our understanding of drug resistance**
  - Identify new mechanisms of drug resistance
  - Improve ability to detect drug resistance
  - Enhance understanding of correlation of mutations with resistance
  - Examine microevolution in the patient
  - Understand contribution of compensatory mutations
Distribution of mutations within patients that acquired resistance to fluoroquinolones

AQ^R 1

AQ^R 2

AQ^R 3

AQ^R 4

0 1 3 10 16

0 1 3 5 6 8 9 10 11 12

0 3 8 10 13 15 18 21

0 1 2 3 4 6 7 8 9 13

D94G S95T

D94Y D94G S95T

G88C D89G A90V D94N D94G S95T

A90V D94N D94A S95T
Upgrade TB Surveillance of Drug-Resistant TB

NATIONAL ACTION PLAN FOR
COMBATING MULTIDRUG-RESISTANT
TUBERCULOSIS

Washington, D.C.

December 2015

NATIONAL ACTION PLAN FOR
COMBATING ANTIBIOTIC-RESISTANT
BACTERIA

March 2015
Advance laboratory capabilities for detection and understanding of drug resistant MTB in support of U.S. TB programs (CARB funded)

- Transition of MDDR to next generation sequencing (NGS) platform
- Enhance informatics capacity for NGS data and laboratory reporting capabilities
- Improve understanding of genetic determinants to drug resistance
- Enhance approaches for early detection of drug resistance
- Improve growth-based and molecular approaches for testing new or repurposed drugs
Global Collaborations for Improving Detection and Interpretation of Drug Resistance

- **ReSeqTB**
  - Globally representative database
    - Funded by Bill & Melinda Gates Foundation (BMGF)
  - Includes WGS, growth-based DST results, and clinical outcome data (when available) from DS and DR isolates
  - Research and clinical utility
RELATIONAL SEQUENCING TB DATA PLATFORM

The Relational Sequencing TB Data Platform (ReSeqTB) catalogs a vast amount of genotypic, phenotypic and related metadata from *Mycobacterium tuberculosis* (Mtb) strains to enable the development of clinically useful, WHO-endorsed *in vitro* diagnostic assays for rapid drug susceptibility testing of Mtb. It provides a standardized and validated whole genome sequencing (WGS) analysis pipeline and a “one stop” source of curated, aggregated, clinically relevant genetic and associated metadata for global Mtb strains. Having these standardized and validated data easily accessible under one platform will accelerate the development of rapid drug susceptibility tests for TB and will significantly improve diagnosis of drug resistant disease in TB patients.

This initiative is led by a collaborative partnership which includes the Bill & Melinda Gates Foundation (BMGF), Critical Path Institute (C-Path), Foundation for Innovative and New Diagnostics (FIND), World Health Organization (WHO), US Centers for Disease Control and Prevention (CDC), New Diagnostics Working Group (NDWG), and the National Institute of Allergy and Infectious Diseases (NIAID).

To request access:
Submit an application

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<tr>
<th></th>
<th>Isolates</th>
<th>Resistant</th>
<th>Susceptible</th>
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<tbody>
<tr>
<td>MDR</td>
<td>270</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>XDR</td>
<td>2</td>
<td>5</td>
<td>54</td>
</tr>
<tr>
<td>Pre-XDR-FQ</td>
<td></td>
<td></td>
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<tr>
<td>Pre-XDR-INJ</td>
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Building Laboratory Capacity in USAPI
Game Changer for Local Labs: GeneXpert® MTB/RIF Assay

- Automated
- Independent module design
- Multi-disease platform
- Results within 2 hours
  - TB or not
  - Drug resistant or not
Acknowledgements

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APHL

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Cortney Stafford, MPH

Jamie Posey, PhD

ReSeqTB Collaborators

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E-mail: cdcinfo@cdc.gov  Web: www.cdc.gov

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.