Tuberculosis Pathogenesis and Transmission

Pamela B. Hackert, MD, JD, MPH
Objectives

• Identify why the paradigm shift identifying the caseating granuloma as the characteristic lesion of all TB occurred with the introduction of effective antibiotics
• Understand how using a three act model can better identify the actual pathology that is occurring in tuberculosis
• Review questions that can be addressed only by using the new paradigm
• Very briefly look at diabetes as a risk factor for progression to active disease
Looking at TB Pathogenesis With a Traditional Eye

### Pathogenesis of LTBI and TB Disease

1. **Area of detail for boxes 2, 4, and 5**
   - Droplet nuclei containing tubercle bacilli are inhaled, enter the lungs, and travel to the alveoli.

2. **Bronchiole**
   - **Tubercle bacilli**
   - **Alveoli**
   - Tubercle bacilli multiply in the alveoli.
Looking at TB Pathogenesis With a Traditional Eye


A small number of tubercle bacilli enter the bloodstream and spread throughout the body. The tubercle bacilli may reach any part of the body, including areas where TB disease is more likely to develop (such as the brain, larynx, lymph node, lung, spine, bone, or kidney).

4. Special immune cells form a barrier shell (in this example, bacilli are in the lungs)

Within 2 to 8 weeks, special immune cells called macrophages ingest and surround the tubercle bacilli. The cells form a barrier shell, called a granuloma, that keeps the bacilli contained and under control (LTBI).
Looking at TB Pathogenesis With a Traditional Eye

5. Shell breaks down and tubercle bacilli escape and multiply

If the immune system cannot keep the tubercle bacilli under control, the bacilli begin to multiply rapidly (TB disease). This process can occur in different areas in the body, such as the lungs, kidneys, brain, or bone (see diagram in box 3).
Unanswered Questions For Traditional Paradigm of Pathogenesis of Tuberculosis

• What protects most adults from disease following infection?
• Why are immunocompetent young adults especially susceptible to disease and death?
• Why does recovery from disease fail to produce immunity, but actually produces increased susceptibility to recurrent disease?
• Why have vaccines that prevent disseminated TB in children, failed to protect adults from pulmonary TB?
• Why does post-primary TB localize in the upper lobes of the lungs?
Then and Now
What Was Old Is New Again

1942

*A Story of Tuberculosis*

Huber the Tuber
by Harry A. Wilmer, M.D.

 Это a must book for everyone — Science News Letter

National Tuberculosis Association

2016

**WANTED**

Manuscript supporting the prevailing paradigm of tuberculosis by any author

DEAD or ALIVE

$1,000 REWARD

For decades, most TB research has developed the paradigm that granulomas are the characteristic lesion of both primary and post-primary (adult type pulmonary) TB. We have not found any original papers that support this paradigm.

Consequently, a reward of $1,000 is offered to the first person to produce a paper written by an investigator who personally studied the pathology of developing human post-primary TB that supports the paradigm that granulomas are the characteristic lesion of both primary and post-primary TB and that cavities arise by erosion of granulomas in to bronchi.

To claim reward, send reprint to Robert L. Hunter@uth.tmc.edu

What Were They Thinking? (and how did they get off track?)

- Antibiotics has reduced the number of cases seen by pathologists of post primary tuberculosis
- Pre-antibiotic era investigators consistently described post primary TB as an exudative reaction
  - A tuberculous lipid pneumonia of foamy alveolar macrophages
  - Undergoes caseation necrosis and fragmentation to produce cavities
- Granulomas in post primary disease arise only in response to old caseous pneumonia and produce fibrosis, NOT cavities
- Concept that cavities arise from caseating granulomas arose from *M.bovis* studies
  - *M.bovis* does not produce post primary tuberculosis in any species
  - Produces an aggressive primary TB that can develop small cavities by erosion of caseating granulomas
Once Infected, It’s All About the Balance

• Protection in TB has traditionally meant containment, not eradication of Mtb
• Once host immunity is affected, the balance is tipped in Mtb’s favor and LTBI progresses to active TB
• The difference between LTBI and active TB is paralleled by different tissue reactions
• Progression from LTBI to active TB (and sometimes back to LTBI) has to be viewed as a continuum and not as a defined step
• Each granuloma represents a disease entity in itself

Stefan H.E. Kaufmann, Introduction, Seminars in Immunology, Volume 26, Issue 6, Pages 429-430
http://dx.doi.org/10.1016/j.smim.2014.09.007
For MTB, Success Is Found Through Elusiveness

- MTB is most successful when it infects a child, then hides for decades before forming a cavity in the lung of a person with sufficient immunity to prevent infection in every other part of the body.
- This person may live for decades expelling infectious organisms into the community without ever becoming seriously ill.
- In several studies, half of the people who expectorate virulent MTB from cavitary tuberculosis have no symptoms of disease and deny that they even have a cough.
- Post primary tuberculosis is a very effective adaption of MTB to the longevity and life styles of its host, namely people.
Pathology of Human TB

Hunter 2016
“Old” Paradigm Thinking

The current paradigm of the pathogenesis of TB considers TB to be a one act play in which the caseating granuloma modulated by cell mediated immunity (CMI) is the characteristic lesion of all TB. While this is an appropriate model for M. bovis and primary TB, it fails to recognize the existence of obstructive lobular pneumonia that initiates and drives all of post-primary TB.
The Three Distinct Stages Hypothesized

**ACT 1: War of Attrition**
- **Immunity:** None → Strong
- Lymphatic/Hematogenous spread
- **Disseminated** → **Caseating Granuloma**
  - Granulomas form, contain and kill MTB as CMI develops

**ACT 2: The Sneak Attack**
- **Effective Systemic Immunity**
- Bronchial spread (tree-in-bud)
- **Resolution (95%)**
  - Obstructive Lipid Pneumonia
  - MTB antigens and host lipids accumulate in obstructive lobular pneumonia
  - **Caseous Pneumonia (5%)**
    - Sudden onset
    - Earliest lesion: Obstructive Lobular pneumonia
  - MTB antigens
  - Tree-in-Bud
  - Obstructed bronchiole

**ACT 3: The Fallout**
- No spread
- **Remain as Fibrocaseous TB**
- Paucibacillary Cavity
- **Mature Cavity**
- MTB Pellicle

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Hunter, 2016
Act I
The War of Attrition

• MTB try to multiply while the host attempts to contain them within granulomas

• With no or little immunity, there is greater lymphatic or hematogenous spread

• Control is through cell mediated immunity

Hunter, 2016
Act II-The Sneak Attack

- Act II Post-primary bronchogenic TB begins asymptptomatically in the apices of the lung, at some distance from the site initial infection
- It is part of latent TB since there are no clinical symptoms
- Few numbers of MTB in modified alveolar macrophages drive accumulation of host lipids and mycobacterial antigens in an isolated section of lung in preparation for a sudden necrotizing reaction sufficient to produce a cavity
Act III-The Fallout

- The stage encompasses the further evolution of necrotic caseous pneumonia
- It is either coughed out to form a cavity or becomes surrounded by epithelioid cells and fibrosis
- This produces granulomatous inflammation and most clinical disease
- Cavities form when caseous pneumonia softens, fragments and is coughed out of the body leaving a hole.
- Pneumonia that is not coughed out remains to induce inflammation. It dries to become fibrocaseous TB
What’s a Pellicle?

"In vitro pellicle, or biofilm mode of growth, where bacteria grow to produce a thick aggregate at the air-liquid interface and exhibit increased phenotypic resistance to antibiotics"
Why does post-primary TB localize in the upper lobes of the lungs?

Hunter 2016
What protects most adults from disease following infection?

Everyone's posting photos with the caption

#tb

Hashtag throwback

Usually used when someone wants to post an old photo on Instagram and expects reactions such as “awe so cute” or “that was so great”

And I'm all like, "Do people know what that is?"

Tuberculosis
Why are immunocompetent young adults especially susceptible to disease and death?
How can multiple pulmonary lesions in a single lung act independently as if the others did not exist?
Why does recovery from post-primary TB NOT produce immunity?
Why have vaccines that prevent disseminated TB in children, failed to protect adults from pulmonary TB?
## Risk of Developing TB Disease

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Risk of Developing TB</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB infection and no risk factors</td>
<td>About 10% over a lifetime</td>
<td>For people with TB infection, no risk factors, and no treatment, the risk is about 5% in the first 2 years after infection and about 10% over a lifetime.</td>
</tr>
<tr>
<td>TB infection and diabetes</td>
<td>About 30% over a lifetime</td>
<td>For people with TB infection and diabetes, and with no treatment, the risk is three times as high, or about 30% over a lifetime.</td>
</tr>
<tr>
<td>TB infection and HIV infection</td>
<td>About 7% to 10% PER YEAR</td>
<td>For people with TB infection and untreated HIV infection and with no LTBI treatment, the risk is about 7% to 10% PER YEAR, a very high risk over a lifetime.</td>
</tr>
</tbody>
</table>
References

- Kaufman S, Introduction, Seminars in Immunology, Volume 26, Issue 6, Pages 429-430 http://dx.doi.org/10.1016/j.smim.2014.09.007

- CDC Core Curriculum Tuberculosis 2013 http://www.cdc.gov/tb/education/corecurr/


- Alexander J. Adami, Jorge L. Cervantes, The microbiome at the pulmonary alveolar niche and its role in Mycobacterium tuberculosis infection, Tuberculosis, Volume 95, Issue 6, December 2015, Pages 651-658, ISSN 1472
The long read

The rats who sniff out tuberculosis

The African giant pouched rat can be trained to sniff out tuberculosis more accurately than most lab tests. So why is the medical profession still sceptical?

by Emma Young
TB Laboratory Testing & Case Studies

April 8, 2016
Angie Schooley, MT
James Sunstrum, M.D.

Beaumont
Objectives

• Review the cascade of laboratory tests a clinician may order to diagnose TB disease
• Integrate molecular assays with culture results
• Demonstrate the proper use of TB diagnostic tests using 3 sample cases of TB disease (easy, medium & difficult)
Disclosures

• None
What do all the words mean?

- Prevent Disease
- Promote Wellness
- Improve Quality of Life

NAA Amplification MGIT MTD
PCR Pyrosequencing Molecular
mutation HPLC MALDI-Tof Gene Xpert
MDDR NAAT Genotyping
WGS

16 S Sequencing

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Status of the tuberculosis problem in 2014.

Madhukar Pai, and Marco Schito J Infect Dis. 2015;211:S21-S28

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Does this patient have TB disease?

CLINICAL CLUES
- Cough > 2 weeks
- Fever > 2 weeks
- Exposure to TB
- Chronic immune suppression
- Endemic country
- Abnormal physical exam

LABORATORY TESTS
- PPD
- IGRA
- Sputum studies:
  - AFB Cultures
  - Molecular studies
- X-rays
- Biopsies
Recommended diagnostic options for pulmonary TB

- **See** the bugs [AFB microscopy]
- **Multiply** the bugs [NAATs]
- **Grow** the bugs [cultures]

• Courtesy of Prof. Madhukar Pai, MD, PhD  Mayo TB Center Webinar March 2016
Mycobacterial Examination

Mycobacterial examination has 6 stages:

1. Proper specimen collection
2. Examination of acid-fast bacilli (AFB) smears
3. Direct identification (NAAT-nucleic acid amplification test)
4. Specimen culturing and final identification
5. Drug susceptibility testing
6. TB genotyping
TB is difficult to diagnose
High Accuracy for Diagnosis of HIV in Contrast to TB DISEASE

HIV

HIV ANTIBODY
HIV RNA

TB DISEASE

AFB SMEAR
CULTURE
Studies Michigan 2015 pulmonary TB cases....N= 87

<table>
<thead>
<tr>
<th>Test</th>
<th>% POSITIVE</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB smear</td>
<td>44%</td>
<td>Negative smear does not rule out TB</td>
</tr>
<tr>
<td>NAAT on AFB+ smear</td>
<td>84%</td>
<td>May be performed on AFB smear negative sputums</td>
</tr>
<tr>
<td>AFB culture confirms <em>M. tb</em></td>
<td>78%</td>
<td>Gold standard, not always positive</td>
</tr>
<tr>
<td>IGRA</td>
<td>89%</td>
<td>May be negative even with positive cultures!</td>
</tr>
</tbody>
</table>

4/5/2016
Specimen Sources

- **Sputum** (primary)
- Pulmonary aspiration (secondary)
- Body fluids (CSF, pleural, peritoneal, etc)
- Tissue biopsy
- Blood
- Urine
- Gastric aspirate
- Stool (special request)
- Other
Sputum and AFB smears

“See the bugs”

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Specimen Collection

Pulmonary Specimen (sputum)

- Early morning specimens = highest yield of AFB
- Collect at least three consecutive specimens at 8-24 hr intervals (at least 1 early morning specimen)
- Recommended volume for testing is 5-10 ml, less may compromise recovery of AFB
- Infection control precautions during specimen collection
- If patient cannot produce sputum by coughing, consider other methods: sputum induction, bronchoscopy, or gastric aspiration
- All persons suspected of TB disease should have sputum cultured
Specimen Collection

- Collect in sterile, leak proof containers
- Seal with tape
- Refrigerate specimen to reduce overgrowth of contaminating bacteria during transit to lab
- Deliver specimen to TB lab within 24 hrs
- Always include patient name on both test request form and the specimen container
Acid-fast Bacilli (AFB) smear

• Least sensitive of all AFB Tests (20-75% positivity)

• Requires 10,000 AFB/ml to be positive

• Positive slide does not differentiate TB from atypical mycobacteria (i.e. *M. avium*)

• Reported within 24 hours of receiving the specimen in the laboratory
Fluorescent AFB Smear Using Auramine-O Staining

• Very sensitive, takes minutes to read

• Not all that is fluorescent is AFB (need a careful eye)

• Chemical fluorescence, **not** an immune stain or Direct Fluorescent Antibody

• Can be confirmed with Ziehl-Neelson (ZN) smear
Nucleic Acid Amplification (NAA) or PCR

“Multiply the bugs”
New CDC Guidelines of Use of NAA
MMWR January 16, 2009

• “NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.”

• NAAT should be performed on all new AFB+ sputum specimens
MTD-Hologic and Gene Xpert-Cepheid are the only FDA approved methods

NAA tests are available that are not FDA approved, such as real time PCR assays

MDHHS performs a real time lab developed PCR test to detect Mtb and MAC using the ABI 7500 Fast DX
GenExpert Assay Procedure for the MTB/RIF Test.

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Printable test result

Time to result, 1 hour 45 minutes

AFB Cultures

“Grow the bugs”

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AFB Culture Test

• More sensitive than AFB smear

• 10 AFB/ml can produce a positive result, whereas AFB smear needs 10,000 AFB/ml

• Culture may be AFB positive even if smear was negative for AFB
Tests Performed on Growth in Mycobacteria Culture

- *Accuprobe* DNA test *(not* amplified)
- HPLC (high performance liquid chromatography)
- MALDI-TOF
- Biochemical Identification Confirmation
- Drug Susceptibility
Susceptibility Testing of *M. tuberculosis*

**When to test**

- All new *M. tb* isolates
- Repeat after 90 days of therapy, if specimens continue to produce *M. tb*
- Relapse or failed therapy
Additional Molecular Tests for TB
CDC – Molecular Detection of TB Drug Resistance (MDDR)

- Rapid testing for DNA mutations associated with drug resistance
- NAAT (+) sputum specimens or culture isolates (prior approval)
- Must meet the following criteria:
  - Known Rifampin resistance
  - Known MDR
  - High risk of Rifampin resistance or MDR-TB
  - High profile patient (e.g. daycare worker, nurse)
  - Mixed or non-viable culture
  - Drug Adverse reaction (e.g. Rifampin allergy)
CDC MDDR

- **First-line** MDDR to detect MDR-TB
  - *rpoB* (Rifampin)
  - *inhA* and *katG* (Isoniazid)

- **Second-line** MDDR to detect XDR-TB
  - *gyrA* (Fluoroquinolones)
  - *rrs* (Kanamycin, Amikacin, Capreomycin)
  - *eis* (Kanamycin)
  - *tlyA* (Capreomycin)
  - *pncA* (Pyrazinamide)
  - *embB* (Ethambutol)
TB DNA Genotyping
Universally Offered by CDC

• DNA “Fingerprint” of each isolate

• Michigan Department of Health & Human Services laboratory runs genotype on all TB cultures in United States and territories
Mycobacterium tuberculosis Genotyping To Prioritize Tuberculosis Outbreak Control Activities
### Demographics of Selected Genotype Clusters in Southeast Michigan, 2008 – 2012

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Race</th>
<th>Ethnicity</th>
<th>Homeless</th>
<th>Alcohol</th>
<th>Drug</th>
<th>Incarceration</th>
<th>HIV positive</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR00012 (MI_0002) n = 58</td>
<td>63% African-American</td>
<td>11% Hispanic</td>
<td>37%</td>
<td>32%</td>
<td>42%</td>
<td>0%</td>
<td>16%</td>
<td>0%</td>
</tr>
<tr>
<td>PCR00291 (MI_0008) n = 48</td>
<td>97% African-American</td>
<td>3% Hispanic</td>
<td>44%</td>
<td>35%</td>
<td>29%</td>
<td>6%</td>
<td>15%</td>
<td>6%</td>
</tr>
<tr>
<td>PCR04678 (MI_0047) n = 23</td>
<td>100% African-American</td>
<td>0% Hispanic</td>
<td>27%</td>
<td>27%</td>
<td>46%</td>
<td>9%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

* All clusters were majority 45 – 64 yrs of age; male and US-born.
Epidemic Curve of Investigation of a Multistate TB Outbreak
3 Sample Cases

Beaumont
From: Current Approaches to Tuberculosis in the United States


Admission chest radiograph showing bilateral lung infiltrates with prominence in the right upper lobe and lingula of the left lung.

Figure Legend:
<table>
<thead>
<tr>
<th>APRIL 2016</th>
<th>“EASY” CASE</th>
<th>1 TB suspected</th>
<th>2 Sputum PPD/IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>AFB smear positive</td>
<td>5 NAAT positive</td>
<td>6 INH, RIF, PZA, EMB</td>
</tr>
<tr>
<td>4</td>
<td>PPD 15 mm</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>AFB in broth DNA probe+</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>21</td>
<td>22 Drug susceptibility</td>
<td>23</td>
</tr>
<tr>
<td>24</td>
<td>25</td>
<td>26 DNA genotype</td>
<td>27</td>
</tr>
<tr>
<td>28</td>
<td>29</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>
#2 case MEDIUM

57 yr male

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm³
<table>
<thead>
<tr>
<th>APRIL 2016</th>
<th>“MEDIUM” CASE</th>
<th>1 HIV+ TB suspected</th>
<th>2 Sputum PPD/IGRA</th>
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</thead>
<tbody>
<tr>
<td>3 AFB smear negative</td>
<td>4 PPD 0 mm 2nd smear negative</td>
<td>5 IGRA negative</td>
<td>7 NAAT positive</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
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<td>17</td>
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<td>24</td>
<td>25</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>4/5/2016</td>
<td>37</td>
<td>Beaumont</td>
<td></td>
</tr>
</tbody>
</table>
Case #3
Difficult

- Patient from Africa
- History of 3 prior episodes of pulmonary TB
- Coughing, sick again
#3 case MDR suspect
<table>
<thead>
<tr>
<th>APRIL 2016</th>
<th>“DIFFICULT” CASE</th>
<th>1 MDR-TB suspected</th>
<th>2 Sputum IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>AFB smear positive</td>
<td>4 IGRA positive</td>
<td>5 NAAT positive</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>MDDR from CDC positive*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11 MDR regimen started

12 13 14 15 16

17 18 19 20 21 22 23

24 25 26 27 28 29 30
### Report Status: Interim

**CLIA ID # 11D0668319**

**Original Submitter:**

**Submission to CDC:**

- **Michigan Dept. of Community Health / Labs**
  - Angie Schooley / Lab
  - Peter Davidson / Program

**CDC Specimen ID:**

**Specimen:** M. tuberculosis complex isolate

**Medium:** MGIT

**Date Collected:** 1/17/2012

**Date Received:** 1/31/2012

**Date Reported:** 2/1/2012

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### Results for Molecular Detection of Drug Resistance; Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Locus (region) examined*</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 254 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (RRDR)</td>
<td>Mutation: TCG&gt;TTG; Ser531Leu</td>
<td>Rifampin resistant. (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are RMP-R.)</td>
</tr>
<tr>
<td>inhA (promoter)</td>
<td>No mutation</td>
<td>Isoniazid resistant. (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are INH-R.)</td>
</tr>
<tr>
<td>katG (ser315 codon)</td>
<td>Mutation: AGC&gt;ACC; Ser315Thr</td>
<td></td>
</tr>
<tr>
<td>emrB (Met308,Gly409)</td>
<td>Mutation: GAC&gt;GCC; Asp354Ala</td>
<td>Probably Ethambutol resistant. (84% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are EMB-R.)</td>
</tr>
<tr>
<td>pncA (promoter, coding region)</td>
<td>No mutation</td>
<td>Cannot rule out PZA resistance.</td>
</tr>
<tr>
<td>gyrA (QRDR)</td>
<td>No mutation</td>
<td>Cannot rule out fluoroquinolone resistance. (86% of FQ-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>rrs (1400 region)</td>
<td>No mutation</td>
<td>Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 254 clinical isolates: 88% of AMK-R isolates have a mutation in the rrs locus; 59% of KAN-R isolates have a mutation in the rrs locus; an additional 29% of KAN-R isolates have a mutation in the elA locus; 49% of CAP-R isolates have a mutation in the rrs locus; an additional 6% of CAP-R isolates have a mutation in the rrs locus.)</td>
</tr>
<tr>
<td>elA (promoter)</td>
<td>No mutation</td>
<td></td>
</tr>
<tr>
<td>ftyA (entire ORF)</td>
<td>No mutation</td>
<td></td>
</tr>
</tbody>
</table>

*A negative result (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

Testing performed using in-house developed assays.
# MDCH Lab Confirmation of 2\textsuperscript{nd} Line Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>R</td>
</tr>
<tr>
<td>Rifampin</td>
<td>R</td>
</tr>
<tr>
<td>PZA</td>
<td>R</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>R</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>S</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>R</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>S</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>S</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>S</td>
</tr>
<tr>
<td>PAS</td>
<td>S</td>
</tr>
</tbody>
</table>
IN CONCLUSION

• **See** the bugs [AFB microscopy]

• **Multiply** the bugs [NAATs]

• **Grow** the bugs [cultures]

• **Kill** the bugs