What’s Lurking Out There?
WSLH Case Studies

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La Crosse, WI

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Case Study: Patient History and Clinical Presentation

- 61 year old male
- History of mild COPD
- Chronic hepatitis C and underlying cirrhosis
- Abnormal CT scan of chest: “bilateral tree-in-bud opacities”
- Clinician is thinking:
  - Indolent infection with non-tuberculous mycobacteria or fungus
  - Sequelae of dust/soil inhalation due to occupation (pouring and setting concrete)
Case Study: Patient History and Clinical Presentation

- Although the patient is asymptomatic, (due to abnormal CT scan), bronchoscopy ordered and BAL collected
- Few AFB seen (smear microscopy)
- TB symptoms and risk factors:
  - Patient denies fever, chills, anorexia, weight loss or night sweats
  - Patient denies TB exposures (girlfriend had latent TB)
  - Skin test/IGRA results unknown
WSLH PCR Testing

- For detection of *M. tuberculosis* complex (TB) and *M. avium* complex (MAC) directly from patient specimens
- Automatically performed on smear positive respiratory and non-respiratory specimens
- Testing takes less than two hours
- Testing is fee-exempt for patients suspected to have active TB
Case Study

- TB PCR result: Positive (low level)
- MAC PCR result: Negative
- TB PCR result confirmed by repeat testing
- “Despite the 2 positive test results, I have great difficulty believing the patient actually has tuberculosis, given the profound paucity of his symptoms and a CT scan which certainly is not strongly suggestive of this possibility”
Possibilities??

- False-positive result
  - Equipment contamination
  - Specimen mix-up
  - Cross contamination during specimen processing
  - False-positive TB PCR result
- Unexpected: “real” TB
How rare are false-positive laboratory results?

  - Reviewed articles and abstracts from Medline, ATS, IUATLD from 1966 to 1999, selected 14 studies evaluating >100 patients
  - False positive culture results are not rare
  - Median false positive rate= 3.1% (range 2.2-10.5%)
  - Clerical errors found to be as common as lab contamination
  - 67% of patients with false positive cultures were treated for TB
How to proceed?

- Patient started on therapy and placed in respiratory isolation
- More (sputum) specimens collected
- Diagnostic laboratory reviews procedures
- Await culture growth (culture confirmation of MTBC)
  - Refer to national center for TB genotyping
TB Genotyping

- Performed at the Michigan Public Health Laboratory and CDC
- Molecular method for distinguishing different strains of *M. tuberculosis* complex
- Used for epidemiology purposes:
  - Discover unsuspected transmission
  - Identify TB outbreaks
  - Detect laboratory cross-contamination event
- Genotyping performed for all culture-positive TB patients in Wisconsin
**TB Genotyping**

- **“Spoligotyping”:** detects presence or absence of 43 spacer oligo sequences in the direct repeat region of TB DNA
- **MIRU-VNTR:** calculates that number of repeats at 24 tandem repeat loci of the TB DNA

<table>
<thead>
<tr>
<th>Spoligotype</th>
<th>MIRU1</th>
<th>MIRU2</th>
</tr>
</thead>
<tbody>
<tr>
<td>777777477760771</td>
<td>2x3226133321</td>
<td>242534233525</td>
</tr>
</tbody>
</table>
TB Genotype Possibilities

- Possible sources of MTBC in laboratory contamination:
  - Contamination with QC strain of *M. tuberculosis* complex (H37Rv)
  - Contamination with another patient specimen that contained MTBC organisms (handled in the laboratory during the same time period)
## TB Genotype Results

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Spoligotype</th>
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<th>MIRU2</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC Strain H37Rv</td>
<td>777777477760771</td>
<td>2x3226133321</td>
<td>242534233525</td>
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<tr>
<td>Patient Isolate</td>
<td>777760077760771</td>
<td>124326153224</td>
<td>323124123226</td>
</tr>
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## TB Genotype Results

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<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Wisconsin TB Patients with exact genotype match</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997-1999</td>
<td>4</td>
</tr>
<tr>
<td>2000-2002</td>
<td>6</td>
</tr>
<tr>
<td>2003-2005</td>
<td>1</td>
</tr>
<tr>
<td>2006-2008</td>
<td>2</td>
</tr>
<tr>
<td>2009-2011</td>
<td>6</td>
</tr>
<tr>
<td>2012-2014</td>
<td>2 (most recent in 2014)</td>
</tr>
</tbody>
</table>
## Case Study: Follow-up Specimens

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Smear Result</th>
<th>Culture Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/2/16</td>
<td>1+ smear positive</td>
<td><em>M. tuberculosis</em> complex (MTBC)</td>
</tr>
<tr>
<td>3/3/16</td>
<td>2+ smear positive</td>
<td>MTBC</td>
</tr>
<tr>
<td>3/4/16</td>
<td>1+ smear positive</td>
<td>MTBC</td>
</tr>
<tr>
<td>3/22/16</td>
<td>2+ smear positive</td>
<td>MTBC</td>
</tr>
<tr>
<td>3/23/16</td>
<td>1+ smear positive</td>
<td>MTBC</td>
</tr>
<tr>
<td>3/24/16</td>
<td>2+ smear positive</td>
<td>MTBC</td>
</tr>
<tr>
<td>4/9/16</td>
<td>2+ smear positive</td>
<td>pending</td>
</tr>
<tr>
<td>4/10/16</td>
<td>2+ smear positive</td>
<td>pending</td>
</tr>
<tr>
<td>4/11/16</td>
<td>1+ smear positive</td>
<td>pending</td>
</tr>
</tbody>
</table>
Result Summary

- Genotype results indicated that the isolation of MTBC from this patient was likely not due to a false positive result or cross contamination event:
  - Not a laboratory QC strain
  - Not a recently-isolated strain or a common strain (last seen in 2014)
- MTBC was isolated from culture of subsequent respiratory specimens, confirming the laboratory diagnosis of tuberculosis.
Summary

- Clinicians in areas with low incidence of tuberculosis don’t always include tuberculosis in their differential diagnosis
- The patient in this case study is now being treated for active tuberculosis disease. He will be in respiratory isolation (at home) until:
  - Two weeks of adequate therapy have been administered and there is evidence of clinical improvement
  - Three consecutive smear negative respiratory specimens are obtained
  - Arrangements for post-isolation care have been made
Summary

- WSLH has access to molecular methods for rapid detection of MTBC and genotyping for epidemiology purposes
- Laboratory contamination events occur and are not as rare as you might think
  - Serious implications for patient management
  - Correlate laboratory results with clinical picture
- If any doubts about laboratory results:
  - Troubleshoot
  - Collect more specimens
  - Open communication with health care provider and health departments is necessary
  - Call WSLH for assistance with genotyping
Contact Information

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Comments or Questions??