Laboratory Testing for Diagnosis and Treatment of TB

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Laboratory diagnosis of TB
- Why do we need the lab?
  - Rule-in TB/Rule-out non-infectious and other infectious causes of disease
  - Need to isolate the organism!
    - Definitive identification
    - Drug susceptibility testing
    - Cluster identification
      - Spoligotyping, RFLP, etc.

Phenotypic methods for identification
- Phenotype can change
  - Example: *Pseudomonas aeruginosa*

Terms
- Phenotype – what something “looks like” or does
- Genotype – DNA, “genes”
- AFB – Acid Fast Bacilli; Mycobacteria fall into this group of bacteria

Morphology
Mycobacterial cell wall

- Mycolic acids make cell wall relatively impermeable
- Slow growth
- “Stable” cells
- Resistance
- Mycolic acid profile is species-specific

Identification by mycolic acid profile

- HPLC – high performance liquid chromatography
- Compare to database
  - (you are only as good as your database)
- Phenotypic – profile can vary by strain

Antibiotic Susceptibility Testing

- How do bacteria “become” resistant?
  - Selection of pre-existing sub-population

- Phenotypic methods:
  - Based on growth in presence of antibiotic
  - Challenges with MTB
    - Slow-growing, cell wall
    - Population

Resistant sub-population already exists

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mutation Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>$10^{-6}$</td>
</tr>
</tbody>
</table>

Drug-resistant mutants in large bacterial population

Multidrug therapy: No bacteria resistant to all 3 drugs

INH  RIF  PZA

Monotherapy: INH-resistant bacteria proliferate

INH

Spontaneous mutations develop as bacilli proliferate to >$10^8$

INH or RIF

INH-resistant bacteria multiply to large numbers

INH mono-resist. mutants killed, RIF-resist. mutants proliferate \(\rightarrow\) MDR TB
Classical Methods

- Agar proportion
  - Plate on media with and without drugs
  - Incubate (3 weeks)
  - Count colonies
  - RESISTANT if number of colonies with drug is >= 1% of the number of colonies without drug
- SLOW, test isolate

“Classical” methods

- Liquid growth, Mycobacteria Growth Indicator Tube (Bactec™ MGIT™)
  - Bactec™ MGIT™ tube +/- drugs
  - If growth with drug, RESISTANT
- Faster...can test culture

Discordant results?

- Testing isolate versus testing culture
- Pure culture?
- Consistent inoculum is difficult
- “Problem strains” – 2 strains sent to >100 labs for analysis

<table>
<thead>
<tr>
<th>Strain</th>
<th>INH-R</th>
<th>RIF-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31/88 (35%)</td>
<td>106/108</td>
</tr>
<tr>
<td>2</td>
<td>112/112</td>
<td>74/117  (82%)</td>
</tr>
</tbody>
</table>

*CDC unpublished data. J. Ridderhof, P. Angra

Newer platforms

- Trek™ Minimum Inhibitory Concentration (MIC) Panel
  - 12 drugs, minimal inhibitory concentration

Genotypic methods for identification

- Nucleic acid-based
- Genotype doesn’t change (mostly)
- 16s rDNA
  - Conserved
  - Housekeeping
  - Encodes fnsI
  - Ribosomal RNA, not protein

Phylogenetics and Identification

- 16s
- rpoB – β subunit of RNA polymerase
  - 139 clinical and reference strains of different species of Mycobacteria
16s not adequate for speciation

<table>
<thead>
<tr>
<th>Identification level</th>
<th>16S rRNA gene sequencing</th>
<th>rpoB gene sequencing</th>
<th>Combined analysis of 16S rRNA and rpoB genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>67 (48)</td>
<td>117 (84)</td>
<td>119 (86)</td>
</tr>
<tr>
<td>Group or complex</td>
<td>68 (49)</td>
<td>10 (7)</td>
<td>10 (7)</td>
</tr>
<tr>
<td>Genus</td>
<td>4 (3)</td>
<td>12 (8)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Possible novel species</td>
<td>7 (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sorsos et al., Journal of Clinical Microbiology, September 2010, Vol. 48, No. 9

Mutations and Drug Resistance

• Change in DNA ->
• Change in rRNA or protein ->
• Change in interaction with antibiotic

“1st Generation” methods

• Amplified MTD® (Mycobacterium tuberculosis Direct) test
  – rRNA target, transcription mediated amplification with hybridization-based detection
  – Can be used to test specimens directly
• AccuProbe®: TB complex, MAI, M. gordonae, M. kansasii
  – Similar technology, can be used on isolates only

“New Generation” Methods

• Detect/Identify MTB (and other mycobacteria)
• Detect mutations associated with antibiotic resistance
• Direct specimens
• Positive MGIT tubes
• Isolates

“New Generation” methods

• Target nucleic acid – GENOTYPE
• Cepheid GeneXpert® MTB/RIF
  – “WHO-endorsed”
• Hain GenoQuick®
• Homebrew assays – various platforms

Molecular Methods

• For identification: hsp65, rpoB
  – Also recA (DNA repair), 16s, ITS
• For antibiotic susceptibility: loci involved in resistance
  – rpoB (β subunit RNA pol) – RIF
  – katG, inhA (catalase peroxidase and NADH reductase) – INH
  – gyrA (DNA gyrase) – FQ
  – rrs (16s) – aminoglycosides, cyclic peptides
  – embB (arabinosyl transferase) – EMB

http://www.trueorigin.org/bacteria01.asp
How to detect specific sequences?

- Amplify DNA target
  - Polymerase Chain Reaction (PCR) with specific primers
- Detect specific nucleotides within that target
  - Probe hybridization
- Detect probe hybridization
  - Color
  - Association of probe with detectable molecule

Probe technology

- Amplify by PCR
- Hybridize probes – specific to mutations of interest
- Detect colors and interpret

Cepheid GeneXpert®

- 2 hours “total”, no processing
- Detects MTB, Rifampin resistance

Cepheid GeneXpert®

- PCR amplification of 81 basepair (bp) region of rpoB
- Five probes, binding pattern interpreted by instrument
- MTB detection, versus culture:
  - Sensitivity 98.0% (90.9% for smear neg)
  - Specificity 98.3%
- Rifampin resistance:
  - Sensitivity 96.7%
  - Specificity 98.6%
  - PPV 93.6%

Data from vendor.

Hain GenoQuick®

- Fast, little hands-on processing
- Can be performed directly on specimens
- VERY EXPENSIVE
  - Instrument is expensive
  - Kits are expensive
- Only identifies Rif R-associated mutations
Hain GenoQuick®

- Fast, but requires some processing
- Not all strips can be used with specimens
- Cheaper, but requires several instruments
- Interpretation more complicated
  - Possibility of error in reading strips
- Gives more information, and information regarding mutations is specific
- Flexible platform

Caveats of Molecular Testing

- Culture is more sensitive
- **You will only find what you are looking for**
  - May miss newly emerging strains
  - Will/May miss mixed infections
- **Negative results mean nothing!**
  - May be resistant through alternate route/mutation
- Reasons for false positive/false negative PCR
Molecular Testing Advantages

• FAST – results in hours to days versus weeks to months
• After extraction steps, no longer BSL3
  – Can use shared molecular platforms
  – Cross-trained staff
• Information regarding mutations conferring resistance