Disclosures

• None
Objectives

• By the end of this session, participants should be able to:

• Describe various tests used for diagnosing active TB

• Prescribe anti-TB regimens for treating drug-susceptible TB

• Articulate major medical management issues in the treatment of active TB
Diagnosis of Active Tuberculosis
TB Diagnostic Modalities

• Stains

• Culture

• Molecular methods for identification of *M. tuberculosis*
  • from culture isolates
  • directly from specimen

• *M. tuberculosis* drug resistance testing
  • rapid broth-based methods
  • molecular markers of resistance
What Happens to the Patient’s Specimen When It is Sent to the Lab?

- **Specimen**
- **Smear**
  - Perform Acid-Fast Smear – Mycobacteria Present?
- **Culture**
  - Perform culture on specialized medium
  - If culture grows, identify mycobacterium using molecular methods (hybridization probes, MALDI-TOF MS, or DNA sequencing)
- **ID**
- **AST**
  - Perform drug resistance testing on isolate

**PCR**

Perform Direct PCR for *M. tuberculosis*
Stains for Mycobacteria

• Why perform a stain?
  • Rapid, inexpensive
  • Fast turn around
    • A stain may take an hour to perform and report
    • A mycobacterial culture requires weeks
    • Molecular methods such as PCR are also quick but cost more and we only have good ones for Mtb
  • May indicate infectiousness

• What kind of stain is done?
  • An “Acid-fast” stain is used (eg., auramine/rhodamine, Ziehl-Neelsen, or Kinyoun stain)
    • Mycobacteria do not stain with the Gram stain
Acid-fast stains for mycobacteria

- mycobacteria are referred to as “acid-fast” bacilli (AFB)
- a complex is formed between mycolic acid and dye used in the stain (e.g., carbol-fuchsin or auramine O)
- this complex is resistant to destaining by mineral acids (this is why mycobacteria are called “acid-fast”)
- so mycobacteria retain the carbol-fuchsin or auramine O stain and other bacteria do not
Staining for Mycobacteria
Acid-fast stains - Issues

• Acid-fast stains are not very specific
  • indicates whether a mycobacterium is present in the specimen
  • does not allow us to know which mycobacteria it is
    • *M. tuberculosis* looks like all the other mycobacterial species on an acid-fast stain

• Acid-fast stains are not very sensitive
  • need 1000-10,000 CFU/ml for a positive AFB smear
2-3 AFB Smears are More Sensitive than 1 Smear
Yield of Serial AFB Smears

<table>
<thead>
<tr>
<th>Study</th>
<th>% of Total Positives Detected by:</th>
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<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; Smear</td>
</tr>
<tr>
<td>Walker et al. (2000), <em>Int J Tuberc Lung Dis</em>, 4:246.</td>
<td>77.1%</td>
</tr>
<tr>
<td>Ipuge et al. (1996), <em>Trans R Soc Trop Med Hyg</em>, 90:258.</td>
<td>83.4%</td>
</tr>
<tr>
<td>Mathew et al. (2002) <em>J Clin Microbiol</em>, 40:3482-4 (low prevalence pop.)</td>
<td>89.4%</td>
</tr>
</tbody>
</table>
Acid-Fast Smears Prepared from Early Morning Sputum Specimens Have Better Sensitivity

<table>
<thead>
<tr>
<th>Study</th>
<th>Spot (Random) Specimen Positive (%)</th>
<th>Early Morning Specimen Positive (%)</th>
</tr>
</thead>
</table>
Mycobacteria Cultures

Necessary to obtain an isolate of the mycobacterium for:

- species identification
- antimicrobial susceptibility testing
Culture of *M. tuberculosis* complex

- Sensitivity of culture is much better than smear
  - a positive AF smear requires 1000-10,000 CFU/ml of specimen
  - a positive mycobacteria culture requires only 10-100 CFU/mL of specimen

Culture

- 2 types of media used:
  - Solid Medium (Lowenstein-Jensen (LJ) or Middlebrook)
  - Broth (Liquid) Medium (FDA-cleared systems - Bactec MGIT and Trek VersaTREK)
  - In general, mycobacteria grow faster in broth but there are some strains that grow better on solid medium
**M. tuberculosis** Colony Morphology on Solid Medium

Note the “rough and buff” morphology typical of *M. tuberculosis*
BACTEC MGIT 960 Culture System

MGIT - Mycobacterial Growth Indicator Tubes (Becton Dickinson)
  - fluorescent indicator in bottom of tube quenched by $O_2$
  - ↑ mycobacterial growth = ↓ $O_2$ and ↑ fluorescence
VersaTREK System

- mycobacterial growth causes changes in bottle headspace pressure which are detected by the instrument; sponges in bottle provide increased surface area for growth

Identification of *M. tuberculosis* complex from culture
Identification of M. tuberculosis complex from culture

- Nucleic Acid Hybridization Probes
- Line probe Hybridization Assays
- DNA sequencing
- MALDI-TOF
Identification of MTB from Culture Isolates: Nucleic Acid Hybridization Probes

- Use for Isolates Grown in Culture
  - no DNA amplification step so need lots of target nucleic acid
  - add probe with unique, complementary sequence to known species; chemiluminescent detection
  - identification within 2-3 hours after growth in culture

If the culture isolate is MTB, the DNA probe will bind isolate’s rRNA and produce a signal
Identification of MTB from Culture Isolates: Nucleic Acid Hybridization Probes

- Hologic Gen-Probe AccuProbes® (nucleic acid hybridization probes) available for:
  - *M. tuberculosis* complex
  - *M. avium* complex
  - *M. gordonae*
  - *M. kansasii*

- FDA-approved for identification of culture isolates

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em> complex</td>
<td>99.2%</td>
<td>99.0%</td>
</tr>
</tbody>
</table>
Identification of MTB from Culture Isolates:
Line Probe Hybridization Assays
(Hain Lifesciences or Innogenetics)

- Genus- and species-specific probes bound to nitrocellulose membrane
- DNA from lysed culture isolate hybridizes to the probe for identification.
- Hain products (as an example):
  - GenoType Mycobacterium CM and AS
    - *M. tuberculosis* complex and 29 nontuberculous mycobacteria on 2 strips
  - GenoType MTBC
    - Differentiation of *M. tuberculosis* complex
  - GenoType MTBDR plus
    - *M. tuberculosis* complex plus wt and mutant *rpoB, katG, inhA*

Not approved for diagnostic use in U.S. at this time
Source: http://www.hain-lifescience.de
Identification of MTB from Culture Isolates: DNA Sequencing

- Sanger dideoxy sequencing is the current gold standard for mycobacteria identification
  - Various targets are useful (rpoB, hsp65, 16S rDNA gene, etc.)
  - Uses broad range primers that will amplify all mycobacteria species
  - Hypervariable region between primers used to distinguish species

Identification of MTB from Culture Isolates: MALDI-TOF MS

BSL3 Activities

10ul loop-ful of organism → Beads+500µl 70% Ethanol → Incubate room temp 10 min → Bead Beat 2 minutes

BSL2 Activities

Centrifuge 5 min → Speed Vac 10 min → 70% Formic Acid & Acetonitrile

MALDI-TOF

Spot 1ul sample + 2ul of Matrix

start to finish takes ~2 hrs for 24 samples

MALDI-TOF Identification of MTB from Culture Isolates: MALDI-TOF MS

start to finish takes ~2 hrs for 24 samples

MALDI-TOF
Drug Resistance Testing of *M. tuberculosis* complex
M. tuberculosis complex Drug Resistance Testing

- agar proportion is the current gold standard for all drugs except pyrazinamide
  - not rapid (14-21 days)
  - labor-intensive, technically complex
  - no FDA-cleared, commercially-available kit

Organism is resistant to drug A in the upper right compartment (>1% of inoculum shown by upper left control quadrant is growing in presence of drug). Organism is susceptible to drugs B & C in the lower compartments. Control quadrant in upper left contains no drugs.
Rapid Broth Susceptibility Testing for MTB
FDA-cleared, semi-automated with MGIT or VersaTREK systems

Compare growth rates in bottles/tubes +/- critical concentrations of drug

CDC goal is results for first-line drugs reported within 15-30 days after receipt of the specimen
M. tuberculosis complex resistant isolates

• If the isolate is resistant to any agent
  • preliminary report issued
  • consider confirming resistance by 2\textsuperscript{nd} method or 2\textsuperscript{nd} lab
  • consider initiating testing of secondary agents to avoid delays

• If the isolate is resistant to only PZA consider
  • speciation
    • M. bovis is mono-PZA-resistant
    • most isolates of M. tuberculosis are PZA-susceptible
Molecular detection of *Mtb* drug resistance markers

**Why?**

Rapid determination of potential drug resistance compared with phenotypic methods

Limited availability at this time except for the CDC MDR TB program
Xpert MTB/RIF and Rifampin resistance

- Target is \( rpoB \): gene encoding beta subunit of bacterial RNA polymerase
- Mutations in an 81bp region of the \( rpoB \) gene are responsible for \(~96\%\) of RIF resistance in \( Mtb \);
- also predicts MDR TB since the majority of RIF-resistant isolates will also be INH-resistant
- Some false positive RIF resistance with Xpert
  - PPV is lower in low prevalence settings
  - CDC recommends reporting Xpert RIF-R as a preliminary result pending confirmation with sequencing; growth-base DST is still required
Molecular Detection of *M. tuberculosis* Drug Resistance at the CDC

- offered for *M. tuberculosis* complex isolates and nucleic-acid amplification-positive (NAAT+) sputum sediments
- perform pyrosequencing and conventional sequencing
- provides rapid identification of mutations associated with resistance to many TB drugs
- limitations include
  - insufficient data to definitively associate all mutations detected with resistance;
  - not all mechanisms of resistance are known
  - not all resistance loci are sequenced
- use in conjunction with conventional DST results
Molecular resistance testing for MTB at the CDC

<table>
<thead>
<tr>
<th>Drug</th>
<th>Locus/Loci examined</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rifampin</td>
<td>rpoB</td>
<td>97.1</td>
<td>97.4</td>
</tr>
<tr>
<td>isoniazid</td>
<td>inhA &amp; katG</td>
<td>86.0</td>
<td>99.1</td>
</tr>
<tr>
<td>fluoroquinolones</td>
<td>gyrA</td>
<td>79.0</td>
<td>99.6</td>
</tr>
<tr>
<td>kanamycin</td>
<td>rrs &amp; eis</td>
<td>86.7</td>
<td>99.6</td>
</tr>
<tr>
<td>amikacin</td>
<td>rrs</td>
<td>90.0</td>
<td>98.4</td>
</tr>
<tr>
<td>capreomycin</td>
<td>rrs &amp; tlyA</td>
<td>55.2</td>
<td>91.0</td>
</tr>
<tr>
<td>ethambutol</td>
<td>embB</td>
<td>78.8</td>
<td>94.3</td>
</tr>
<tr>
<td>pyrazinamide</td>
<td>pncA</td>
<td>86.0</td>
<td>95.9</td>
</tr>
</tbody>
</table>

What Happens to the Patient’s Specimen When It is Sent to the Lab?

- Specimen
  - Perform Acid-Fast Smear – Mycobacteria Present?
  - Smear
    - Culture
      - Perform culture on specialized medium
      - ID
        - If culture grows, identify mycobacterium using molecular methods (hybridization probes, MALDI-TOF MS, or DNA sequencing)
      - AST
        - Perform drug resistance testing on isolate
  - PCR
    - Perform Direct PCR for *M. tuberculosis*
Direct Identification of *M. tuberculosis* complex from patient specimen *without waiting* for growth in culture

In general, we have good molecular methods for direct detection of MTB but not for NTMs.
Nucleic Acid Amplification-based (NAA) tests for MTB

- CDC recommendation:
  - NAA testing be performed on at least one (preferably the first) respiratory specimen from each patient with suspected pulmonary TB
    - if it would alter case management
    - If it would alter TB control activities
  - NAA testing does not replace the need for culture
NAA Tests for Direct Detection of MTB

- FDA-cleared
  - Hologic/Gen-Probe MTD
  - Cepheid GeneXpert MTB/RIF
- CE-marked/RUO in U.S.
  - Hain LineProbe
- Laboratory Develop Tests (LDTs)
  - Rapid cycle/real-time PCR
Direct Detection of MTB from Patient Specimens
*Mycobacterium tuberculosis* Direct Test (MTD)
(Hologic Gen-Probe)

- people frequently refer to this as the “TB probe” assay but that is not correct; this is a PCR-like amplification method
  - transcription-mediated amplification of *M. tuberculosis* complex rRNA directly from respiratory specimens
- clinical specificity: 99-100%
- clinical sensitivity:
  - smear positive: 91-95%
  - smear negative: 83-100%
- technically challenging test
  - inhibition from specimen components a concern;
  - open PCR system so false positives due to cross-contamination of specimens are possible.
  - cross-reactions occur w/ some rare mycobacteria: *M. celatum*, *M. terrae*-like organisms, *M. holsiatricum*
Direct Detection of MTB from Patient Specimens
Cepheid Xpert® MTB/RIF Test

• WHO-endorsed
• Runs on the Cepheid GeneXpert platform
• FDA-approved for respiratory specimens
• Detects *M. tuberculosis* complex and provides information about RIF resistance
• Results in about 2 hrs; minimal hands-on needed

Source: www.finddiagnostics.org
Xpert MTB/RIF accuracy for detection of *Mtb* complex

- Limit of Detection is 131 CFU/ml (package insert)

- Chang et al, 2012, J Infect 64:580-8:
  - Meta-analysis of 18 studies with 10,224 patients total
  - Pulmonary TB:
    - Sensitivity, Smear positive disease – 98.7%
    - Sensitivity, Smear negative disease – 75.0%
    - Specificity - 98.2%
  - Extrapulmonary TB:
    - Sensitivity - smear positive, 95.2%; smear negative 70.7%
    - Specificity – 82.6%

- Time to diagnosis comparison:
  - Smear microscopy = same day (but non-specific)
  - Broth culture took an average of 16 days
  - Solid media plate cultures took an average of 20 days
  - Xpert MTB/RIF – same day diagnosis
Direct Detection of MTB from Patient Specimens
Line Probe Assays (Hain Lifesciences)

M. tuberculosis complex direct detection

Not approved for diagnostic use in the U.S.

Source: http://www.hain-lifescience.de
Direct Detection of MTB from Patient Specimens

Laboratory-developed PCR Tests (LDTs)

Example of Real-time PCR Workflow in our Laboratory

- specimen or culture lysis, inactivation and processing
- DNA extraction
- PCR amplification and detection

Approximate turn-around time = 4h
Direct Detection of MTB from Patient Specimens

Laboratory-developed PCR Tests (LDTs)

Advantages

• closed PCR system – reduced opportunity for false-positives
• good sensitivity and specificity but it can vary since each test developed/verified independently
• often less expensive
• some can be used on a wider variety of specimen types included smear negative specimens and formalin-fixed, paraffin-embedded tissue blocks

Limitations

• Often not as well-characterized as FDA-cleared tests
  • How does sensitivity and specificity compare to cleared tests?
• Payer reimbursement and regulatory issues for LDTs
General Limitations of NAA tests for Direct Detection of *M. tuberculosis*

- Inhibition from specimen components a concern for falsely negative results
  - Inhibition control needed unless system lab has checked for inhibitors in all specimen types
- PCR detects presence of nucleic acid but doesn’t indicate if the organism is still viable
  - Patient could be being treated successfully but may still have a positive PCR result
- Culture is more sensitive so always perform culture too
  - Negative PCR result does not rule out *M. tuberculosis* infection
  - Culture isolate is needed for drug susceptibility testing
Summary

• AFB stains are rapid but insensitive and nonspecific
• Mycobacterial culture should always be ordered together with AFB stain
• Identification after growth in culture is rapid using molecular methods
• Direct identification of MTB using molecular methods most often uses smear-positive respiratory specimens; certain methods allow for other specimens
• Molecular detection of some drug resistance markers is available for Mtb culture isolates and directly for smear-positive respiratory specimens
Treatment of Active Tuberculosis
### Treatment of Drug-Susceptible TB Disease

<table>
<thead>
<tr>
<th>Phase</th>
<th>Details</th>
</tr>
</thead>
</table>
| **Initial Phase** | • First 8 weeks of treatment  
• Most bacilli killed during this phase  
• 4 drugs used |
| **Continuation Phase** | • After first 8 weeks of TB disease treatment (18 or 31 weeks duration)  
• Bacilli remaining after initial phase are treated with at least 2 drugs |
Current Preferred Regimens for Drug-Susceptible TB disease:

- Isoniazid, Rifampin, Pyrazinamide, Ethambutol

- **Isoniazid & Rifampin are the cornerstones**
  - Both are **bactericidal** against rapidly dividing mycobacteria
  - **Rifampin** also exhibits excellent late **sterilizing** effect on semi-dormant organisms
    - Non-INH based regimen = usually 9 months
    - Non-Rifampin regimen = 12-18 months (variable)

- **Pyrazinamide**
  - Potent **sterilizing** ability
  - Non-pyrazinamide based regimen = 9 months

- **Ethambutol**
  - Hedge against resistance
Standard TB Therapy for Drug-Susceptible Disease

• **Initial Phase:**
  - 4 drugs for 2 months (8 weeks)
  - Rifampin, isoniazid, pyrazinamide, ethambutol
    - Okay to stop ethambutol, once it is known that isolate is susceptible to rifampin, isoniazid, and pyrazinamide

Standard TB Disease Continuation Therapy

- **Continuation Phase:**
  - Rifampin & Isoniazid for 4 months (18 weeks)
  - Six months (26 weeks) total course of therapy
  - If PZA not used in initiation, then 7 months (31 wk) continuation

- **Continuation Phase: for cavitary disease AND positive cultures after initiation phase**
  - Rifampin & Isoniazid x 7 months (31 weeks) if cavitary disease at diagnosis and positive cultures after initiation phase at 2 months
  - Nine months (39 weeks) total course of therapy

Noncompliance or Abandonment of Therapy is Major Impediment of TB Treatment

• Directly observed therapy (DOT) has been shown to:
  • Facilitate treatment completion rates and bacteriologic evidence of cure
  • Decrease acquired and primary drug resistance
  • Decrease relapse rates

• CDC and American Thoracic Society (ATS) recommend consideration of DOT for all and
  • Especially for those with drug resistant organisms, cavitary disease, or HIV infection

TABLE 2. Drug regimens for culture-positive pulmonary tuberculosis caused by drug-susceptible organisms

<table>
<thead>
<tr>
<th>Initial phase</th>
<th>Continuation phase</th>
<th>Range of total doses (minimal duration)</th>
<th>Rating* (evidence)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Drugs</td>
<td>Interval and doses‡ (minimal duration)</td>
<td>Regimen</td>
</tr>
<tr>
<td>1</td>
<td>INH</td>
<td>Seven days per week for 56 doses</td>
<td>1a</td>
</tr>
<tr>
<td></td>
<td>RIF</td>
<td>(8 wk) or 5 d/wk for 40 doses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PZA</td>
<td>(8 wk)¶</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td>EMB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>INH</td>
<td>Seven days per week for 14 doses</td>
<td>1c</td>
</tr>
<tr>
<td></td>
<td>RIF</td>
<td>(2 wk), then twice weekly for 12 doses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PZA</td>
<td>(6 wk) or 5 d/wk for 10 doses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EMB</td>
<td>(2 wk), then twice weekly for 12 doses (6 wk)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>INH</td>
<td>Three times weekly for 24 doses</td>
<td>2a</td>
</tr>
<tr>
<td></td>
<td>RIF</td>
<td>(8 wk)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PZA</td>
<td></td>
<td>2b</td>
</tr>
<tr>
<td></td>
<td>EMB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>INH</td>
<td>Seven days per week for 56 doses</td>
<td>3a</td>
</tr>
<tr>
<td></td>
<td>RIF</td>
<td>(8 wk) or 5 d/wk for 40 doses</td>
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<td></td>
<td></td>
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<td>4b</td>
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</tbody>
</table>

Evidence Ratings:
A=preferred, B=acceptable alternative, C= when A&B cannot be given, E=never
I=randomized controlled trial, II=Clinical trials, not randomized or done in other populations

ATS; CDC; IDSA. Treatment of Tuberculosis. MMWR 2003;52(RR-11):1-77.
Key Points: Treatment of TB Disease

• **Initiation:**
  - RIF/INH/PZA/EMB until susceptibilities confirmed
  - Can stop EMB if susceptible to RIF/INH/PZA
  - RIF/INH/PZA for 8 weeks

• **Continuation:**
  - RIF/INH for 18 weeks
  - If PZA not used in initiation or if patient has cavitary disease + positive cultures at 8 wks, then RIF/INH continued for 31 weeks
Treatment of Extrapulmonary TB Disease

- Generally the same treatment as for pulmonary TB
- Addition of corticosteroids for:
  - TB pericarditis
  - TB meningitis
- Recommended that duration of therapy be extended to 9-12 months for TB meningitis
  - May extend to 18 months for tuberculoma

ATS; CDC; IDSA. Treatment of Tuberculosis. MMWR 2003;52(RR-11):1-77.
Sputum Culture Monitoring During Pulmonary TB Treatment

- Serial sputum smears every 2 weeks to assess early response
- Monthly sputum for AFB smear and culture (until 2 consecutive cultures negative)
- Repeat drug-susceptibility tests if culture-positive after 3 months of treatment

ATS; CDC; IDSA. Treatment of Tuberculosis. MMWR 2003;52(RR-11):1-77.
Clinical Monitoring During Pulmonary TB Treatment

• Periodic (minimum monthly) evaluation to review adherence and identify adverse reactions

• Repeat chest x-ray:
  • After 2 months treatment for patients with negative cultures
  • As clinically indicated for worsening
  • At end of treatment
Diagnostic Monitoring During Pulmonary TB Treatment

• Liver enzymes at baseline; HIV testing at baseline; hepatitis testing if indicated; monthly liver enzymes if indicated

• Renal function and CBC if abnormalities at baseline

• Visual acuity and color vision at baseline if EMB used and monthly
  • If EMB used > 2 months or
  • EMB dose > 15-20 mg/kg or
  • EMB with renal failure
TB Treatment in Pregnancy/Breastfeeding

- **INH considered safe in pregnancy/breastfeeding**
  - Risk of hepatitis increased in peripartum period
  - Pyridoxine (25 mg/day) recommended if INH is administered during pregnancy, administer to infant if breastfeeding

- **RIF & EMB considered safe in pregnancy & breastfeeding**

- **PZA - little information in pregnancy, generally avoided in US**
  - Safe for breastfeeding
  - Benefits of PZA may outweigh the risk (drug resistant cases)
  - WHO & IUATLD recommend this drug for use in pregnant women with tuberculosis
Treatment of Culture-negative Pulmonary TB

Continuation phase is shortened to 2 months

ATS; CDC; IDSA. Treatment of Tuberculosis. MMWR 2003;52(RR-11):1-77.