Currently Available
Drug Susceptibility Testing Methods

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Susceptibility Testing of *M. tuberculosis* complex

- Guide in choice of chemotherapy – provide the best chance of cure
- Confirm the emergence of drug resistance when a patient fails to show a satisfactory bacteriologic response to treatment and guide the choice of further treatment with different drugs
- Offer appropriate treatment to contacts
- Use to estimate the prevalence of primary and acquired drug resistance in a community
Susceptibility Testing of *M. tuberculosis* complex

Agar Proportion Method

- The method of proportion using Middlebrook 7H10 agar has been considered the “gold standard” method in the U.S. for several decades – used in Reference Laboratory, DTBE, CDC
Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard

This standard provides protocols and related quality control parameters and interpretive criteria for the susceptibility testing of mycobacteria, Nocardia spp., and other aerobic actinomycetes.

A standard for global application developed through the NCCLS consensus process.
Susceptibility Testing of *M. tuberculosis* complex - Definitions

- **Resistance** = Growth of >1% of an inoculum of bacterial cells in the presence of a “critical” concentration of anti-TB drug

- **Critical concentrations** represent the lowest concentrations of drugs that inhibit 95% of “wild strains” of Mtb (have never been exposed to the drugs), while at the same time do not inhibit strains that have been isolated from patients who are not responding to therapy and are considered resistant; concentration that best discriminates between S and R strains.
NCCLS* Standard M24-A (2003) Primary Antituberculosis Drugs

• Use a rapid method (e.g. BACTEC); perform on all initial isolates
• Test (equivalent) critical concentration for:
  – Isoniazid (INH)
  – Rifampin (RIF)
  – Ethambutol (EMB)
  – Pyrazinamide (PZA)
• Test additional higher concentration of INH

*now called CLSI
Rationale
Primary Antituberculosis Drugs

• Testing 4 drugs (INH, RIF, PZA, and EMB) provides comprehensive information for the standard regimen in U.S.

• Testing a higher concentration of INH provides additional therapeutic information for frequently encountered drug resistance.

• Testing a reduced panel (INH, RIF, and EMB) recognizes cost issues and low prevalence areas, but requires more frequent reflex testing of additional primary and secondary drugs.
Broth Systems

- Selection of critical (testing) concentrations based on comparison of results with agar proportion = “equivalent critical concentrations”
- Much more rapid results (5-7 days) than agar proportion (21-28 days)
- FDA approval for first-line drugs
- Published evaluations of second-line drugs
BACTEC 460 Instrument

- Semi-automated; needles
- Laboratory work-horse
- 12B media
- Radiometric
- Detects CO₂ production by mycobacteria
- DST for INH, RIF, EMB, STR, PZA
Mycobacteria Growth Indicator Tube (MGIT)

- Fluorescence quenched by $O_2$ in $O_2$-rich media
- If mycobacteria present, $O_2$ used up, no quench, fluoresces under UV light
- DST for INH, RIF, EMB, STR, PZA
VersaTrek
TREK Diagnostic Systems

• Unique growth matrix – cellulose sponge material
• Monitors rate of $O_2$ consumption
• INH, RIF, EMB, PZA DST available
Susceptibility Testing of *M. tuberculosis* complex

- Critical concentrations of drugs may differ among test systems
  - This causes confusion

- Not necessarily “equivalent”
  - This causes confusion
M. tb Drug Susceptibility Performance Evaluation Participating U.S. Laboratories Primary Antituberculosis Drugs (July 1999)

- All 5 "Primary" Drugs (INH, SM, EMB, RIF, PZA)
  - 90 (67.6%)

- 4 "Primary" Drugs (no PZA)
  - 36 (27.1%)

- 2-3 "Primary" Drugs
  - 7 (5.2%)

n = 133
M. tb Drug Susceptibility Performance Evaluation Participating U.S. Laboratories Primary Antituberculosis Drugs (June 2006)

4 “Primary” Drugs (INH, EMB, RIF PZA)
86 (80.4%)

“Primary” Drugs (no PZA)
21 (19.6%)
n = 107
Problem – have rapid first-line drug results, but if R detected, takes at least an additional 21 d to get second line results when tested by agar proportion!!!! Therefore, labs need plan to test/refer known/suspected drug-resistant isolates rapidly
Second-Line Drug Classes

- **Aminoglycosides**: Amikacin, Kanamycin
- **Polypeptides**: Capreomycin
- **Fluoroquinolones**: Ciprofloxacin, Ofloxacin, others
- **Thioamides**: Ethionamide, Prothionamide
- **Serine analogues**: Cycloserine
- **PAS**

**First line drugs**

WHO. Guidelines for the programmatic management of drug-resistant TB. 2006.
Revised WHO Case Definition for XDR TB (Oct 10, 2006)

Resistance to at least isoniazid and rifampin (MDR) plus resistance to fluoroquinolones and one of the second-line injectable drugs (amikacin, kanamycin, or capreomycin)

Goals
- Public health surveillance
- Reliable DST methodology
- Clinical relevance
Susceptibility Testing of *M. tuberculosis* Complex
– Recommended Secondary Testing Panel (NCCLS M24-A)

- Secondary Drugs (test if R to RIF or any 2 primary drugs)
  - Ethambutol hydrochloride (higher concentration)
  - Capreomycin
  - Ethionamide
  - Kanamycin (class for amikacin)
  - Ofloxacin (class for quinolones)
  - *p*-Aminosalicylic acid
  - Rifabutin
  - Streptomycin (2 concentrations)
  - (Cycloserine testing not recommended – hard to reproduce)
Participant U.S. Laboratories
SLD testing capacity
June 2006

• 30 Laboratories test at least 1 SLD
• 9 Laboratories test all 6 SLD classes
• 16 test Kanamycin and/or Amikacin, FQ, and Capreomycin
Participant U.S. Laboratories (CDC MPEP)
Frequency of Secondary Antituberculosis Drugs
June 2006

Percentage of Laboratories

- Ethionamide: 29
- Kanamycin: 25
- Capreomycin: 18
- p-Amino-Salicylic Acid: 20
- Ciprofloxacin: 15
- Ofloxacin: 15
- Cycloserine: 12
- Amikacin: 14
- Other FQ: 6

n = 407
2nd-line Drug DST Constraints

- Technically complex
- Drug powders often unstable *in vitro*
- Critical drug concentrations defining resistance often close to drug MIC and/or attainable serum levels, results therefore inconsistent
- Cross-resistance not fully understood
- Methodology not standardised
- Lack of correlation with clinical response
- Very few laboratories with technical capability
Problems/Concerns with Current Practices

- Most testing algorithms based on referrals of specimens/isolates
- Lack of confidence/reluctance of labs to report resistance prior to confirmation
- Discordant results – inter- and intra-lab, different methods, etc.
- Manpower/training issues
Testing Algorithms

• Work is often dispersed and piecemeal
  – specimens or isolates referred from one lab to another
  – communication between labs may be a problem

• Communication with care-giver/TB program often a problem especially when testing becomes further removed from originating lab

• It is important that labs communicate doubts (e.g., unexpected drug resistance)
Reasons for Discordant DST Results

• “human error/lab error”
• Bacterial population (isolate vs. subculture)
• Differential growth kinetics
• Different inoculation methods (size, clumps)
• Different methods or media
• Cross-contamination
• Transcription, labeling errors
• The “bug” - the MIC ≈ critical concentration
Laboratory Methods for *Mycobacterium tuberculosis*

- **LJ Culture**
  - Drug Susceptibility
  - Identification
  - Isolation

- **7H10 Middlebrook**
  - Drug Susceptibility
  - Identification

- **Liquid Radiometric**
  - Drug Susceptibility

- **PCR Based**
  - Drug Susceptibility

**Weeks**

0 2 4 6 8 10 12 14
Principle of molecular beacons

• Probe target is amplified in a “real time PCR”
• Molecular Beacon Probe forms a hairpin
  • No fluorescence because of close proximity of reporter dye to quencher dye
• If target present, hybridization results in dsDNA and separation of reporter dye from quencher dye results in fluorescence
• Thus, the hybridization is detected as fluorescence
Detection of INH and RIF Resistance by Molecular Beacon Assays
State of CA Microbial Diseases Laboratory

- Turnaround time about 4 hours
  - “real time PCR”
- Accuracy so far > 96%

Information from Ed Desmond, PhD, CMDL
INNO-LIPA Rif.TB
Innogenetics, Belgium

- Strip assay (line probe assay)
- PCR-based
- Detect MtbC
- Detect RIF R ($rpoB$ gene)
- Not FDA-approved for US
GenoType MTBDR
Hain Lifescience, Germany

- Strip technology (line probe assay)
- PCR based
- Detect MtbC
- Detect RIF R ($rpoB$ gene)
- Detect INH R ($katG$ gene)
- Not FDA-approved for US
Summary

• DST results must be available as soon as possible to guide/validate treatment choices

• Even when a laboratory performs rapid broth-based first-line drug testing or even molecular testing, if the testing/referral algorithm is not optimized, there will be substantial delays in obtaining second-line drug results when needed