TB in Nebraska, New Challenges & Solutions
Mycobacterium TB in Nebraska, New Challenges & Solutions
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Challenges:

1. Accurately diagnose infections
2. Prevent transmission
3. Provide appropriate treatment
4. Correctly classify the organism
1. Accurately diagnose infections

• Tuberculosis (TB) is a serious re-emerging bacterial illness that usually affects the lungs.

• TB bacteria are spread from person to person through the air.
  – *Mycobacterium tuberculosis* complex
There are two forms of TB:

- **TB infection (latent TB)** – not contagious
- **TB disease (active TB)** – are contagious

People with TB infection (latent TB) can take drugs to prevent them from getting TB disease (active TB).
Therefore:

• **Prevention of TB involves:**
  – **Identification of latent TB infections**
However:

• People with latent TB infection:
  – Have no symptoms
  – Don’t feel sick
  – Have a normal chest x-ray
  – Have a negative sputum smear
  – Have circulating blood cells (lymphocytes) that recognize mycobacterial proteins (antigens)
QuanFERON –TB Gold Assay

- Alternative to tuberculin skin test (TST)
- *in vitro* vs. *in vivo*
- M. tb complex-specific antigens used
- 1 visit to clinic
- Less subject to errors
- Fewer false-positives
QuanFERON –TB Gold Assay

• Principle:
  – Tests for infected lymphocyte’s ability to respond to mycobacterial antigens
    • ESAT-6 (Early Secretory Antigenic Target – 6)
    • CFP-10 (Culture Filtrate Protein – 10)
  – By secreting a cytokine
    • IFN-γ (interferon-gamma)
  – And measured by ELISA
    • (Enzyme-Linked Immunosorbent Assay)
Stage 1 – Incubation of Blood
Stage 2 – Detection of IFN-γ

- Negative control (not stimulated)
- ESAT-6 stimulated
- CFP-10 stimulated
- Positive control (mitogen)
Recommended for:

- Groups more likely to be exposed to TB
  - People from countries where TB is common
  - People in close contact with active TB case
  - People with HIV
  - People in nursing homes, prisons or homeless shelters
  - Laboratory personnel
2. Prevent transmission

- Identifying suspected sources
- Understanding transmission patterns

Genotyping provides tool
Genotyping Analysis

Isolate A

Isolate B

Likely Related
Genotyping Analysis

Isolate A

Isolate B

Not Related
Genotyping Methods

- Two PCR-based methods:
  - Spoligotyping
  - MIRU-VNTR

- Results converted to numeric code
- Matches can be further investigated by other technologies
Spoligotyping

• **Spacer Oligonucleotide Typing**

• Presence or absence of 43 spacer regions found in the Direct Repeat region of *M. tb* genome.

• Results converted to 15 digit code
Spoligotyping

Original banding pattern

Binary code

14 + 1 grouping

Designation (15 digits)
MIRU-VNTR

- Mycobacterial Interspersed Repetitive Units – Variable Number of Tandem Repeats

- Identifies strains by the difference in copy number of tandem repeats at 12 different locations of the genome
MIRU-VNTR

MIRU locus name 02, 04, 10, 16, 20, 23, 24, 26...

# of repeats 2 3 2 2 3 4 2 5

MIRU designation (12 digits) 23223425....
## Genotyping Results

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Genotyping Program:

• Laboratory component
  – Specimen submission to genotyping lab
    • Atlanta, GA
    • Richmond, CA
    • Ann Arbor, MI
  – Tests performed in reference lab
    • > 32,000 isolates tested
  – Results sent back to state lab
Genotyping Program:

• Program component
  – Share patient information with laboratory
  – Receive and interpret genotyping reports
  – Decision to act on genotyping results
Genotyping results:

• Identifying suspected sources
  – Some “source cases” identified by epi investigation have been different strains

• Understanding transmission patterns
  – Unsuspected sources have been identified
3. Provide appropriate treatment

- People infected with TB can take medications to prevent active TB disease.
- People with active TB disease can usually be cured with anti-TB drugs.
- The drugs must be taken exactly as prescribed.
- Some new TB strains are resistant to anti-TB drugs.
M. Tb Treatment

• Long term treatment
• Multi-drug regimen
  – Primary drugs
    • Rifampin, Isoniazid, pyrazinamide, ethambutol
  – Secondary drugs
    • Streptomycin, cycloserine, macrolides, quinolones
Drug resistance

- **MDR TB**: Multi - drug resistant
  - Resistant to Rifampin & Isoniazid
  - About 5% of all TB infections (average)
  - Highest rate in former Soviet republics

- **XDR TB**: Extensively drug resistant
  - Resistant to all primary and at least one secondary
  - 45 countries report at least one case
Drug resistance testing

• Antimycobacterial Susceptibility Tests (ASTs)

• Two methods
  – Agar based
  – Broth based

• Creighton University does NE surveillance
ASTs by Agar proportion method

- Gold standard
- Dilutions of standardized inoculum onto control and drug containing agar
- Compare growth in absence or presence of drug
- >1% colony growing on the drug containing agar suggests resistance
Limitations of method

• Organism must be identified to the species level before reporting AST data

• Results take about 3 weeks
4. Correctly classify organism

• Non-TB mycobacteria are cause of disease
  – *Mycobacterium avium* - respiratory disease
  – *M. kansasii* – respiratory / cutaneous disease
  – *M. marinum* – “fish tank granuloma”
  – *M. leprae* – skin disease
  – *M. gordonae* - contaminate
• Greater than 90 species known to exist

• Treatments vary by species

• Conventional methods of ID can be lengthy

• Molecular methods can be utilized
MycoAlign

- Developed as a collaboration between UNO and UNMC
- Combination molecular and web-based computational system
- ID of *Mycobacterium* spp.
Molecular Target – rDNA gene

- Composed of multiple genes that code for ribosomes
  - Bacteria 16S and 23S
- Contains a variable region to discriminate among species
- Internal transcribed spacer regions (ITS)
Molecular Target – rDNA gene

- Stable within species
- Contains conserved sequence areas
  - Create universal primer sets
  - Small sequence is manageable
Gel electrophoresis

600 bp→
Mycobacterium
database

Test Number
78

Sequence
GGTTTTCCGAGTCTGCGGAGTATTGTGCGCGGCGGATGATGTGCGCGGCGG

Comparison Regions

ITS

Compare
Clear
Mycobacterium

Sequence Comparison Results

Test Number  78

Similarity results for sequence Mycobacterium abscessus
ITS Similarity Percent: 96.23%

Similarity results for sequence Mycobacterium chelonae seq II/Mche-C
ITS Similarity Percent: 84.956%

Similarity results for sequence Mycobacterium chelonae seq I/Mche-A
ITS Similarity Percent: 83.628%

Similarity results for sequence Mycobacterium chelonae seq I/Mche-B
ITS Similarity Percent: 82.743%

Similarity results for sequence Mycobacterium triviale
ITS Similarity Percent: 82.24%
# MycoAlign results

<table>
<thead>
<tr>
<th>MycoAlign Result</th>
<th>MycoAlign Score</th>
<th>TOT MycoAlign Lab</th>
<th>TB Lab Result</th>
<th>Probe TOT</th>
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</table>

3.77 7.17
Challenges:

1. Accurately diagnose infections

Solution:

Use QuantiFERON-Gold Assay
Challenges:

2. Prevent transmission

Solution:

Use National Genotyping Program
Challenges:

3. Provide appropriate treatment

Solution:

Conduct AST Surveillance
Challenges:

4. Correctly classify the organism

Solution:

Use MycoAlign software