Genotyping: process and application

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Overview

• Definition of TB genotyping
• Application of genotyping to tuberculosis (TB) control
• TB genotyping techniques
• Steps in obtaining genotyping results
• TB clusters
• Steps in a TB cluster investigation
TB genotyping

• Laboratory approach used to determine if *Mycobacterium tuberculosis* isolates are genetically related

• Centers for Disease Control and Prevention’s National TB Genotyping Service began in 2004
  – Two contract labs (California and Michigan)

Application of genotyping to TB control

• Identify false-positive cultures
• Detect outbreaks
• Detect transmission
  – Unsuspected relationships between patients and new/unusual transmission settings
  – Occurring between patients who reside in different jurisdictions
• Evaluate routine TB control activities
Three commonly used genotyping techniques

- **RFLP analysis**: IS6110 restriction fragment length polymorphism analysis

- PCR (polymerase chain reaction)-based methods
  - **Spoligotyping**: spacer oligonucleotide typing
  - **MIRU analysis**: mycobacterial interspersed repetitive unit variable number tandem repeat analysis

**RFLP analysis**

- Based on measuring the number and length of specific DNA fragments that are cut using specific restriction enzymes
- Insertion sequence **6110**
  - Genetic marker unique to members of the *M. tuberculosis* complex
- Results
  - Pattern and number of copies of IS6110 (each copy = one band)
  - Image scanned for computer analysis

Source: [http://www.cdc.gov/tb/programs/genotyping/Chap3/3_CDCLab_2Description.htm](http://www.cdc.gov/tb/programs/genotyping/Chap3/3_CDCLab_2Description.htm)
**Spoligotyping**

- Based on spacer sequences found in the direct repeat region in the *M. tuberculosis* chromosome
  - Detects the presence or absence of 43 spacers

**Results**

Original banding pattern

7 7 7 7 7 7 4 7 7 7 6 0 7 7 1

Source: http://www.cdc.gov/tb/programs/genotyping/Chap3/3_CDCLab_2Description.htm

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**MIRU analysis**

- Segments of *M. tuberculosis* DNA contain tandem repeated sequences
  - Number of copies of repeated sequence varies among strains

- Determines the number of repeats at defined regions (loci) of the TB chromosome
  - Total of 41 loci, some of which are genotyped (e.g., 12, 15, 24)

- Results (example for 12-loci MIRU analysis)
  - 12-character designation (each character corresponds to the number of repeats at one of the loci)

<table>
<thead>
<tr>
<th>MIRU locus name</th>
</tr>
</thead>
<tbody>
<tr>
<td>02</td>
</tr>
<tr>
<td>No. of repeats</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

MIRU-12 designation: 232234253322

Source: http://www.cdc.gov/tb/programs/genotyping/Chap3/3_CDCLab_2Description.htm
Evaluation of genotyping methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFLP analysis</td>
<td>• Gold standard</td>
<td>• Slow turnaround (4-8 weeks)</td>
</tr>
<tr>
<td></td>
<td>• Most discriminatory (&gt;6)</td>
<td>• Laborious and expensive</td>
</tr>
<tr>
<td></td>
<td>• Less discrimination (&lt;6)</td>
<td>• Less discrimination (&lt;6)</td>
</tr>
<tr>
<td></td>
<td>• No standard nomenclature</td>
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</tr>
<tr>
<td>Spoligotyping</td>
<td>• Little DNA needed</td>
<td>• Less discriminatory than RFLP</td>
</tr>
<tr>
<td></td>
<td>• Quick turnaround</td>
<td>• Less informative</td>
</tr>
<tr>
<td></td>
<td>• Cost effective</td>
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<td></td>
<td>• Standard nomenclature</td>
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</tr>
<tr>
<td></td>
<td>• Better resolution than spoligotyping</td>
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</tbody>
</table>

Which genotyping method(s) should my TB control program use?

- Different methods provide different information
  - Different jurisdictions use one or more methods
  - Using more than one method is more informative than any single method

- CDC provides free routine spoligotyping and MIRU analysis as part of its national genotyping service
  - RFLP is done under certain circumstances

- New York City (NYC) uses all three methods
Key steps in obtaining genotyping results

Clinical, hospital, commercial laboratories

State laboratory

Genotyping laboratory

TB isolate

TB GIMS

Genotyping results

TB Genotyping Information Management System

TB Control Program

Using genotyping results in TB control
TB cluster definition

- Two or more TB patients that have isolates with the same genotype

  - PCR cluster: Two or more patients with isolates with matching spoligotype and MIRU result \(\text{(CDC definition)}\)

  - NYC cluster: Two or more patients with isolates with matching spoligotype and RFLP result

What does a TB cluster mean?

- Clustering \textbf{may} indicate recent transmission
  - Cannot rule-out remote transmission without additional information

- Need to determine if patients are epidemiologically linked
**Epidemiologic link**

Indicates that patients are linked by person, place or time

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**NYC epidemiologic link definition**

<table>
<thead>
<tr>
<th>Possible</th>
<th>Probable</th>
<th>Definite</th>
</tr>
</thead>
</table>
| **Possible** | • Patients live/spend time in area within approximately 0.5 miles of each other  
• Patients have similar social environment (e.g., similar social networks or high-risk behaviors)  | • Patients frequent the same location during the same date range, regardless of the infectious period of either patient  |
| **Probable** | • Patients frequent the same location during the same date range, regardless of the infectious period of either patient  | • Patients name each other as contacts  
• Patients share a common contact without naming each other as direct contacts  
• Patients frequent the same location during the infectious period of at least one of the patients  |
Steps in conducting a cluster investigation

Assign cluster
Collect and analyze data
Develop cluster hypothesis
Final report

Communicate with case managers
Re-interview patient
Communicate results

Collect data

- Review data from existing data sources
  - Demographic and clinical information for patients and contacts
  - Contact investigation results
  - Previous cluster investigation results (if available)
  - Surveillance and treatment data
  - Potential transmission sites identified
    - Residence
    - Worksite
    - School
    - Treatment facilities
    - Hangout spots
  - Social characteristics
    - Homelessness
    - Incarceration
    - Substance use
Analyze data

- Create tables
  - Patient characteristics
  - List of contacts
  - Potential sites of transmission

- Map data
  - Use software to map potential sites of transmission

- Develop timeline
  - Review the infectious periods of all cluster patients
  - Consider date of entry into US (foreign-born patients)

Develop hypothesis

- Use existing data to develop transmission hypothesis for each cluster patient

- Example:

  ![Diagram showing TB Patient 1 and TB Patient 2 in Cluster A with a thought bubble asking if they know each other and did not name each other.](image)
Develop cluster-specific questionnaire

• Cluster questionnaire template
  – Demographics
  – Work or school history
  – Potential sites of transmission
  – Homeless and incarceration history
  – Social activities

• Modify questionnaire and add additional cluster specific questions as needed

Re-interview patient

• Re-interview patient using cluster questionnaire during treatment (ideal) or within 12 months of treatment completion

• Interview methods
  – In-person (ideal)
  – Phone

• Three attempts to interview
Create final report

- Overview of cluster
- Individual patient summaries (narrative)
- Epidemiologic curve (larger clusters)
- Tables
  - Patient characteristics
  - Related persons
- Map of potential transmission sites
- Timeline
- Transmission hypothesis
  - For each cluster patient
- Transmission diagram
  - Illustrates epidemiologic links identified during contact or cluster investigation

Communicate results

- Cluster rounds
  - Present investigation findings to case managers
  - Encourage discussion and new ideas for cluster investigation
  - Brainstorm possible interventions
- “Cluster snapshot”
  - One-page description of cluster that highlights key characteristics and findings
Conclusions

- Different genotyping methods
  - All have advantages and disadvantages
  - Using a combination of methods is more informative than any single method
- Applying genotyping results helps improve TB control
- Genotyping results are used to define TB clusters
  - Clustering may indicate recent transmission
- TB genotyping results combined with epidemiologic data help determine whether patients are involved in the same transmission chain
- Cluster investigations provide TB controllers with detailed information about TB transmission
  - Helps identify interventions to prevent future transmission

Example of a “cluster snapshot”
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