# Molecular Epidemiology of Extrapulmonary Tuberculosis

## A Maryland State Review

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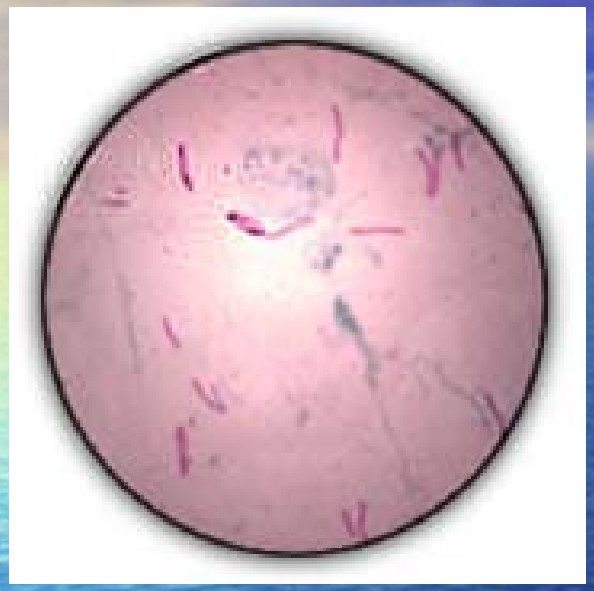
# Outline

- Background
- Research Topic
- Methods
- Results
- Discussion
- Future Studies

# *Mycobacterium tuberculosis*



Acid fast bacterium (AFB)

Reproduces very slowly

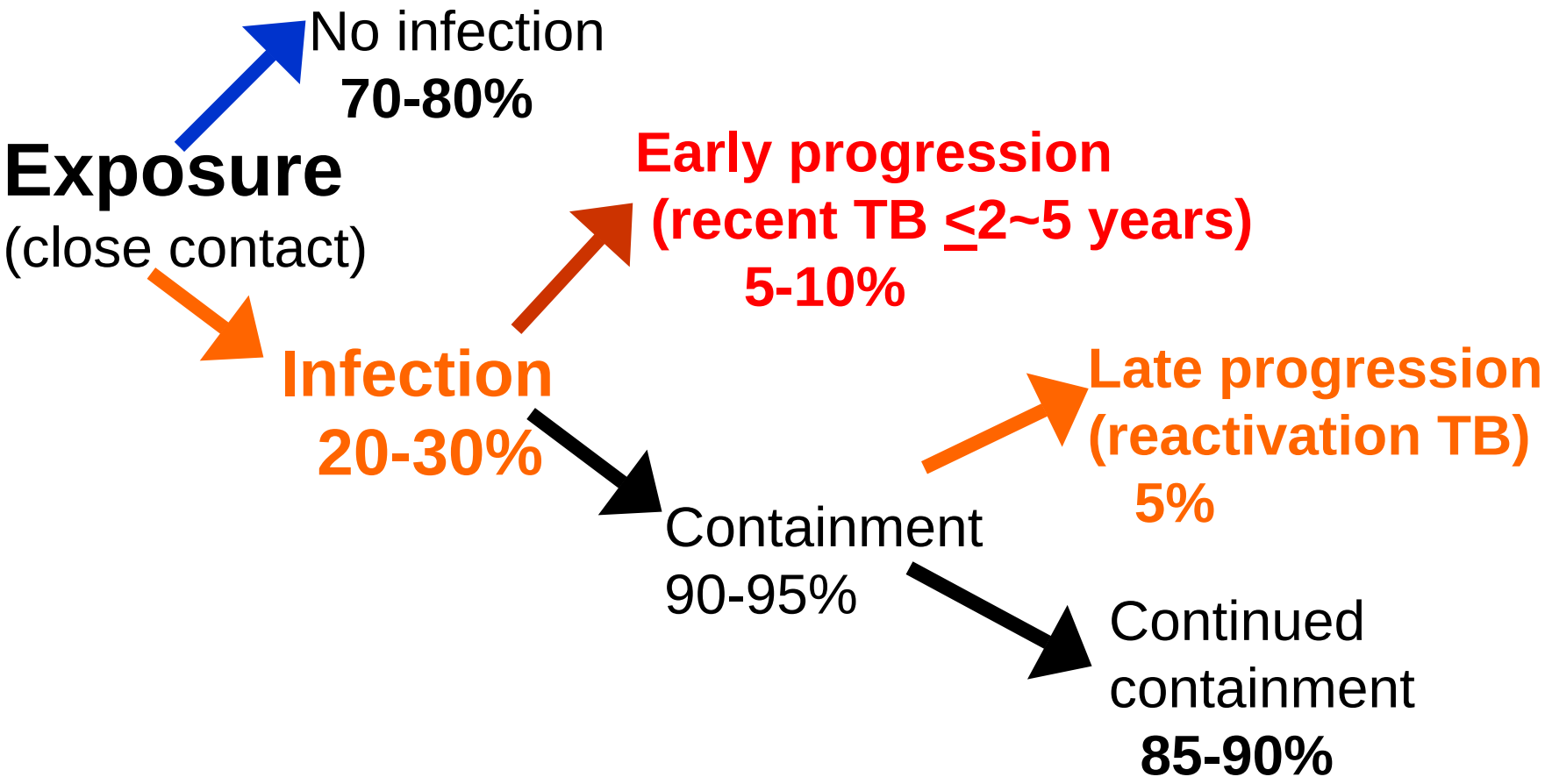
- ✓delays in laboratory reports
- ✓prolonged treatment

TB can remain **dormant** in body tissue for years after initial infection takes place

# Tuberculosis in brief

- Airborne communicable disease, spread by *M. tb* carried within droplet nuclei
- Droplet nuclei produced when a TB case coughs, sneezes, or sings
- Pulmonary TB is the most common presentation, accounting for approximately 80% of cases
- Extrapulmonary TB occurs in any part of body (bone, meninges, kidney, lymph nodes,etc.)

# Risk of TB infection and disease among exposed individuals



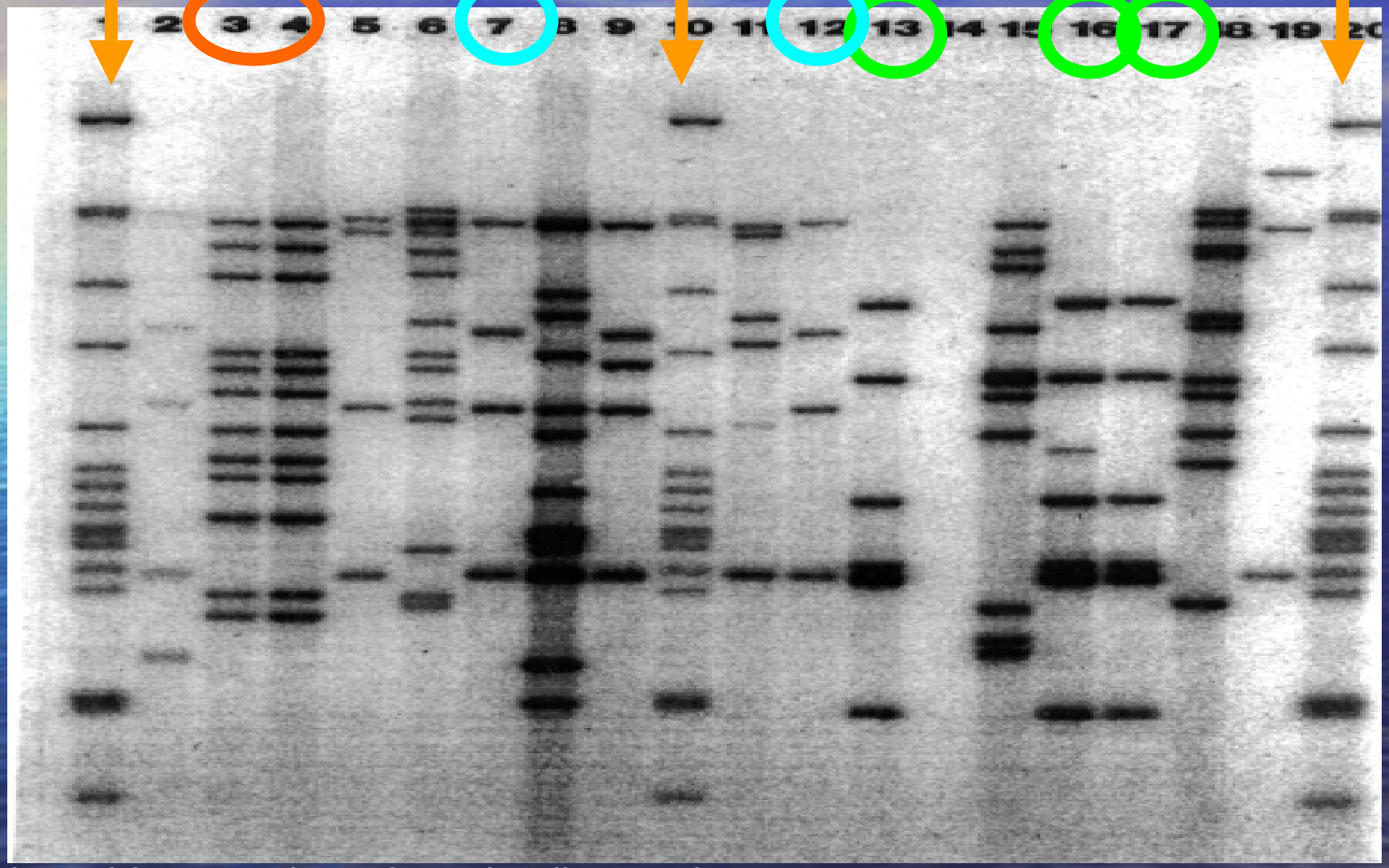
# US populations with high TB incidence

- Foreign-born
- Alcohol and/or drug abusers
- HIV-infected
- Homeless
- Incarcerated
- Poor

# DNA Fingerprinting

- IS6110-based RFLP
  - Insertion sequence is a mobile element present in the TB genome at different sites and in variable copy numbers
  - Restriction enzymes cut DNA at specific sequences
  - Can characterize the TB strain by position and number of the IS6110 bands

# DNA fingerprinting of *M. tuberculosis* isolates from different patients in a population study




Adapted from Braden Infect Dis Clin Practice 1997; 6:89-95

# Current uses

- Laboratory contamination detection or confirmation
- Outbreak investigation
- Population-based studies of ongoing (recent) TB transmission

# Research Topic

- Is pleural TB associated with recent transmission?
  - Logic: Pleural TB is often a manifestation of primary TB; primary TB can represent recent transmission.

- Acquisition
  - Extrapulmonary TB, including pleural TB, is not infectious
    - If recent transmission is occurring, these cases would represent the secondary infections
  - We can estimate recent transmission with clustering data:
    - DNA cluster: $\geq$2 cases whose isolates had matching DNA fingerprints
  - Of concern as proportion of extrapulmonary cases has increased over the years

# San Francisco Study

- A recent study by Ong et al. showed clustering (crude proxy for recent transmission) was more likely to be seen with pleural TB, as a single site of disease, than all other sites of TB.[1]

- SF population is very different from the MD population with respect to age, race and foreign-born.

- Will MD data show the same?

(1) Ong A, et al. A Molecular Epidemiological Assessment of Extrapulmonary Tuberculosis in San Francisco. Clin Infect Dis 2004; 38:25-31.

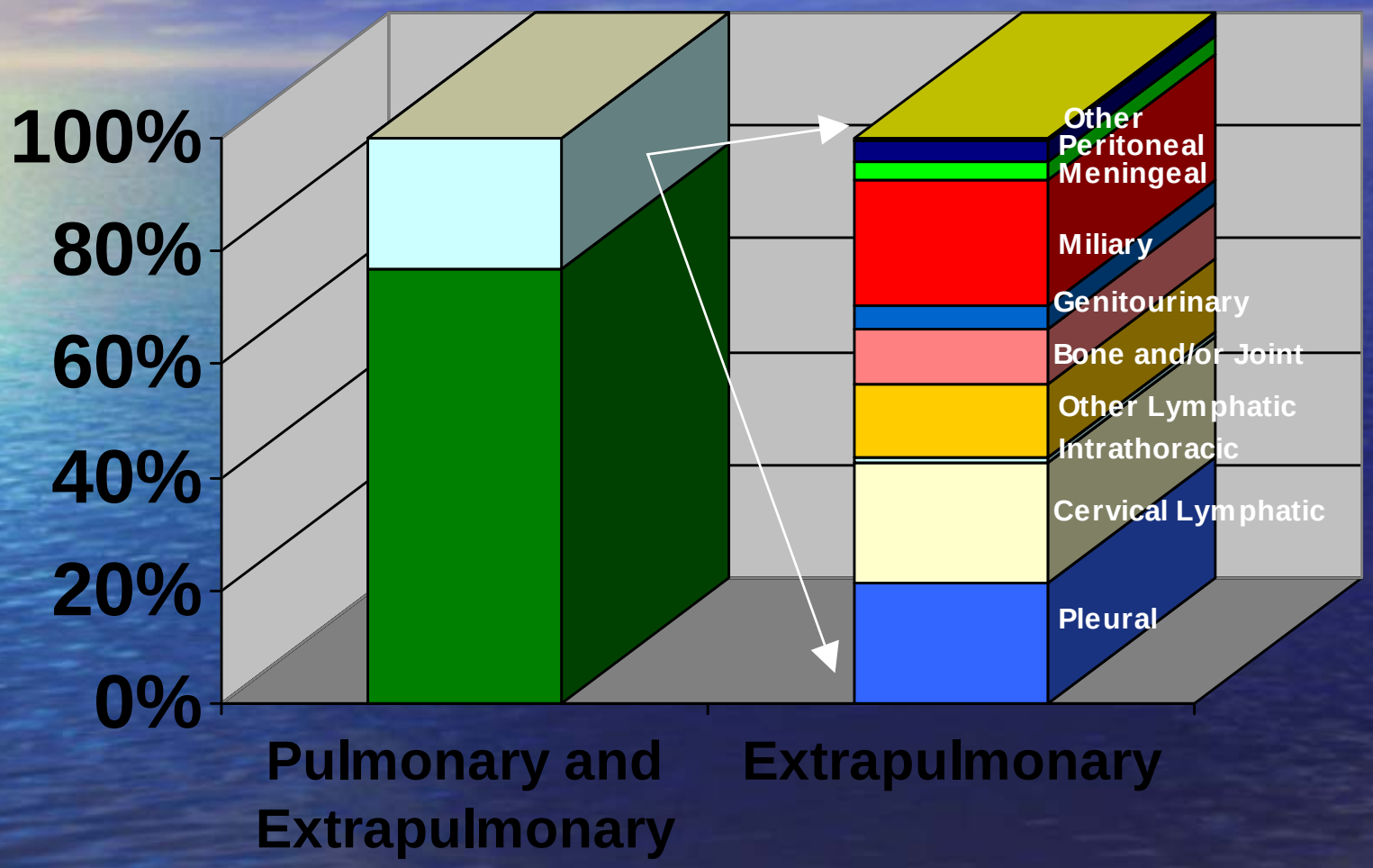
# Methods

- Population based survey of report verified TB cases identified in MD between 1 January 1996 and 31 December 2001.
- Culture positive isolates were characterized with IS6110-based RFLP, spoligotyping
- Analysis was limited to cases with a single site of disease and isolates with more than five IS6110 bands.

# Methods

- Data collected on:
  - Race
  - Age
  - Sex
  - US/Foreign Birth
  - HIV serostatus
  - Genotype
- Patients were considered clustered if isolates had identical banding patterns, +/- one band and were identified within five years

# Proportion of Patients by Disease Site



# Study Population

1811 reported TB cases

1411 (77.9%) cases were culture positive

1256 (89.0% ) culture positive cases with a single site of disease

1222 (86.6%) single site isolates fingerprinted

944 (77.3%) $\geq$6 IS6110 bands

299 (31.7%) cases defined as clustered

645 (68.3%) cases defined as unique

# Results

- Multivariate logistic regression used to generate adjusted odds ratios and confidence intervals

- All analyses are adjusted for race, age, sex, foreign birth, HIV and cluster status.

| | Pleural TB vs. Pulmonary TB | |
|---|---|---|
| | **Maryland** | **San Francisco** |
| | Pleural = 46<br>Pulmonary = 728 | Pleural = 43<br>Pulmonary = 1376 |
| Characteristic | AOR (95% CI) | AOR (95% CI) |
| **HIV status** | | |
| Seropositive | 0.7 (0.2, 1.9) | **0.2 (0.04, 0.8)** |
| Seronegative | 1.0 (referent) | 1.0 (referent) |
| **Genotype** | | |
| Clustered | 0.8 (0.4, 1.6) | **2.3 (1.2, 4.6)** |
| Unique | 1.0 (referent) | 1.0 (referent) |

| | Nonrespiratory TB vs. Pulmonary TB | |
|---|---|---|
| | **Maryland** | **San Francisco** |
| | Nonrespiratory = 170<br>Pulmonary = 728 | Nonrespiratory = 252<br>Pulmonary = 1376 |
| Characteristic | AOR (95% CI) | AOR (95% CI) |
| **Age, years** | | |
| 0-24 | 1.3 (0.7, 2.4) | **2.0 (1.2, 3.5)** |
| 25-44 | 1.3 (0.8, 2.2) | **2.0 (1.4, 3.0)** |
| 45-64 | 1.2 (0.7, 2.0) | **1.7 (1.1, 2.5)** |
| 65+ | 1.0 (referent) | 1.0 (referent) |
| **Sex** | | |
| Female | 1.5 (1.0, 2.1) | 2.7 (2.0, 3.6) |
| Male | 1.0 (referent) | 1.0 (referent) |

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| Characteristic | AOR (95% CI) | AOR (95% CI) |
| **Place of Birth** | | |
| Outside US | 1.4 (0.9, 2.3) | **1.8 (1.01, 3.0)** |
| US | 1.0 (referent) | 1.0 (referent) |
| **HIV status** | | |
| Seropositive | 0.7 (0.4, 1.4) | **1.8 (1.1, 2.9)** |
| Seronegative | 1.0 (referent) | 1.0 (referent) |
| **Genotype** | | |
| Clustered | 0.5 (0.3, 0.8) | 0.6 (0.4, 0.8) |
| Unique | 1.0 (referent) | 1.0 (referent) |

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| 25-44 | 0.6 (0.2, 1.7) | **0.2 (0.2, 0.7)** |
| 45-64 | **0.2 (0.0, 0.6)** | 0.6 (0.3, 1.3) |
| 65+ | 1.0 (referent) | 1.0 (referent) |
| **Sex** | | |
| Female | 0.5 (0.2, 1.02) | **0.2 (0.18, 0.7)** |
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| Characteristic | AOR (95% CI) | AOR (95% CI) |
| **HIV status** | | |
| Seropositive | 0.8 (0.2, 3.0) | **0.12 (0.1, 0.5)** |
| Seronegative | 1.0 (referent) | 1.0 (referent) |
| **Genotype** | | |
| Clustered | 1.5 (0.6, 3.8) | **3.4 (1.5, 7.1)** |
| Unique | 1.0 (referent) | 1.0 (referent) |

# Discussion

- Our data do not support the hypothesis that pleural TB is associated with recent transmission.
  - While genetic clustering is less likely with nonrespiratory sites of disease than pulmonary sites, this association is not seen with pleural sites of infection.
- Implications:
  - Associate Contact Investigations

# Discussion

- Possible reasons for discrepancies:
  - Study definitions
    - Cluster, time frame
  - Genotyping methods
    - RFLP/spoligo vs. RFLP/PGRS
  - Variations in study populations
    - "Maryland, America in Miniature"
  - Selections bias

# Future Studies

- Multi-site infection
  - Pulmonary/pleural vs. pulmonary/nonrespiratory
    - Is there an association with recent transmission?
- Transmission Studies
    - Cluster variable as outcome
      - What factors influence transmission within our population?

# Acknowledgments


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