Basic concept of drug resistance in tuberculosis

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By the end of this presentation, participants would be able to describe…

- *Mycobacterium tuberculosis*
- Natural resistance
- Wild type mutant of *M. tuberculosis*
- Acquired resistance
- Primary resistance
- Resistance among new cases
- Resistance among previously treated cases
- Combined Resistance
- Transient resistance
- Mono and Poly resistance
- Multidrug-resistance (MDR)
- Extensive-resistance (XDR)
- Failure/Relapse/Default and resistance
Die Aetiologie der Tuberkulose

von Dr. R. Koch, Geh. Regierungsrath.
Natural resistance: naturally resistant to a specific drug

- **M. tuberculosis** is naturally resistant to many antibiotics
  - the highly hydrophobic cell envelope acting as a permeability barrier
  - many potential resistance determinants encoded in the genome.
    - hydrolytic or drug-modifying enzymes such as b-lactamases and aminoglycoside acetyl transferases,
    - potential drug–efflux systems, such as 14 members of the major facilitator family and numerous ABC transporters.


- **M. bovis** isolates are naturally resistant to pyrazinamide.
Acquired Resistance to Anti-tuberculosis Drugs

• Unlike several bacterial species that obtain resistance through mobile genetic elements (such as plasmids and transposons), resistance to anti-tuberculosis drugs in *M. tuberculosis* is caused by *spontaneous chromosomal mutation*
Isoniazid (INH): a prodrug that requires the activation of bacterial catalase-peroxidase enzyme (\textit{katG}).

- \textit{katG} mutation
  - Reduce ability \textbf{to activate the prodrug}
  - cause INH resistance (low or high level)
  - Could be restored by transformation with a functional \textit{katG} gene

Rifampicin (RMP)

- Interferes with the synthesis of mRNA by binding to the bacterial DNA-dependent RNA polymerase.

- All bacteria achieve resistance to RMP by mutation in RNA polymerase subunit β (rpoB), the gene encoding the β-subunit of the DNA-dependent RNA polymerase of *Mycobacterium tuberculosis*.

Zhang Y, et al. ASM 2000
Bedaquiline

- A diarylquinoline drug: inhibits the proton pump of *M. tuberculosis* adenosine triphosphate (ATP) synthase

- **Target based resistance**: mutation in the subunit c of ATP synthase encoded by the *atpE* gene
  - the proportion of resistant mutants that emerged was $5 \times 10^{-7}$ at 4 times MIC, and $5 \times 10^{-8}$ at 8 times MIC, for *M. tuberculosis*

- **Non-target based resistance** to BDQ (ross-resistance to clofazimine): mutations in Rv0678, a transcriptional repressor of the genes encoding the MmpS5-MmpL5 efflux pump.

Mechanism of bedaquiline and clofazimine resistance in Rv0678 mutants

Efflux Inhibition with Verapamil Potentiates Bedaquiline in \textit{Mycobacterium tuberculosis}

- Drug efflux is an important resistance mechanism in \textit{Mycobacterium tuberculosis}. We found that verapamil, an efflux inhibitor, profoundly decreases the MIC of bedaquiline and clofazimine to \textit{M. tuberculosis} by 8- to 16-fold.

- Efflux inhibition is an important sensitizer of bedaquiline and clofazimine, and efflux may emerge as a resistance mechanism to these drugs.

Probability of drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis*

- The highest proportions of mutants observed in fluctuation test
  - Isoniazid    \(3.5 \times 10^{-6}\)
  - Streptomycin \(3.8 \times 10^{-6}\)
  - Rifampin     \(3.1 \times 10^{-8}\)
  - Ethambutol   \(0.5 \times 10^{-4}\)  
    David HL Appl Microbiol 1970;20:810-4

- Fluoroquinolone \(2 \times 10^{-6} - 1 \times 10^{-8}\)

- The larger a bacterial population, the higher was the probability that resistant mutants were present
- The number of viable organisms in a cavity was likely to be in excess of \(10^8\) (Canetti G 1964)
Emergence of drug resistance

British Medical Research Council, 
BMJ 1948; 2:769-783
Cumulative percentage of Streptomycin resistant isolates

Days since start of treatment

BMRC. Br Med J 1948
Fall and rise phenomenon

Fall and rise phenomenon

Toman K, 1979
Acquired drug resistance

• Anti-tuberculosis drugs impose selection pressure resulting in predominant replication of drug-resistant strain.

• Anti-tuberculosis drugs do not cause mutation of *M. tuberculosis*. 
Acquired drug resistance

Acquired resistance can be demonstrated if

1. drug susceptibility patterns of tuberculosis bacilli are determined before anti-tuberculosis treatment and repeated at a later point in treatment

2. genotyping of tuberculosis strains is available.
   - reinfection with a resistant strain may result in the observation of different susceptibility patterns between pre-treatment and post-treatment strains.

Chiang C-Y, 2012
Primary drug resistance in tuberculosis

• Primary resistance in tuberculosis indicates patients infected with *M. tuberculosis* that is resistant to anti-tuberculosis drugs from the outset prior to anti-tuberculosis treatment

• is caused by transmission of drug-resistant bacilli followed by development of drug-resistant tuberculosis among those who are primarily infected with drug-resistant strains
Drug Resistance among New Tuberculosis Cases

• the presence of resistant isolates of *M. tuberculosis* in patients who deny having had any prior anti-TB treatment (for as much as 1 month)

• Proxy for the prevalence of primary drug resistance

• Drug resistance among new cases is used to evaluate transmission of tuberculosis.
Misclassification of drug resistance among new tuberculosis cases

• patients may not remember whether they have been previously treated with anti-tuberculosis drugs, or may not be informed that they were treated with anti-tuberculosis drugs

• health care workers may not carefully obtain history of previous tuberculosis treatment.

➤ misclassification of previously treated cases as new cases may over-estimate drug resistance among new patients.

Chiang C-Y 2012
Drug Resistance among Previously Treated Tuberculosis Cases

- the presence of resistant isolates of *M. tuberculosis* in patients who have been treated for tuberculosis for 1 month or more

- a useful proxy for truly acquired resistance?
Drug Resistance among Previously Treated Tuberculosis Cases

- Patients who have acquired resistance,
- Patients have been primarily infected with a resistant strain and subsequently failed therapy,
- Patients who have been re-infected.

- Not a useful proxy for truly acquired resistance
What is transient drug resistance?

- The resistant culture is composed of a few colonies—rarely more than five—usually obtained shortly before complete sputum conversion occurs.
- There is no justification for any change of treatment.

Toman K 1979
Mitchison DA, BMJ 1965
Type of resistance

• Mono resistance: resistant to one drug

• Poly resistance: resistant to 2 or more drugs

• Multidrug-resistant tuberculosis (MDR-TB): resistant to at least isoniazid and rifampin
Extensively Drug Resistant Tuberculosis (XDR-TB)

• XDR-TB is defined as TB with resistance to at least isoniazid and rifampicin (MDR-TB) with further resistance to a fluoroquinolone and a second line injectable agent (amikacin, kanamycin, or capreomycin).
Totally drug-resistant tuberculosis?

- In 2007, two patients with strains having resistance to all first and second-line anti-TB drugs which were tested were reported from Italy (1)
- In 2009, 15 TB patients in Iran were reported to be resistant to all anti-TB drugs tested (2)
- In December 2011, clinicians in Mumbai, India, described four patients with “TDR-TB” (3). A few weeks later, the Times of India reported another eight cases in Mumbai.

Challenges and Controversies in Defining Totally Drug-Resistant Tuberculosis

• resistance to all drugs tested: the number of drugs tested varies widely between laboratories

• cross-resistance among different drugs within a class of drugs (e.g., the fluoroquinolones) or closely related classes of drugs (e.g., the aminoglycosides and polypeptides) is not 100%
Challenges and Controversies in Defining Totally Drug-Resistant Tuberculosis

- Access for group 5 drugs has been increasing, such as amoxicillin/clavulanic acid, clofazimine, linezolid, macrolides, and monobactams (imipenem, meropenem)
- several new anti-TB drugs are under development
- must avoid the unintended implication that patients with total drug resistant TB should not or cannot be treated
- global laboratory capacity for DST of *M. tuberculosis* isolates remains limited
Failure and Relapse

- **Failure**: remains sputum positive after a certain period of anti-tuberculosis treatment

- **Relapse**: become sputum positive again after cure

- **Recurrent tuberculosis**:
  - Relapse
  - Reinfection
Diagram of 2 populations of tubercle bacilli and their evolution during chemotherapy

Grosset J. Amsterdam: Excerpta Medica. 1977:1-11
Basic mechanisms of chemotherapy

Figure 2. Hypothesis of special populations of the bacterial population in lesions killed by different drugs.

## Grading of activities of anti-tuberculosis drugs

<table>
<thead>
<tr>
<th>Extent of Activity</th>
<th>Prevention of resistance</th>
<th>Early bactericidal</th>
<th>Sterilizing</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Isoniazid</td>
<td>Isoniazid</td>
<td>Rifampin Pyrazinamide</td>
</tr>
<tr>
<td></td>
<td>Rifampin</td>
<td>Ethambutol</td>
<td>Isoniazid</td>
</tr>
<tr>
<td></td>
<td>Ethambutol</td>
<td>Rifampin</td>
<td>Ethambutol</td>
</tr>
<tr>
<td></td>
<td>Streptomycin</td>
<td>Streptomycin</td>
<td>Streptomycin</td>
</tr>
<tr>
<td>Low</td>
<td>Pyrazinamide</td>
<td>Pyrazinamide</td>
<td>Pyrazinamide</td>
</tr>
<tr>
<td></td>
<td>Thiacetazone</td>
<td>Thiacetazone</td>
<td>Ethambutol</td>
</tr>
</tbody>
</table>

Mitchison DA. *Tubercle* 1985; 66:219-225
Early, two-day bactericidal activity of anti-tuberculosis drugs (reduction in colony-forming units in sputum)

Jindani A. 1980. Graph by Rieder HL, 2002
Table 2. Efficacy of anti-tuberculosis drugs in preventing the emergence of resistance to isoniazid in patients with severe disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Isoniazid with</th>
<th>No. of patients</th>
<th>Failures of treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Africa [3-7]</td>
<td>Rifampicin</td>
<td>183</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Streptomycin</td>
<td>96</td>
<td>2</td>
</tr>
<tr>
<td>Madras [8-11]</td>
<td>Ethambutol*</td>
<td>105</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>PAS</td>
<td>309</td>
<td>12</td>
</tr>
<tr>
<td>E. Africa</td>
<td>Thiacetazone</td>
<td>423</td>
<td>16</td>
</tr>
</tbody>
</table>

*With initial supplement of streptomycin daily for 2 weeks.
Basic mechanisms of chemotherapy

Figure 2. Hypothesis of special populations of the bacterial population in lesions killed by different drugs.

Failure, Resistance

Relapse
Failure, Relapse, Default and Drug Resistance

• Failure: not always resistant
• Relapse: not necessarily susceptible
• Default: depending on anti-tuberculosis treatment before treatment interruption

➢ Is there acquired resistance (amplification of resistance) during treatment?
Prevalence of anti-tuberculosis drug resistance among relapse, Taipei

• Number of patients = 93
• Prevalence of resistance to any drug, 33.3%

Prevalence of anti-tuberculosis drug resistance among treatment after default, Taipei

- Number of patients = 57
- Prevalence of resistance to any drug 42.1%

Prevalence of anti-tuberculosis drug resistance among treatment after failure, Taipei

- Number of patients = 33
- Prevalence of drug resistance to any drug 69.7%

Proportion of MDR-TB among new and retreatment TB, Taiwan, 2007-2013

<table>
<thead>
<tr>
<th>Year</th>
<th>New MDR-TB (%)</th>
<th>Retreatment MDR-TB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>1.0%</td>
<td>5.3%</td>
</tr>
<tr>
<td>2008</td>
<td>1.0%</td>
<td>6.2%</td>
</tr>
<tr>
<td>2009</td>
<td>1.0%</td>
<td>6.3%</td>
</tr>
<tr>
<td>2010</td>
<td>1.1%</td>
<td>8.2%</td>
</tr>
<tr>
<td>2011</td>
<td>1.0%</td>
<td>6.7%</td>
</tr>
<tr>
<td>2012</td>
<td>1.0%</td>
<td>5.3%</td>
</tr>
<tr>
<td>2013</td>
<td>0.9%</td>
<td>5.1%</td>
</tr>
</tbody>
</table>

Courtesy: Taiwan CDC
Define Failure by Smear

Possible causes of failure:

- True failure with positive culture
- Delayed sputum conversion
- Did not receive treatment (very poor adherence)
- Culture negative: dead bacilli
- Non-tuberculosis mycobacterium
Sputum smear conversion during directly observed treatment for tuberculosis

Rieder HL. Tuber Lung Dis 1996;77:124-9
Smear to Culture Ratio during Chemotherapy, by Treatment Regimen

INH + RMP

INH + SM

Courtesy: Rieder HL.
Culture Positivity among Smear-Positives during Chemotherapy, British Columbia, Canada, 1988-1995

Week of treatment
0 4 8 12 16 20 24 40
Per cent
0
20
40
60
80
100


Courtesy: Rieder HL.
Pulmonary non-tuberculous mycobacterial (NTM) infections

• NTM is a broad array of organisms that have been isolated from soil and water

• exposure to these reservoirs is thought to be the source of human infection
Table 1  Slowly and rapidly growing non-tuberculous mycobacteria that have been reported to cause lung disease

<table>
<thead>
<tr>
<th>Slowly growing mycobacteria</th>
<th>Rapidly growing mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. arupense</td>
<td>M. abscessus</td>
</tr>
<tr>
<td>M. asiaticum</td>
<td>M. alvei</td>
</tr>
<tr>
<td>M. avium</td>
<td>M. boenickei</td>
</tr>
<tr>
<td>M. branderi</td>
<td>M. bolletti</td>
</tr>
<tr>
<td>M. celatum</td>
<td>M. brumae</td>
</tr>
<tr>
<td>M. chimaera</td>
<td>M. chelonae</td>
</tr>
<tr>
<td>M. florentinum</td>
<td>M. confluentis</td>
</tr>
<tr>
<td>M. heckeshornense</td>
<td>M. elephantis</td>
</tr>
<tr>
<td>M. interjectum</td>
<td>M. fortuitum</td>
</tr>
<tr>
<td>M. intermedium</td>
<td>M. goodii</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td>M. holsaticum</td>
</tr>
<tr>
<td>M. kansasii</td>
<td>M. mageritense</td>
</tr>
<tr>
<td>M. kubicae</td>
<td>M. massiliense</td>
</tr>
<tr>
<td>M. lentiflavum</td>
<td>M. mucogenicum</td>
</tr>
<tr>
<td>M. malmoense</td>
<td>M. peregrinum</td>
</tr>
<tr>
<td>M. palustre</td>
<td>M. phocaicum</td>
</tr>
<tr>
<td>M. saskatchewanse</td>
<td>M. septicum</td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>M. thermostresistible</td>
</tr>
<tr>
<td>M. shimodei</td>
<td></td>
</tr>
<tr>
<td>M. simiae</td>
<td></td>
</tr>
<tr>
<td>M. szulgai</td>
<td></td>
</tr>
<tr>
<td>M. triplex</td>
<td></td>
</tr>
<tr>
<td>M. xenopi</td>
<td></td>
</tr>
</tbody>
</table>

Marras TK. Thorax 2007;8:661–666.
Increasing Incidence of Nontuberculous Mycobacteria, Taiwan, 2000–2008

A) Annual number and rate of isolates of nontuberculous mycobacteria (NTM) (triangles) and Mycobacterium tuberculosis (circles). B) Annual incidence of isolates of NTM, M. avium complex (MAC), and rapidly growing mycobacteria (RGM).

The epidemiologic relationship between tuberculosis and nontuberculous mycobacterial disease: a systematic review

inverse relationship: an increase in the proportion of mycobacterial disease caused by NTM in many parts of the world due to a simultaneous reduction in TB and increase in NTM disease.