Transmissibility, virulence and fitness of resistant strains of \textit{M. tuberculosis}
Transmissibility, Virulence and Fitness of resistant strains of *M. tuberculosis*

• For infectious pathogens, fitness is a composite measure of an organism’s ability to survive, reproduce, and be transmitted.

• Question: will drug-resistant TB outcompete drug susceptible TB? Will TB become resistant to all antibiotics?
Fitness and drug resistance

• Darwinian fitness is often defined as “the likelihood to survive and reproduce”.

• Relative fitness: the fitness of a drug-resistant bacterial strain relative to a drug-susceptible strain

• Reproductive fitness (absolute fitness): the number of secondary cases generated (basic reproductive rate Ro)

Fitness and drug resistance

• Acquisition of drug resistance in bacteria often carries a “cost” of reduced bacterial growth in absence of the drug.

• However, there is
  – Low- or no-cost mutation
  – compensatory evolution

Drug resistance and reproductive fitness

- Laboratory approach
- Epidemiologic approach
- Mathematical modeling
Some observation on the pathogenicity of isoniazid-resistant variants of tubercle bacilli

• The Vallee strain (Bovine) and H37Rv strain were exposed to INH in vitro, variants were subculture through 3 passage in INH containing medium.

• 2 guinea pigs injected for each strains
  – isoniazid sensitive Vallee strain died at 12 and 19 days,
  – isoniazid-resistant Vallee strain died at 33 and 43 days

✓ isoniazid sensitive H37Rv strains died at 19 and 25 days
✓ isoniazid-resistant H37Rv strains remained alive at 60 days

*Middlebrook G, Cohn ML. Science 1953; 118:297-299*
Some observation on the pathogenicity of isoniazid-resistant variants of tubercle bacilli

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Culture results</th>
<th>Pathogenicity for normal guinea pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary isolation</td>
<td>Subcultures from animals</td>
</tr>
<tr>
<td></td>
<td>$S$ INH $&lt; 1 \mu g/ml$</td>
<td>$S$ INH $&lt; 1 \mu g/ml$</td>
</tr>
<tr>
<td></td>
<td>$OA^+; \text{ATS}^+$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$R$ INH $&gt; 1 \mu g/ml$</td>
<td>$R$ INH $&gt; 10 \mu g/ml$ (2 cases)</td>
</tr>
<tr>
<td></td>
<td>$OA^+; \text{ATS}^+$</td>
<td>$R$ INH $&gt; 1 \mu g/ml$ (5 cases)</td>
</tr>
<tr>
<td></td>
<td>$R$ INH (not known)</td>
<td>Negative cultures from abscesses and spleens</td>
</tr>
<tr>
<td></td>
<td>$OA^-$; $\text{ATS}^+$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$R$ INH (not known)</td>
<td>Negative cultures from abscesses and spleens</td>
</tr>
<tr>
<td></td>
<td>$OA^-$; $\text{ATS}^+$</td>
<td></td>
</tr>
</tbody>
</table>

*Note.* $S$, sensitive; $R$, resistant; $OA$, unmodified oleic acid albumin agar medium; $\text{ATS}$, American Trudeau Society egg yolk potato medium; $+$, growth; $-$, no growth; $\pm$, growth rare or absent; AFB, acid-fast bacilli.
Some observation on the pathogenicity of isoniazid-resistant variants of tubercle bacilli

- We wish to emphasize that these data refer only to pathogenicity of tubercle bacilli for normal guinea pig

- we have no direct, conclusive evidence that these INH-resistant strains of tubercle bacilli are equally nonpathogenic for normal human tissue.

Middlebrook G, Cohn ML. Science 1953; 118:297-299
Drug-Resistant Strains of *M. tuberculosis* Exhibit a Range of Virulence for Mice

- Avirulent: growth rate slower than Erdman laboratory strain
- Virulent: growth rate equivalent to Erdman laboratory strain
- Fast-growing: growth rate faster than Erdman laboratory strain

Drug-Resistant Strains of *Mycobacterium tuberculosis* Exhibit a Range of Virulence for Mice

- The panel exhibited a wide range of virulence, regardless of the degree of resistance to conventional drugs.

**katG S315T mutation**

- Causes a reduction in the activation of isoniazid whilst maintaining katG activity and virulence in mice.

Relationship between the isoniazid-resistant mutation katGS315T and the prevalence of MDR/XDR-TB in Osaka, Japan

• Five hundred and sixty isolates (34.4%) shared an RFLP pattern.

• Isolates belonging to the RFLP cluster had katGS315T
  – INH-resistant isolates, 18/48 (37.5%)
  – MDR-TB cases, 18/29 (62.1%)
  – XDR-TB cases, 17/21 (80.9%)

Compensatory mutation

- Bacterial antibiotic resistance mutations that incur an initial fitness cost may be compensated by later mutations that restore an organism’s reproductive potential.

Evolution of drug resistance on bacterial fitness

- Reversion to the drug-susceptible state can occur when drug pressure is removed. However, compensatory evolution is more likely, even in the absence of drug pressure, as more evolutionary targets exist in the bacterial genome; true reversion can only occur through a back-mutation at the exact position of the original drug resistance-conferring mutation.
Relative competitive fitness of laboratory-derived rifampin-resistant mutants of *M. tuberculosis*

All mutants had a statistically significant fitness cost. This cost was less in *rpoB* S531L mutants than in other *rpoB* mutants, irrespective of the strain background.

Relative competitive fitness of clinically derived rifampin-resistant mutants of *M. tuberculosis*

Four of the five mutants with the rpoB S531L mutation (light gray bars) had no fitness cost compared with their rifampin-susceptible ancestors. All mutants with other rpoB mutations (dark gray bars) had significant fitness defects.

Prolonged patient treatment can result in multidrug-resistant strains with no fitness defect and that strains with low- or no-cost resistance mutations are also the most frequent among clinical isolate.
Tuberculosis Infection of young high-risk contact by drug susceptibility of organism from the index patients

<table>
<thead>
<tr>
<th>Index patients’ susceptibility test result</th>
<th>Examined No.</th>
<th>Infected No.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>601</td>
<td>239 (39.8)</td>
</tr>
<tr>
<td>Susceptible</td>
<td>751</td>
<td>252 (33.6)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1,352</td>
<td>491 (36.3)</td>
</tr>
</tbody>
</table>

Effect of Drug Resistance on the Generation of Secondary Cases of Tuberculosis

• Assumed that drug-susceptible and drug-resistant strains were transmitted to secondary case patients if the drug-resistance and genotype patterns were identical.

• Calculated the number of secondary cases for each drug-resistance pattern and determined the relative secondary-case rate ratio (SR) of drug-resistant TB to drug-susceptible TB.

Effect of Drug Resistance on the Generation of Secondary Cases of Tuberculosis

• Secondary case rate ratio of drug-resistant to drug-susceptible TB cases
  – Overall 0.51 (95% confidence interval [CI], 0.37–0.69).
  – H-resistant 0.29 (95% CI, 0.15–0.57)
  – HS-resistant 0.10 (95% CI, 0.02–0.42),
  – S-resistant 0.88 (95% CI, 0.53–1.47)
  – no secondary cases caused by MDR-TB.
  – R-resistant 2.33 (95% CI, 1.04–5.25).

Evidence for transmission of multidrug-resistant *M. tuberculosis* in HIV-negative individuals

<table>
<thead>
<tr>
<th>City, country</th>
<th>Study setting</th>
<th>HIV-negative %</th>
<th>Cases involved in transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York, USA</td>
<td>Hospital</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>Cape Town, South Africa</td>
<td>Community</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>Rio de Janeiro, Brasil</td>
<td>Hospital</td>
<td>100</td>
<td>23</td>
</tr>
<tr>
<td>France</td>
<td>Family</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Texas and Mexico, USA</td>
<td>Community</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>Buenos Aires, Argentina</td>
<td>Family, community and hospital</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>Spain</td>
<td>Community</td>
<td>100</td>
<td>38</td>
</tr>
<tr>
<td>Tunisia</td>
<td>Community</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>Thailand</td>
<td>Refugees</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Hungary</td>
<td>Community</td>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td>Rwanda</td>
<td>Hospital</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Galicia, Spain</td>
<td>Hospital</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>

Molecular Epidemiology of MDR-TB in Eastern Taiwan

- 73 of 77 MDR-TB patients enrolled from May 2007 to February 2009 in Eastern Taiwan had isolates available for genotyping by spoligotyping and MIRU-VNTR.
  - 28 (38.4%) had unique pattern
  - 45 (61.6%) were clustered pattern strains.

- Epidemiological links could be established in 21 (46.7%) of the 45 patients with clustered pattern strain patients.

Patient characteristics and epidemiological links of the largest cluster in Hsiulin Village.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Month of birth</th>
<th>Month of diagnosis of TB</th>
<th>Month of diagnosis of MDR-TB</th>
<th>Epidemiological link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 8-1</td>
<td>M</td>
<td>12-1957</td>
<td>11-2000</td>
<td>04-2004</td>
<td>Index case</td>
</tr>
<tr>
<td>Case 8-2</td>
<td>F</td>
<td>10-1970</td>
<td>03-2002</td>
<td>12-2008</td>
<td>Sister of case 8-4, friend of index case</td>
</tr>
<tr>
<td>Case 8-4</td>
<td>M</td>
<td>11-1962</td>
<td>08-2004</td>
<td>06-2007</td>
<td>Colleague of index case</td>
</tr>
<tr>
<td>Case 8-5</td>
<td>M</td>
<td>07-1964</td>
<td>06-2005</td>
<td>11-2007</td>
<td>Neighbor of case 8-4</td>
</tr>
<tr>
<td>Case 8-6</td>
<td>M</td>
<td>11-1970</td>
<td>07-2006</td>
<td>07-2006</td>
<td>Neighbor &amp; colleague of index case</td>
</tr>
<tr>
<td>Case 8-7</td>
<td>M</td>
<td>05-1962</td>
<td>08-2006</td>
<td>12-2006</td>
<td>Cousin &amp; neighbor of index case</td>
</tr>
<tr>
<td>Case 8-8</td>
<td>M</td>
<td>02-1993</td>
<td>11-2006</td>
<td>02-2007</td>
<td>Son of index case</td>
</tr>
</tbody>
</table>

Essential to prevent between hosts transmission!
Modeling epidemics of multidrug-resistant *M. tuberculosis* of heterogenous fitness

- Even when the average relative fitness of MDR strain is low and a well-functioning control program is in place, a small subpopulation of relatively fit MDR strain may eventually outcompete both the drug sensitive strain and the less fit MDR strains.

Evidence for transmission of XDR *M. tuberculosis*

<table>
<thead>
<tr>
<th>Country</th>
<th>Study setting</th>
<th>HIV-positive %</th>
<th>Total XDR cases in the study</th>
<th>Clustered XDR strains n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Africa</td>
<td>Hospital</td>
<td>100</td>
<td>53</td>
<td>39/46 (85)</td>
</tr>
<tr>
<td>Iran</td>
<td>Family/community</td>
<td>25</td>
<td>12</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>South Africa</td>
<td>Hospital/community</td>
<td>ND</td>
<td>41</td>
<td>15/41 (37)†</td>
</tr>
<tr>
<td>Uzbekistan</td>
<td>Hospital</td>
<td>ND</td>
<td>10</td>
<td>7/10 (70)</td>
</tr>
</tbody>
</table>

Factors associated with the spread of drug resistance tuberculosis

1. the production rate of mutations that code for resistance;
2. the proportion of patients carrying resistant genotypes (strains) who are treated
3. the different survival and multiplication rates of resistant and susceptible genotypes in patients on various treatment regimens
4. the relative transmissibility of resistant and susceptible genotypes among individuals
5. the proportion of infections with resistant strains that leads to active TB.

Infection with drug-susceptible strain

Exposed to DS-TB

Exposed to DR-TB

Risk factors

Risk factors

Disease

Drug-resistant tuberculosis

Drug-susceptible Tuberculosis

Risk factors


In conclusion

• Drug-resistant and MDR-TB will be increasing if principles in protecting rifampin is not strictly applied

• Drug resistant TB may not necessarily be less virulent with reduced fitness – transmission happens and can become the driving force of the epidemic of drug-resistant TB.

• MDR and XDR-TB could be everywhere if we do not act properly now.