Drug Susceptibility of Mycobacterium tuberculosis Beijing Genotype and Association with MDR TB

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To determine differences in the ability of Mycobacterium tuberculosis strains to withstand antituberculosis drug treatment, we compared the activity of antituberculosis drugs against susceptible Beijing and East-African/Indian genotype M. tuberculosis strains. Beijing genotype strains showed high rates of mutation within a wide range of drug concentrations, possibly explaining this genotype’s association with multidrug-resistant tuberculosis.

The emergence of Mycobacterium tuberculosis resistance to antituberculosis (anti-TB) drugs is a major public health challenge that is threatening World Health Organization targets set for the elimination of TB (1). Approximately 500,000 cases of multidrug-resistant TB (MDR TB) are diagnosed annually, but the true magnitude of the MDR TB problem is not known because adequate laboratory tools are lacking. Multiple factors contribute to low cure rates, treatment failures, and relapses: poor-quality guidance regarding treatment, HIV co-infection, transmission of resistant forms of TB, underdeveloped laboratory services, and unavailability of alternative drug treatments. However, the evolution of M. tuberculosis is an additional factor that presumably fuels the worldwide problem of emerging resistance. The Beijing genotype is significantly associated with drug resistance (2,3), especially in geographic areas where prevalence of resistance to anti-TB drugs is high, and it is associated with recent TB transmission (2–6). There are also indications that the population structure of M. tuberculosis in areas with a high prevalence of anti-TB drug resistance is changing rapidly toward an increase in Beijing genotype strains (2,6–8).

The World Health Organization target rates for detecting and curing TB in Vietnam have been met; however, the rate of TB infection is not decreasing as expected (4,5). Earlier in this country, the Beijing genotype was strongly correlated with MDR TB and treatment failures (9). Extensive molecular epidemiologic studies showed that the Beijing and East-African/Indian (EAI) genotypes are predominating in Vietnam; each lineage causes ≈40% of the TB cases. According to the single-nucleotide polymorphism typing described by Hershberg et al. (10), the Beijing genotype is a representative of the modern lineage, and the EAI genotype is believed to represent an evolutionary lineage more closely related to the common ancestor of the M. tuberculosis complex.

We compared the in vitro activity of anti-TB drugs against susceptible Beijing and EAI M. tuberculosis isolates from Vietnam and determined the in vitro mutation frequency of these strains during drug exposure. We also determined time-kill kinetics of anti-TB drugs and assessed the emergence of resistant mutants and the concentration range within which resistant mutants and no susceptible mycobacteria were selected. The concentration at which resistant mutants did not emerge (the mutant prevention concentration) was also ascertained. By using this approach, we established an in vitro model for determining differences in the ability of M. tuberculosis strains to resist anti-TB drug treatment.

The Study

Results of a liquid culturing system (BD BACTEC MGIT 960 System; BD Diagnostics, Sparks, MD, US) (for details, see the online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-0912-Techapp.pdf) showed that all 5 Beijing and 5 EAI genotype strains were susceptible to isoniazid (INH), rifampin (RIF), moxifloxacin (MXF), and amikacin (AMK). MICs were determined by using the agar proportion method (11), which showed that ranges were small for the Beijing and EAI genotype strains: INH, 0.062–0.125 mg/L; RIF, 0.125–1 mg/L; MXF, 0.125–0.5 mg/L; and AMK, 0.5–2 mg/L. Duplicate values showed only minor differences.

We determined the mutation frequencies of the Beijing and EAI genotype strains by using previously defined critical drug concentrations of 1 mg/L for INH, RIF, and...
Low concentrations of RIF were needed to achieve >99% activity toward low-density cultures of the 2 strains (Figure 1). We compared Beijing and EAI genotype strains that were duplicates. Cultures with low and high densities of Beijing genotype strains originated from Vietnam. By antituberculosis drug concentrations had to be increased substantially (Table 2). However, to achieve 100% killing, especially for Beijing-1585, RIF concentrations had to be increased substantially (Table 2). Compared with the low-density culture for Beijing-1585, a substantial increase in RIF concentrations was needed to achieve 100% killing of the high-density culture (Table 2). This finding may be relevant in the clinical context because high-density mycobacteria populations are expected to exist in infected tissues of TB patients.

RIF-resistant mutants did not emerge in low-density cultures of Beijing-1585 and EAI-1627. However, RIF-resistant mutants were selected at relatively high numbers from high-density Beijing-1585 cultures compared with high-density EAI-1627 cultures. In Beijing-1585 cultures, mycobacterial killing; differences between Beijing-1585 and EAI-1627 were minor (Table 2). However, to achieve 100% killing, especially for Beijing-1585, RIF concentrations had to be increased substantially (Table 2). Compared with the low-density culture for Beijing-1585, a substantial increase in RIF concentrations was needed to achieve 100% killing of the high-density culture (Table 2). This finding may be relevant in the clinical context because high-density mycobacteria populations are expected to exist in infected tissues of TB patients.

Figure 1. Frequency of rifampin (RIF)-resistant mutants in Mycobacterium tuberculosis Beijing and East-African/Indian (EAI) genotype strains (5 strains each) originating from Vietnam. Mutation frequencies were determined in duplicate. Statistical analysis was performed by using an unpaired Mann-Whitney test.

Table 1. Mutation frequency of Mycobacterium tuberculosis genotype strains originating from Vietnam, by antituberculosis drug

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Isoniazid</th>
<th>Rifampin</th>
<th>Moxifloxacin</th>
<th>Amikacin</th>
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<tbody>
<tr>
<td>Beijing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1585</td>
<td>5.7 × 10⁻⁶</td>
<td>3.0 × 10⁻⁵</td>
<td>3.3 × 10⁻⁵</td>
<td>4.3 × 10⁻⁶</td>
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<td>1607</td>
<td>8.6 × 10⁻⁶</td>
<td>1.5 × 10⁻⁵</td>
<td>5.4 × 10⁻⁵</td>
<td>6.9 × 10⁻⁶</td>
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<td>7.3 × 10⁻⁶</td>
<td>1.0 × 10⁻⁵</td>
<td>9.2 × 10⁻⁶</td>
<td>1.0 × 10⁻⁵</td>
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<tr>
<td>2121</td>
<td>6.8 × 10⁻⁶</td>
<td>2.9 × 10⁻⁵</td>
<td>9.5 × 10⁻⁵</td>
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</tr>
<tr>
<td>2145</td>
<td>9.1 × 10⁻⁶</td>
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<td>5.5 × 10⁻⁵</td>
<td>7.9 × 10⁻⁶</td>
</tr>
<tr>
<td>East-African/Indian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1627</td>
<td>3.7 × 10⁻⁶</td>
<td>4.1 × 10⁻⁵</td>
<td>2.8 × 10⁻⁵</td>
<td>9.3 × 10⁻⁶</td>
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<td>6.3 × 10⁻⁸</td>
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</table>

* Determined in duplicate.
exposure to RIF concentrations of 2–32 mg/L selected resistant mutants only; this was not observed in EAI-1627 cultures. Analysis of RIF-resistant Beijing mutants showed the following altered rpoB gene sequences: CAC → GAC (H526D), CAC → TAC (H526Y), and TCG → TTG (S531L), as assessed by using the GenoType MTBDRplus (Hain Lifescience, Nehren, Germany) assay (for details, see the online Technical Appendix).

For 3 of the 4 anti-TB drugs, the difference in the range of mutant prevention concentrations for the Beijing and EAI genotype strains was small: INH, 128–256 mg/L; RIF, 256–1,024 mg/L; and MXF, 2–8 mg/L. The mutant prevention concentration for AMK was >1,024 mg/L for all strains tested.

Conclusions

We showed that the currently used anti-TB drug susceptibility assays do not discriminate between the in vitro susceptibility, as determined by the methods used in this study, of the M. tuberculosis Beijing and EAI genotype strains. We also showed that the determination of mutation frequencies might be more informative than results of anti-TB drug susceptibility assays. For RIF, mutation frequencies in Beijing genotype strains were high compared with those in EAI genotype strains, and the selection of RIF-resistant mutants among Beijing strains, but not EAI strains, occurred within a wide range of RIF concentrations.

In addition, the killing capacity of RIF toward the Beijing genotype is dependent on the density of mycobacteria: high concentrations of RIF are required to achieve 100% killing of high-density Beijing genotype populations but not of high-density EAI genotype populations. These in vitro characteristics might contribute to the less favorable treatment outcome of Beijing genotype TB infections and their significant association with drug resistance. Our findings demonstrate the need for anti-TB drug treatments that will prevent resistance among M. tuberculosis Beijing genotype TB cases, and they suggest that the development of genotype-specific TB therapy might be justified.

Figure 2. Concentration- and time-dependent bactericidal effect of rifampin (RIF) toward low-density cultures of Mycobacterium tuberculosis BE-1585 (5.1 × 10^5 CFU/mL) (A) and M. tuberculosis EAI-1627 (6.8 × 10^5 CFU/mL) (B). Cultures were exposed to RIF at 2-fold increasing concentrations for 6 days at 37°C. After 1, 3, or 6 days of exposure, subcultures were performed on solid media to count CFUs. *Accurate CFU counting could not be performed because complete outgrowth of mycobacteria occurred on the sixth day of RIF exposure, leading to aggregation.

Table 2. Concentration- and time-dependent bactericidal effect of rifampin toward Mycobacterium tuberculosis genotypes in low- and high-density cultures*

<table>
<thead>
<tr>
<th>Day</th>
<th>Lowest RIF concentration resulting in killing of M. tuberculosis, mg/L</th>
<th>Beijing-1585 genotype</th>
<th>EAI-1627 genotype</th>
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<tr>
<td></td>
<td>&gt;99% killing Low† High‡</td>
<td>100% killing Low† High‡</td>
<td>&gt;99% killing Low§ High¶</td>
</tr>
<tr>
<td>1</td>
<td>1 0.001 0.008 2 64</td>
<td>256 ND</td>
<td>64 1024</td>
</tr>
<tr>
<td>3</td>
<td>1 0.008 64 1024</td>
<td>256 ND</td>
<td>64 1024</td>
</tr>
<tr>
<td>6</td>
<td>1 0.008 64 1024</td>
<td>64 1024</td>
<td>1 2</td>
</tr>
</tbody>
</table>

*Cultures were exposed to RIF at 2-fold increasing concentrations for 6 days at 37°C; at indicated time-points, subcultures were performed on solid media for counting. Low, low-density culture; high, high-density culture; ND, not determined.
†Density of 5.1 × 10^5 CFU/mL.
‡Density of 4.4 × 10^6 CFU/mL.
§Density of 6.8 × 10^5 CFU/mL.
¶Density of 3.0 × 10^6 CFU/mL.
Acknowledgments

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Dr de Steenwinkel is a medical doctor, resident in training for medical microbiologist, and a PhD student in clinical microbiology and antimicrobial therapy at Erasmus University Medical Center. His interests include research on improving therapy for TB and fundamental exploration of resistance formation.

References


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