Transmission of Drug-Resistant Tuberculosis among Treated Patients in Shanghai, China

Xia Li,1,2,3 Ying Zhang,1,2,3 Xin Shen,1 Guomiao Shen,1,2,3 Xiaohong Gui,1 Bin Sun,1 Jian Mei,1 Kathryn DeRiemer,2 Peter M. Small,6,7 and Qian Gao1,2,3

1Key Laboratory of Medical Molecular Virology, Shanghai Medical College, 2Institute of Medical Microbiology, and 3Institute of Biomedical Sciences, Fudan University, and 4Department of Tuberculosis Control, Shanghai Municipal Centers for Disease Control and Prevention, Shanghai, China; 5School of Medicine, University of California, Davis; 6Institute for Systems Biology and 7Bill and Melinda Gates Foundation, Seattle, Washington

We sought to determine whether patients who had therapy failure with increasingly drug-resistant strains of tuberculosis had primary or acquired drug resistance, by genotyping the initial and subsequent drug-resistant clinical isolates of Mycobacterium tuberculosis collected from patients by the Shanghai Centers for Disease Control and Prevention over the course of a 5-year period. The vast majority of patients (27/32) had primary drug resistance, indicating transmission of a drug-resistant strain of M. tuberculosis. Only 16% (5/32) had acquired drug resistance because of a poor treatment regimen or nonadherence to an adequate regimen. Our findings highlight the urgency of increasing efforts to interrupt the transmission of drug-resistant tuberculosis in communities and facilities in Shanghai, China.

Tuberculosis is one of the most serious infectious diseases in China; it causes an estimated 1.3 million new cases and kills an estimated 0.2 million persons annually [1]. Recent surveys in China reported a prevalence of multidrug-resistant (MDR) tuberculosis among patients with diagnoses ranging from 4.5% in Zhejiang Province to 10.8% in Henan Province [2, 3] and from 5.3% among persons with new cases of tuberculosis to 27% among persons previously treated for tuberculosis nationwide [1]. Given the cost of treating drug-resistant tuberculosis, the poor outcomes with available drugs, and the adverse events of the second-line drugs, it is imperative to make every effort to prevent this disease.

It has been known for decades that a subset of patients who are treated for tuberculosis and who have clinical failure of therapy or relapse are found to harbor bacterial strains that are resistant to antimicrobials. Before the application of molecular genotyping techniques, the clinical history was often used to classify patients with “drug resistance among new cases” versus “drug resistance among previously treated cases” [3, 4]. However, it is more informative to consider the mechanism by which resistance developed. Resistance can arise from the initially infecting strain acquiring resistance-conferring mutations (termed “acquired drug resistance”). This phenomenon is fostered by health systems prescribing inappropriate antimicrobials, by patients’ failure to adhere to appropriately prescribed drugs, or, in some cases, for no apparent reason. Alternatively, patients who have been successfully cured of their initial bout of tuberculosis may be reinfected with a novel strain that is already drug resistant (termed “primary resistance”). Molecular epidemiologic studies of serial isolates from cohorts of patients provided a refined approach to the classification of these treatment failures. If the initial and subsequent isolates have the same genotype pattern, it can generally be concluded that they have developed acquired drug resistance. If the genotypes differ, it can be inferred that the patient has developed tuberculosis as a consequence of exogenous reinfection with a different strain of M. tuberculosis—primary drug resistance. There are some caveats to this approach: laboratory results may be spurious because of laboratory errors, and genotypes may change over time, creating the appearance of a new strain [5]. It is important to distinguish between these 2 mechanisms of clinical failure, because the control of the former focuses on better management of patients after their tuberculosis is diagnosed, whereas controlling the latter requires enhanced efforts to find and treat infectious cases, to interrupt transmission.

In the present study, we determined the probable cause of drug resistance among a cohort of patients with pulmonary tuberculosis in Shanghai. Directly observed short-course treatment strategy was introduced in Shanghai in 1990, and the tuberculosis program has reported high success rates of treatment [6]. The incidence of pulmonary tuberculosis in Shanghai is ~38 cases/100,000 persons/year (2004), which is much lower...
than the average incidence in China (~102 cases/100,000 persons/year). We used clinical and epidemiological data, together with molecular genotyping methods, to evaluate pairs of clinical isolates of \textit{M. tuberculosis} from each patient and to determine whether drug resistance occurred because of an inadequate regimen or nonadherence to an adequate regimen or because of transmission of drug-resistant strains of \textit{M. tuberculosis}. To our knowledge, no previously published report has addressed this issue.

\textbf{Patients, materials, and methods.} We performed a retrospective study using an existing data set from the Shanghai Municipal Centers for Disease Control and Prevention (CDC) from January 1999 through September 2004. The initial sampling frame consisted of all new patients with pulmonary tuberculosis who received their diagnosis and were treated in 31 designated district tuberculosis hospitals in Shanghai during this period. Patients with tuberculosis were treated using standardized regimens that included isoniazid, rifampin, streptomycin, ethambutol, and pyrazinamide in a control program with high success rates of treatment [6]. During treatment, if the drug-susceptibility test showed that the patient was resistant to certain drugs or if the patient was not responding well to the initial regimen, the initial regimen could be changed. Table 1 shows the initial regimens of each patient.

The Shanghai CDC collected data on the social and demographic characteristics, clinical characteristics, treatment regimens and adherence, and the results of drug-susceptibility testing and species identification for each patient. We used the database to identify patients who had at least 2 available clinical isolates with discordant drug-susceptibility test results.

Three sputum samples were initially collected from each patient with suspected tuberculosis for routine diagnostic procedures at different times before tuberculosis treatment was initiated; 1 of the sputum samples, usually the first one, unless it was of poor quality, was used for culture. Pulmonary tuberculosis was diagnosed if the patient was sputum smear positive for acid-fast bacilli based on light microscopy and/or culture positive for \textit{M. tuberculosis}. Additional specimens were routinely collected for sputum smear examinations and culture at the end of the second, fifth, and sixth months of treatment. All of the pretreatment positive cultures and some of the sequential cultures were sent to the Tuberculosis Reference Laboratory of the Shanghai CDC, which participated in the World Health Organization/International Union against Tuberculosis and Lung Disease Global Project on Anti-Tuberculosis Drug Resistance Surveillance [3], and drug-susceptibility testing for first-line antituberculosis drugs and species identification was performed. The isolates were stored in freezers at −70°C.

All isolates of \textit{M. tuberculosis} included in the study were tested for susceptibility to antimicrobial agents using the absolute concentration method [7]. The drugs tested included isoniazid (1 and 10 μg/mL), rifampin (50 and 250 μg/mL), streptomycin (10 and 100 μg/mL), and ethambutol (5 and 50 μg/mL). MDR tuberculosis was defined as resistance to at least isoniazid and rifampin. Species identification was performed using biochemical methods.

We used the mycobacterial interspersed repetitive unit (MIRU) method to genotype the clinical isolates of \textit{M. tuberculosis} from selected patients. We followed the protocol described by Kwara et al. [8], with several modifications [9]. Twelve pairs of primers were synthesized by Sangon Biochemical, and polymerase chain reaction (PCR) mixtures were prepared using the 2× Taq PCR MasterMix (Tianweiidai). The PCR products were analyzed by 2.5% agarose gel electrophoresis. For those patients whose pair of isolates had identical MIRU patterns, IS6110-based restriction fragment length polymorphism (RFLP) analysis was also performed in accordance with a standardized international protocol [10].

\textbf{Results.} From January 1999 through September 2004, the Shanghai CDC collected 6622 clinical isolates from patients with pulmonary tuberculosis whose mycobacterial infection was bacteriologically confirmed by sputum smear and/or culture. Of these, 100 had a pair of isolates with discordant drug-susceptibility test results and 222 had a pair of isolates with identical drug-susceptibility test results (figure 1). Some isolates were missing, and others could not be recovered. However, we compared the age, sex, smear grade, and treatment regimens of patients whose isolates were lost and those whose isolates were recovered; we found no statistically significant differences (data not shown), and we concluded that the losses occurred randomly. We analyzed the data for 38 patients for whom isolates were available and their 76 clinical isolates (figure 1). Of the 38 patients, the initial isolate was drug susceptible in 22 (58%), and the remaining 16 (42%) patients each had an initial isolate that was resistant to at least 1 antituberculosis drug (table 1).

All 76 clinical isolates were genotyped using the MIRU method. Of the 38 patients, 14 had pairs of isolates with identical MIRU patterns and 24 had pairs of isolates with different MIRU patterns. Of the 14 patients whose pairs of isolates had identical MIRU patterns, 5 had pairs of isolates with identical IS6110 RFLP patterns and 9 had pairs of isolates with different IS6110 RFLP patterns. Patients whose pairs of isolates had different MIRU or IS6110 RFLP patterns were infected by different strains of \textit{M. tuberculosis}, whereas patients whose isolate pairs had identical MIRU and IS6110 RFLP patterns were infected by a single strain of \textit{M. tuberculosis}. Eighty-seven percent (33/38) of the patients had a pair of isolates with different MIRU or IS6110 RFLP genotype patterns, which was likely the result of ongoing transmission and exogenous reinfection or mixed infection by different strains of \textit{M. tuberculosis}.

To determine the relative proportion of primary versus acquired drug resistance, we excluded 6 patients with initial iso-
Table 1. Information about patients and their clinical isolates.

<table>
<thead>
<tr>
<th>Patient sex (age, in years)</th>
<th>Interval, months</th>
<th>Treatment type(^a)</th>
<th>Drug susceptibility</th>
<th>MIRU genotypes</th>
<th>IS6110 RFLP analysis</th>
<th>Drug resistance</th>
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<td>Male (64) 16</td>
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<td>Identical</td>
<td>Acquired</td>
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<td>Identical</td>
<td>Acquired</td>
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<td>HSR</td>
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<td>Identical</td>
<td>Acquired</td>
</tr>
<tr>
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<td>HSRE</td>
<td>5</td>
<td>Identical</td>
<td>Identical</td>
<td>Acquired</td>
</tr>
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<td>HSRE</td>
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<td>Identical</td>
<td>Identical</td>
<td>Acquired</td>
</tr>
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<td>S</td>
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<td>Different</td>
<td>Primary</td>
</tr>
<tr>
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<td>HS</td>
<td>1</td>
<td>Identical</td>
<td>Different</td>
<td>Primary</td>
</tr>
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<td>Different</td>
<td>Primary</td>
</tr>
<tr>
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<td>Different</td>
<td>Primary</td>
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<td>HSR</td>
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<td>Different</td>
<td>Primary</td>
</tr>
<tr>
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<td>HSR</td>
<td>1</td>
<td>Identical</td>
<td>Different</td>
<td>Primary</td>
</tr>
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<td>Different</td>
<td>Primary</td>
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<td>Different</td>
<td>Primary</td>
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<td></td>
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<tr>
<td>Male (46) 1</td>
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<td></td>
</tr>
<tr>
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<td>R</td>
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<td>Different</td>
<td>Primary</td>
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</tr>
<tr>
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<td>Primary</td>
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<td>Primary</td>
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<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
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<td>1</td>
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<td>Primary</td>
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<td>HR</td>
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<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Female (65) 17</td>
<td>N</td>
<td>HR</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (63) 39</td>
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<td>HS</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
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<tr>
<td>Male (79) 4</td>
<td>N</td>
<td>RE</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (46) 29</td>
<td>N</td>
<td>SR</td>
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<td>Different</td>
<td>Primary</td>
<td></td>
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<tr>
<td>Male (48) 2</td>
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<td>HSR</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (51) 4</td>
<td>N</td>
<td>SR</td>
<td>5</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (58) 1</td>
<td>S</td>
<td>HS</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (44) 40</td>
<td>R</td>
<td>HS</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Female (42) 9</td>
<td>SR</td>
<td>HSR</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Female (22) 1</td>
<td>H</td>
<td>N</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (69) 4</td>
<td>H</td>
<td>N</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (40) 5</td>
<td>H</td>
<td>N</td>
<td>3</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (52) 1</td>
<td>S</td>
<td>N</td>
<td>5</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Male (71) 1</td>
<td>HS</td>
<td>N</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
<tr>
<td>Female (41) 5</td>
<td>HSRE</td>
<td>HSRE</td>
<td>1</td>
<td>Different</td>
<td>Primary</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** E, ethambutol; H, isoniazid; MIRU, mycobacterial interspersed repetitive unit; N, susceptible to all first-line drugs tested; R, rifampin; RFLP, restriction fragment length polymorphism; S, streptomycin.

\(^a\) 1, the 2 isolates were collected during 1 treatment period; 2, the 2 isolates were collected during 2 successive episodes of tuberculosis, with cure as the outcome of the previous episode.

\(^b\) Regimen 1, H, R, pyrazinamide (Z), E, or S daily for 2 months; regimen 2, H, R, Z, E, or S 3 times a week for 2 months, then H and R daily for 4 months; regimen 3, H, R, Z, E, or S daily for 2 months, then H and R daily for 3 months; regimen 4, H, R, and Z daily for 2 months, then H and R daily for 4 months; regimen 5, other regimens, including second-line drugs; regimen 6, H, R, Z, E, and S 3 times a week for 2 months, H, R, and E 3 times a week for 6 months.
lates that were drug resistant and subsequent isolates that were drug susceptible or resistant to fewer drugs. The remaining 32 patients had initial isolates that were drug susceptible or resistant to at least 1 drug and became resistant to ≥1 drugs. Of these 32 patients, 27 (84%) had a pair of isolates with different genotype patterns. Five patients (16%) had identical genotype patterns. On the basis of both the drug-susceptibility and genotyping results, we conclude that 27 patients (84% [95% confidence interval {CI}, 67.2%–94.7%]) had drug resistance due to transmission of a drug-resistant strain of *M. tuberculosis*, and 5 patients (16% [95% CI, 5.3%–32.8%]) had acquired drug resistance from an inadequate treatment regimen or nonadherence to an adequate regimen. There were 5.4 times (27/5) as many cases with primary drug resistance caused by ongoing transmission of drug-resistant strains of *M. tuberculosis*, compared with cases of acquired drug resistance.

Eighty-seven percent (33/38) of the patients were male, and the median age was 51 years (range, 22–84 years). The median time between the first isolate and the next isolate was 4 months (range, 1–40 months). For the same patient, the isolate became resistant to a median of 1 additional drug (range, lost resistance to 2 drugs to gained resistance to 3 drugs). Patients who developed acquired drug resistance were younger (median, 35 years) than patients who had primary drug resistance (median,

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**Figure 1.** Selection of patients and the genotyping results of their successive clinical isolates. TB, tuberculosis.
53 years (P = .088). Similarly, the interval between 2 isolates from patients who had acquired drug resistance (median, 10 months) was longer than the interval between 2 isolates of patients who had drug resistance as a result of transmission of a drug-resistant strain of M. tuberculosis (median, 4 months) (P = .083). Although the differences in age and interval between clinical isolates with discordant drug-susceptibility test results were not statistically significant, the results suggest that younger people are more likely to develop acquired drug resistance and that it takes a longer time for the strains to acquire drug resistance.

The initial isolate from 2 of the 32 patients indicated MDR tuberculosis and remained MDR. In addition, 10 patients had MDR tuberculosis in their second isolate. On the basis of genotyping results, 1 of the patients developed MDR tuberculosis during treatment, and this indicated acquired drug resistance. Nine of the 10 patients with MDR tuberculosis who had different genotype patterns had the illness as result of transmission of drug-resistant strains of M. tuberculosis, and they represented 33% (9/27) of all cases of tuberculosis with transmission of a drug-resistant strain of M. tuberculosis.

**Discussion.** Historically, when patients had progressively drug-resistant tuberculosis while receiving therapy, it was assumed that their initial isolate had become resistant because of acquired drug resistance presumably caused by inadequate therapy. With the advent of molecular techniques, it was demonstrated that some patients could develop drug-resistant tuberculosis as a consequence of exogenous reinfection with a novel drug-resistant strain [11–14].

In the present study, we have integrated drug-susceptibility testing and genotyping to determine the proportion of patients in Shanghai who become progressively drug resistant during therapy because of exogenous reinfection with drug-resistant strains. Of the 6622 patients with tuberculosis treated by the Shanghai CDC during a 5-year period, 100 had serial isolates that were increasingly drug resistant. Eighty-four percent (27) of 32 who were fully evaluated had exogenous reinfection with drug-resistant isolates. Although several studies have shown that reinfection with different strains can occur in treated patients, the comprehensive sampling used in the present study provides an estimate of the frequency of this phenomena in Shanghai [15, 16].

There are some limitations to the study. Many patients were excluded from analysis because their isolates could not be found. However, available demographic data did not detect a bias between those analyzed and excluded, which suggests that their inclusion may not have changed the results significantly. We relied on previously collected data, and some retreated patients may have been misclassified as new cases (or vice versa). Similarly, we relied on programatically generated and recorded drug-susceptibility data, and some of these results may have been erroneous. Finally, mixed infections can occur, although the frequency of this in a largely HIV-seronegative population such as ours is unlikely to be high [17]. We have begun to validate and extend our conclusion in a prospective population-based study.

In Shanghai, the vast majority of drug resistance during therapy was not due to poor response to the initial treatment regimen or to an inadequate treatment regimen but rather to ongoing transmission of drug-resistant strains of M. tuberculosis. This suggests that drug-resistant tuberculosis is being transmitted in the facilities and communities in which these patients are being treated, and accelerated efforts to interrupt transmission are needed. Disturbingly, more than one-quarter of the new infections we identified were with MDR strains, which suggests the ongoing transmission of highly resistant organisms in the community. Local existing programs in infection control and the management of patients with MDR tuberculosis should also be reviewed to identify further means of blocking the transmission of drug-resistant tuberculosis. Most concerning is the possibility that there is undetected transmission of extremely drug-resistant tuberculosis, which leaves patients virtually untreated with currently available antituberculosis drugs [18–20].

Clearly, new tools—such as rapid diagnostics, new antituberculosis therapies, and effective vaccines—are needed to control drug-resistant tuberculosis. However, these results suggest that we cannot wait to implement measures to block the transmission of MDR and extremely drug-resistant tuberculosis.

**References**