

Recurrent Tuberculosis and Exogenous Reinfection, Shanghai, China

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Of 52 patients with recurrent tuberculosis in Shanghai, People's Republic of China, 32 (61.5%) had isolates in which genotype patterns of *Mycobacterium tuberculosis* differed between first and second episodes. This result indicates that exogenous reinfection is common in an area with a high incidence of tuberculosis.

Elucidating the role of reinfection in tuberculosis (TB) recurrence is important in the People's Republic of China because this country has the second highest incidence of TB in the world, an estimated rate in 2004 of 101 cases/100,000 persons/year (1). After effective short-course therapy for active TB, some patients experience another, recurrent TB episode. The recurrent episode may be due to endogenous reactivation or exogenous reinfection. The role of exogenous reinfection has been debated for decades (2,3). Understanding the cause for recurrence helps clinicians evaluate the effectiveness of therapeutic regimens and TB prevention and control programs to assess strategies and interventions.

DNA fingerprinting techniques provide excellent tools to address whether recurrent TB is caused by endogenous reactivation or exogenous reinfection. Different *Mycobacterium tuberculosis* strains can be differentiated by genotyping methods that use information about genetic markers and their distribution in the genome (4). Among persons with recurrent TB, if the isolates from 2 TB episodes have the same genotype, the episode is defined as an endogenous relapse; otherwise, it is defined as exogenous reinfection. Previously, researchers have tried to assess the relative importance of endogenous relapse versus exogenous reinfection, with varied results (2,5–10). Our study helps elucidate the role of reinfection in TB recurrence in China.

The Study

Shanghai is 1 area in China with high TB treatment success rates. Persons with TB symptoms (mainly cough for at least 2 weeks, chest pain, weight loss, and fever) can go to any hospital or community health center in Shanghai. They are first screened by chest radiograph. All patients with suspected TB are referred to a TB hospital, where sputum is examined by smear and culture. TB is bacteriologically confirmed if ≥ 1 sputum smear examination result was positive for acid-fast bacilli or if the culture was positive. The TB hospital sends all mycobacteria-positive cultures to the TB reference laboratory at the Shanghai Municipal Centers for Disease Control and Prevention (Shanghai CDC), which participated in the World Health Organization/International Union against Tuberculosis and Lung Disease global drug resistance surveillance project, for species identification and drug susceptibility testing. TB patients are treated in the TB hospital during the intensive phase. On the basis of the sputum smear and culture examination 1 or 2 months after TB therapy is initiated, the patient is discharged from the hospital and finishes treatment as an outpatient. The community health center physician trains family members to supervise and observe the TB patient's remaining doses and treatment. Completion of anti-TB therapy is based on the examination of sputum smear, culture, and chest radiographic results. Shanghai CDC collects and manages patient information, such as social and demographic characteristics, clinical data, TB treatment regimens, and the result of drug susceptibility testing and species identification.

From January 1999 through September 2004, Shanghai CDC collected 6,442 clinical isolates from a total of 6,960 persons with bacteriologically confirmed (by smear or culture) TB. Of these case-patients, 5,688 were cured, and 202 (164 male and 38 female) had a recurrence, defined by the following criteria: 1) their TB episode was confirmed by culture; and 2) they experienced 2 successive TB episodes, with cure as the outcome of the first episode. Cure was defined as the completion of a standard course of combination therapy and successive negative sputum cultures during treatment. At the same time, chest radiography showed resolution of the focus of infection. On the basis of the selection criteria, 54 patients with recurrent TB were included in the study (Figure 1).

The mycobacterial interspersed repetitive unit (MIRU) typing method (11) was used to genotype strains from these patients. This method is relatively easier to perform and less technically demanding than IS6110 restriction fragment length polymorphism (IS6110-RFLP), which was used in many previous molecular epidemiologic studies of TB (12). We followed the protocol described by Kwara et al. (13) with modifications. PCR products were analyzed by 2.5% (w/v) agarose gel electrophoresis

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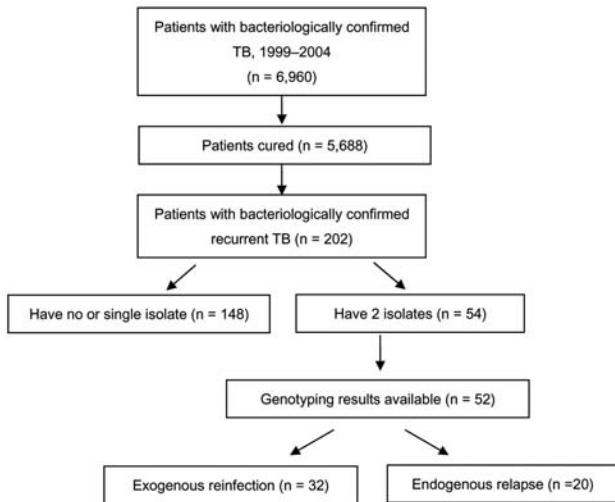


Figure 1. Selection of patients in the study, Shanghai, People's Republic of China, 1999–2004. TB, tuberculosis. The total number of patients from 1999 through 2004 was 6,960; among these patients, 5,688 were cured.

(Figure 2A). Genotyping was performed for 2 isolates. We analyzed the data for the remaining 52 patients and found MIRU patterns for both episodes to be the same for 20 patients and different for 32. Of these 32 patients, 13 had 1 MIRU locus change between the 2 isolates, 10 had a change in 2 loci, and 9 had ≥ 3 loci changes in their isolates (online Appendix Table, available from http://www.cdc.gov/ncidod/EID/vol12no11/05-1207_appT.htm). These results indicate that 32 (61.5%) of 52 (95% confidence interval 47.0%–74.4%) of the recurrent cases were due to reinfection. To further validate the MIRU genotype result, the IS6110 RFLP genotyping method was performed; results showed that the isolates with 1 or 2 MIRU locus changes had very different RFLP patterns (a difference in ≥ 4 bands in the IS6110-RFLP, Figure 2B).

We further used patient age group and intervals between the 2 episodes to classify recurrent TB. We found that the percentage of TB patients with an exogenous reinfection decreased with age from 100% (TB patients <30 years of age) to 66.7% (TB patients 30–60 years) and 53.3% (TB patients >60 years). We also found that the frequency of exogenous reinfection increased with the amount of time that elapsed between the end of TB treatment for the first episode of TB and the date that the second episode was diagnosed. Exogenous reinfection accounted for 7 (46.7%) of the 15 recurrent episodes that occurred within 6 months after treatment for the first episode; the percentage of recurrent cases due to exogenous reinfection increased to 73.9% (17/23) among TB patients whose second episode occurred ≥ 1 year after treatment for the first episode.

Conclusions

We analyzed genotypes of 104 isolates from 52 patients who experienced 2 TB episodes from 1999 through 2004. Thirty-two of 52 patients had different MIRU genotype patterns in clinical isolates from their 2 episodes, which indicates that exogenous reinfection accounted for 61.5% of the recurrent cases in Shanghai during the study period. The high proportion of exogenous reinfection in recurrent TB patients indicates that high levels of transmission of *M. tuberculosis* are an important cause of TB in Shanghai, China.

Although several reports have indicated that exogenous reinfection may occur after successful treatment, the proportion of TB cases that are actually caused by exogenous reinfection may vary dramatically for several reasons, such as the patients' HIV infection status, different facilities, and healthcare providers' various definitions (some used different numbers of days elapsed between the first and second episode to define a recurrent case of TB), and particularly the small sample size (2). Several studies have reported that HIV may be a risk factor for exogenous reinfection (2,14,15). Unfortunately, we do not have data on each patient's HIV infection status, and we cannot totally exclude the effect of HIV infection. However, considering the low incidence of HIV infection among residents of Shanghai (≈ 0.6 cases/100,000 persons/year), we consider it unlikely that HIV is a major factor in our findings. The criteria used to define recurrent TB cases differ; various

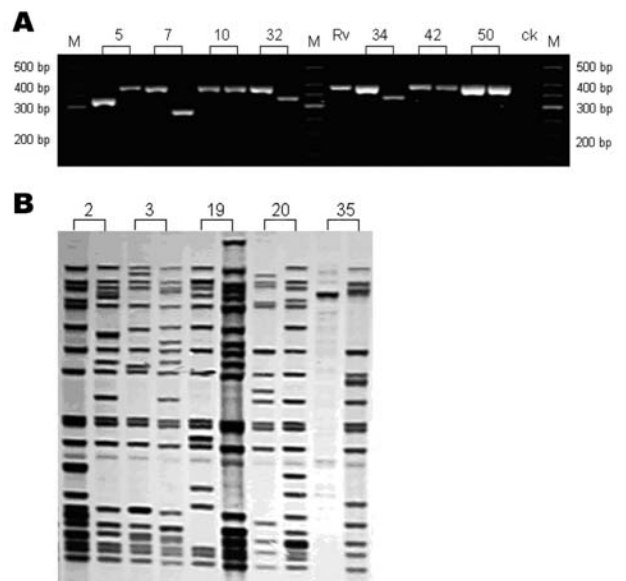


Figure 2. Genotyping analysis of clinical isolates from patients with recurrent tuberculosis. Numbers represented the patients' codes. A) Gel electrophoresis analysis of the PCR products of the mycobacterial interspersed repetitive unit (MIRU) locus 10, base pair; M: DNA marker; Rv, H37Rv positive control; ck, negative control. B) IS6110 restriction fragment length polymorphism analysis of some patients with different MIRU patterns.

studies defined the interval between the end of TB treatment and a new episode (recurrent TB) as 3–12 months (5,7,8). Our study did not define the interval and determined that 61.5% of the recurrent TB cases were due to exogenous reinfection. If we chose a 6-month interval to define a recurrent TB case, then we would find an even higher percentage of recurrent TB cases were due to exogenous reinfection (67.6%, 25/37).

Previous studies and our study demonstrate that TB patients can be reinfected with a new strain of *M. tuberculosis* after treatment, which indicates that the immunity evoked by the primary infection does not protect the patient against a later infection. A recent study from South Africa demonstrated that the rate of TB reinfection after successful treatment is even higher than the rate of new TB infection (5). Such results suggest major challenges for the development of a new vaccine that will be effective against *M. tuberculosis*.

In summary, our study showed that 61.5% of recurrent TB cases in Shanghai from 1999 through 2004 were due to exogenous reinfection and confirmed that reinfection may be common in areas with a relatively high incidence of TB. This finding provides important implications for TB control. To prevent recurrent TB, more attention should be paid to the interruption of TB transmission.

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