The frequency and determinants of exogenous reinfection and of endogenous reactivation of tuberculosis in patients previously treated are poorly understood. In Gran Canaria Island, Spain, between 1991 and 1996, 962 tuberculosis cases were confirmed by culture. Drug susceptibility testing was performed on available bacterial isolates and IS6110-based RFLP genotyping was carried out. Twenty-three patients (2.4%) had two positive cultures separated by at least 12 mo, 18 of whom had bacterial DNA available for genotypic analysis. The initial and final isolates from eight (44%) were different genotypes, indicating exogenous reinfection. Six of them were retreated after cure and two retreated after default. Six were HIV seronegative and two were HIV seropositive. Endogenous reactivation was seen in the remaining 10 patients of whom eight were retreated after default and two after cure. Three of the eight (38%) being retreated after default developed multidrug resistance. One genotype was responsible for a second episode of tuberculosis in five cases, three exogenous reinfections and two endogenous reactivations. In the context of a moderate incidence of tuberculosis, exogenous reinfection is an important cause of TB recurrence, even in HIV-seronegative patients.

The Island of Gran Canaria in Spain has a population of 713,768 inhabitants with annual notification rates of new cases of tuberculosis of 28–32/100,000 inhabitants and cure rates of 84–86% in 1991–1996 (16). In this community we have studied consecutive cases to determine the DNA fingerprinting patterns of bacilli isolated from patients with recurrent tuberculosis. We have used this opportunity to determine whether recurrent disease was related to exogenous reinfection or to endogenous reactivation.

**METHODS**

All patients diagnosed with tuberculosis (TB) who were confirmed by culture between January 1, 1991 and December 31, 1996 were studied. They were identified by active surveillance, or by review of TB control registries and hospital and health center charts. All patients had the standard treatment recommended for new patients consisting of isoniazid (H), rifampin (R), and pyrazinamide for the initial intensive phase (usually 2 mo) and H and R for 4 mo for a total of 6 mo of treatment. All patients with recurrent tuberculosis received directly observed therapy (17) with a regimen adjusted according to the results of drug susceptibility testing. The outcome of treatment of the cases was evaluated.

Patients who took more than 80% of all prescribed antimicrobial agents and had negative cultures at the end of the Months 4 and 6 were designated cured. If they remained or became again bacteriologically positive at 5 mo or later during the course of their treatment, the outcome of treatment was defined as failure. If they did not complete the full treatment prescribed, the outcome of their treatment was defined as default. Patients with two cultures of *Mycobacterium tuberculosis* separated by a minimum period of 12 mo were defined as cases of recurrent tuberculosis. These patients were then classified as retreatment after cure, after default, or after failure.

In each patient, information was gathered at the time of their recurrence by chart review and personal interview concerning age, sex, and the results of testing for human immunodeficiency virus (HIV) infection. HIV tests were performed routinely in all patients with tuberculosis, with pre- and posttest counseling, and the results were kept confidential. HIV tests were carried out during both the first and the second episode. In addition, the clinical course of each patient was determined.

All isolates of *M. tuberculosis* included in the study were tested for susceptibility to antimicrobial agents according to the proportional method (18). The drugs tested included isoniazid (0.2 μg and 1 μg/ml), rifampin (1 μg/ml), streptomycin (2 μg and 10 μg/ml), and ethambutol (5 μg and 10 μg/ml).

Isolates of *M. tuberculosis* from each of the patients included in the study were frozen at −40°C in Dubos liquid media prior to being subcultured on Lowenstein–Jensen media and incubated at 37°C in an aerobic atmosphere with 5–10% CO2. Cultivated bacteria then underwent genotyping with IS6110-based restriction fragment length polymorphism (RFLP) analysis (19). Resulting RFLP patterns were analyzed with the Gel Compare 4.0 (Applied Math) software program.

Laboratory cross-contamination of bacterial cultures was considered unlikely. The samples from the patients with the same strain processed in the laboratory during the time period of the study were processed more than 3 wk apart. Each of the patients had at least two positive cultures in each disease episode. Each culture consisted of a
large number of colonies on solid culture media (20). For every patient, at least two isolates from each episode of tuberculosis were genotyped, and these genotypes were identical.

Those patients in whom the initial and subsequent isolates were identical were classified as having disease due to “endogenous reactivation.” Those in whom isolates were different were classified as having disease due to “exogenous reinfection.”

Comparisons between categorical variables were made using the chi-square test, Fisher’s exact test with relative risk, and 95% confidence limits calculated.

RESULTS

Of the total of 962 patients with cultures positive during the study period, 23 (2.4%) had cultures that yielded M. tuberculosis on two occasions, separated by at least 12 mo. Twelve of these cases were retreated after default and 11 retreated after cure. There were no treatment failures. The median number of days of therapy received in the initial episode by those retreated after default was 103 (range 27–163).

Of the 962 patients, 682 patients (70.9%) had fingerprinting results. Of the 23 patients with two cultures positive at least 12 mo apart, five patients were excluded from further analysis because adequate bacterial DNA was not available for genotyping (two of whom were retreated after default and three retreated after cure). The clinical charts of the remaining 18 patients were carefully reviewed and all of them had signs and symptoms consistent with tuberculosis, both in the first and in the second episode of the disease. At least two cultures were positive in each episode of disease evaluated. In the first episode, three cultures were positive in 14 patients and two in the remaining 4. In the recurrent episode, three cultures were positive in 16 and two in the remaining 2.

Clinical characteristics of the 18 patients are shown in Table 1. The majority of patients (13) were male and HIV seronegative. The median time between the first and second cultures was 19 mo (range 12 to 40). The median age of the cases was 38 yr (range 22 to 56). All the patients had pulmonary disease. Isolates from patients in each of the first episodes of disease were susceptible to all antimicrobial agents tested.

Bacterial DNA fingerprinting of the isolate from the episode of recurrent disease in eight patients (44%) identified a different RFLP pattern, indicating exogenous reinfection (Figure 1). Among these eight cases, six were retreated after cure and two retreated after default. The two patients retreated after default had experienced a good clinical response in the initial treatment (the reason given for why they defaulted) and later returned with signs and symptoms of tuberculosis (19 and 12 mo respectively).

The remaining 10 recurrent cases (55%) had identical initial and final genotypes indicating endogenous reactivation. Two of the cases were retreated after cure and eight retreated after default.

Cases judged to have exogenous reinfection were not significantly more likely to be HIV seropositive (RR 0.87; 95% CI 0.25–2.95) but were more likely to have been retreated after cure (RR 3.75; 95% CI 1.02–13.80). The median time to recurrence was 18.5 mo (range 12 to 31) for those with exogenous reinfection as compared with 19.5 mo (range 12 to 40) for those with endogenous reactivation (p > 0.05). The median age of those with exogenous reinfection was 36.5 yr (range 32 to 45) as compared with 43.5 yr (range 22 to 56) for those with endogenous reactivation (p > 0.05).

There were five patients whose isolate of M. tuberculosis from the episode of recurrent disease shared the same RFLP pattern (two who had endogenous reactivation and three who were exogenously reinfectected). These isolates were of a strain frequently isolated in our island and the “Beijing” genotype was confirmed by spoligotyping (21). No epidemiological connections were found among these five patients despite a thorough investigation. Two of these five patients, both with endogenous reactivation, had developed resistance to isoniazid and rifampin by the time they presented for retreatment.

An additional patient was found to have become resistant to isoniazid and rifampin, for a total of three patients (Table 1). Specimens from each of these patients were recultured and rereviewed to rule out an error due to incorrect labeling of samples in the laboratory. All three patients were found to have identical initial and final bacterial DNA fingerprints (en-
convincingly demonstrated instances of reinfection in immunocompromised patients (22, 25). Further studies, using molecular fingerprint methods, confirmed reinfection, primarily in AIDS patients (4, 8, 11), but also in occasional patients without demonstrable immunosuppressive conditions (6, 11–14, 27–30). The same techniques have demonstrated the occurrence of mixed initial infection (31), which, in some scenarios, would be indistinguishable from reinfection. The possibility of multistain infection could be ruled out in our study because at least two isolates per patients in each episode were genotyped and had identical RFLP patterns.

To date, there is only one study reported that describes the relative contribution of reactivation and reinfection in a whole population. This study, conducted in an area with one of the world’s highest rates of tuberculosis (estimated at 251 cases per 100,000 inhabitants from January 1996 to May 1998), showed that 75% of recurrent cases had been exogenously reinfected (14).

The current study demonstrates that the likelihood of reinfection is high even in a community with relatively low rates of tuberculosis and good measures to control tuberculosis (16). The implication of our study is that exogenous reinfection may be a significant cause of tuberculosis in many more communities than was suggested by the previous studies. The exogenous reinfection was not related to HIV infection in this community.

Our study also provides a new perspective on the phenomenon of acquired drug resistance. It has long been acknowledged that the rate of drug resistance is much greater in patients who are being retreated than it is in those never previously treated, generally ascribed to the acquisition of drug resistance in the strain during the course of chemotherapy. However, prior studies have not distinguished between endogenous reactivation and exogenous reinfection. Consequently, the reported rates of acquired drug resistance actually reflect a combination of newly acquired resistance and of primary resistance of strains causing reinfection. The traditional approach to analysis of our data would suggest that 3 of 18 (16%) retreatment cases had acquired drug resistance. With the use of bacterial genotyping we have been able to show that the true rate at which resistance was acquired among reactivated strains was greater (30%).

This population-based study provides insight into the manner in which patients who present for retreatment develop tuberculosis. It demonstrates that the majority of those who return for retreatment after default develop disease caused by the organism that caused the initial episode of disease, which had frequently become drug resistant. In contrast, those who returned for retreatment after being cured generally developed disease caused by a new organism. Tuberculosis control programs in this and in similar communities must focus on ensuring completion of therapy of the initial episode of tuberculosis, thereby minimizing endogenous reactivation and acquired drug resistance while simultaneously preventing transmission to previously cured cases.

**References**


