JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1999, p. 3078–3079 Vol. 37, No. 9
0095-1137/99/$04.00+0

Letters to the Editor

Stability of IS*6110* Restriction Fragment Length Polymorphism Patterns of Multidrug-Resistant *Mycobacterium tuberculosis* Strains

We read with interest the recently published study by Alito et al. (1) concerning the stability of IS*6110* restriction fragment length polymorphism (RFLP) patterns of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains. The authors analyzed the IS*6110* RFLP patterns and spoligotypes of two groups of MDR *M. tuberculosis* strains which they discuss to represent two tuberculosis (TB) outbreaks. Within the first group, both IS*6110* fingerprint patterns and spoligotypes of the MDR strains have been found to be identical, whereas among the strains of the second group spoligotypes were identical but the IS*6110* patterns showed variations. Based on this, the authors conclude that the rate of change of IS*6110* RFLP patterns in particular MDR *M. tuberculosis* strains may be too fast for a reliable interpretation of strain typing results over a period of a few years.

In our recent paper (3), we also elucidated the stability of IS*6110* patterns of drug-resistant *M. tuberculosis* strains by analyzing 165 serial isolates obtained from 56 patients with drug-resistant TB. We did not observe a higher level of instability of IS*6110* patterns in these isolates in comparison with the rates of changes described in other studies comprising mainly drug-susceptible isolates (e.g., references 2, 4, and 6). In addition, no particular *M. tuberculosis* genotypes showing a higher rate of IS*6110* changes have been identified. From these data we conclude that the stability of IS*6110* patterns in drug-resistant *M. tuberculosis* strains does not seem to differ from that of drug-susceptible isolates. The IS*6110* changes observed, however, occurred only in MDR isolates (37 of the 56 patients were infected with MDR strains), which is likely to be due to the longer time intervals between the times of retrieval of the serial isolates in the patient group with MDR TB (a mean of 300 days for the MDR isolates compared to a mean of 60 days for the resistant but not MDR isolates). Moreover, we have not found a higher instability of IS*6110* patterns in several cases of recent transmission of MDR strains.

It is well known that the IS*6110* patterns of unrelated *M. tuberculosis* strains generally show a high degree of variability. However, some *M. tuberculosis* strain families displaying similar IS*6110* fingerprint patterns and identical spoligotypes have been described, e.g., the "Beijing family" (5). Hence, the differences in IS*6110* patterns among the strains of the second MDR group observed by Alito et al. (1) may be due not to a recent outbreak of a MDR strain showing a higher rate of IS*6110* change but to false clustering of strains of an *M. tuberculosis* family in one fingerprint group. This notion is further supported by the fact that in our German strain collection we found two *M. tuberculosis* isolates showing the same spoligotype as and an IS*6110* pattern similar to those of the strains of the second group of MDR strains described by Alito et al. (1) (Fig. 1).

In conclusion, we believe that the data presented by Alito et al. (1) do not give evidence to confirm higher instability of IS*6110* patterns among particular MDR *M. tuberculosis* strains. On the contrary, our results indicate that the evolutionary clock of IS*6110* RFLP seems to be identical among drug-susceptible and drug-resistant *M. tuberculosis* isolates.

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Authors' Reply

In response to the letter by Niemann et al. commenting on our recent publication (1), we have the following remarks. Niemann et al. stated that they did not find a higher rate of change in IS*6110* RFLP patterns of serial isolates from patients with drug-resistant tuberculosis in comparison with the rate of change found in isolates from patients with mainly drug-susceptible tuberculosis in other studies. They conclude that instability of the IS*6110* RFLP pattern is not associated with drug resistance. At face value, this seems to be true. In an extended study of serial isolates from 546 patients in The Netherlands (2), we also found that changes in the IS*6110*-based RFLP pattern of *M. tuberculosis* occurred as often in drug-resistant as in susceptible strains. Yet, Niemann et al. state in their letter that all changes they observed in IS*6110* RFLP patterns in their recent study (3) were found in MDR strains. Their explanation for this phenomenon is that the time intervals at which their serial MDR strains were isolated were longer than the ones for isolates resistant to only one drug. However, their findings may also indicate that instability of the IS*6110* RFLP pattern is higher in particular (MDR) strains. In our laboratory we have recently observed an overrepresentation of changing RFLP patterns in drug-resistant *M. tuberculosis* strains. We hope to present these results within a few months.

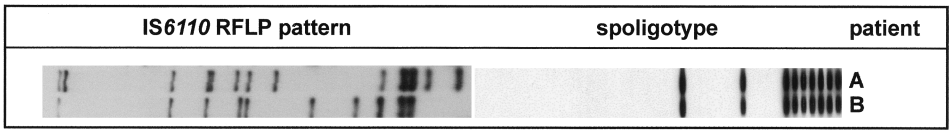

FIG. 1. IS*6110* RFLP patterns and spoligotypes of two *M. tuberculosis* strains obtained from two patients living in Germany.

The second issue Niemann et al. raise is that the minor differences in the IS*6110* RFLP pattern of the second-outbreak strain may be due not to the instability of IS*6110* RFLP but to the predominance of a conserved genotype family in the area of Buenos Aires. As no others, we are aware that there are predominant *M. tuberculosis* genotypes circulating in particular areas and that this may interfere with the interpretation of strain typing results in molecular epidemiology. However, our conclusions are not based solely on strain typing results. As stated in our paper (1), based on conventional contact tracing we are quite sure that all of the isolates from the patients involved in the second outbreak were derived from a common ancestor within a period of 4 years. Nosocomial outbreaks caused by other *M. tuberculosis* strains have been recorded several times in Buenos Aires in the past years (4). Therefore, it is highly unlikely that the second outbreak described in our paper is a coincidental combination of circumstances resulting in the isolation of MDR strains with identical spoligopatterns and highly similar IS*6110* RFLP types from patients with overlapping hospitalization dates.

In conclusion, we agree that our study (1) provides only an indication that the instability of IS*6110* RFLP may be higher in resistant strains. Yet, our statement that the IS*6110* RFLP may evolve too fast in particular MDR strains is based on a solid observation. Particular *M. tuberculosis* genotypes may well have different molecular clocks.

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