

Genotyping of rifampin-resistant *Mycobacterium tuberculosis* isolates from western Turkey

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Background: Although the rate of multiple drug resistance is high, there is no published data on the transmission rate of drug-resistant strains of *Mycobacterium tuberculosis* in the Aegean region of western Turkey that are based on molecular methods.

Methods: IS6110 and pTBN12 restriction fragment length polymorphism (RFLP) methods were used for typing *M. tuberculosis* strains isolated from 26 sputum samples from 26 patients.

Results: Nineteen of the rifampin-resistant isolates (73.1%) contained 6 to 11 copies of IS6110. Eighteen different IS6110 DNA fingerprint patterns were observed in the 26 rifampin-resistant isolates. Twenty-three of the 26 rifampin-resistant isolates were also resistant to isoniazid. When evaluated together, both methods yielded 21 (80.9%) different banding patterns and the level of clustering was 34.6%. The average number per pattern was 1.23 (26/21).

Conclusions: IS6110 fingerprinting suggests that the rifampin-resistant isolates obtained from the Aegean region had a relatively high clustering rate and were clonally related. These findings showed that the rifampin-resistant isolates are actively transmitted between patients. Urgent measures should be taken to prevent the spread of these resistant strains.

Key words: *Mycobacterium tuberculosis*, multidrug-resistant tuberculosis, multiple drug resistance, DNA fingerprinting, rifampin, Turkey

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Ann Saudi Med 24(2):102-105

Newer molecular methods constitute powerful tools for identifying outbreaks and tracing contacts in studies of infectious diseases. In the early 1990s, IS6110 restriction fragment length polymorphism (RFLP) became the standard typing method for tuberculosis (TB) epidemiology.¹ IS6110, used as a probe for RFLP, is an insertion sequence containing 1.355-base pairs. It is present in different copy numbers (between 0 and 25 copies) in the *Mycobacterium tuberculosis* complex and is located at various chromosomal sites.^{2,3} A DNA fingerprinting method based on the IS6110-RFLP has been used widely to type *M. tuberculosis* isolates in epidemiologic studies of TB. IS6110 results are stable and reproducible, and have a high discriminatory power. Therefore, IS6110 is useful for population-based epidemiologic studies and the control of laboratory cross-contamination.^{4,5} However, it is widely recognized that strains with low copy numbers (fewer than six) show little polymorphism, and identical patterns are commonly found for strains from apparently unconnected patients.⁶ A second probe, referred to as pTBN12, is useful for fingerprinting mycobacteria. This recombinant plasmid contains an insert characterized as a polymorphic GC-rich repetitive sequence. The polymorphic GC-rich repetitive

sequence is a short sequence that is repeated multiple times in the genomes of *M. tuberculosis* and other mycobacteria. It has proven useful in distinguishing *M. tuberculosis* strains with fewer than six copies of IS6110.⁴

The aim of this study was to determine the epidemiological relationship between rifampin-resistant *M. tuberculosis* strains isolated from different patients in the Aegean region of Turkey. We also intended to obtain information about the origin and transmission of the drug-resistant strains and the efficiency of regional control programs.

Methods

The 26 clinical *M. tuberculosis* isolates used in this study came from 26 different patient sputum samples from the Regional Tuberculosis Laboratory and Ege University Mycobacteriology Laboratory, Izmir, Turkey. Previously, rpoB mutations and antituberculosis susceptibilities had been determined by DNA sequencing and proportional methods.⁷ The critical concentrations for defining resistance to rifampin, isoniazid, streptomycin, and ethambutol were 1 µg/mL, 0.2 µg/mL, 2 µg/mL and 5 µg/mL, respectively.⁸ Patient data were collected from the records of the laboratories. If necessary, additional data were obtained from the clinicians.

Molecular typing was performed at the Inonu University Molecular Microbiology Laboratory, Malatya, Turkey, using the IS6110 and pTBN12-RFLP methods. Two micrograms of genomic DNA was digested with PvuII. Restriction fragments were separated by gel electrophoresis and transferred to a nylon membrane. DNA fragments were detected with a IS6110 probe.¹ Isolates with copy numbers less than six were typed by use of a pTBN12 probe. Genomic DNA was restricted by AluI. Restriction fragments were separated by gel electrophoresis and transferred to a nylon membrane. DNA fragments were detected with a chemoluminescent-labeled pTBN12 probe.⁹

Isolates were considered in the same cluster if they met any one of the following criteria: (i) a copy number of six or greater with identical IS6110-RFLP patterns, (ii) a copy number of six or greater with only one banding pattern that differed and which had the same pTBN12 fingerprinting pattern, (iii) a copy number below six but with identical banding patterns by both methods.¹⁰

Results

The drug susceptibility patterns and rifampin-resistance genotypes of the 26 *M. tuberculosis* isolates are shown in Table 1. Twenty-three of the 26 rifampin-resistant isolates were also resistant to isoniazid. Nineteen of 26 (73.1%)

rifampin-resistant isolates contained between 6 and 11 copies of IS6110. As shown in Table 1, 18 different IS6110 DNA fingerprint patterns were observed in the 26 rifampin-resistant isolates. Of the 18 patterns, five were shared by more than one isolate (a total of 13 isolates were in the cluster); the remaining 13 (50%) isolates gave unique fingerprinting patterns. IS6110-RFLP patterns of the isolates are shown in Figure 1. Four isolates with identical two copies of IS6110 were found as clonally unrelated by the pTBN12 fingerprinting method. However, the two isolates with identical four copies of IS6110 were also in the same cluster by the pTBN12 probe. When both methods were evaluated together, the level of clustering was 34.6 % in rifampin-resistant *M. tuberculosis* isolates. Twenty-one (80.8 %) different DNA fingerprinting profiles were determined and the average number of isolates per pattern was 1.23 (26/21).

Discussion

The Aegean region in the western part of Turkey has about 8 million inhabitants. The social security system is mainly public and is financed through taxes. The standard of living is above average for Turkey. In this region, 8.2 % of the *M. tuberculosis* strains isolated between 1999 and 2001 were found to be resistant to rifampin. During the

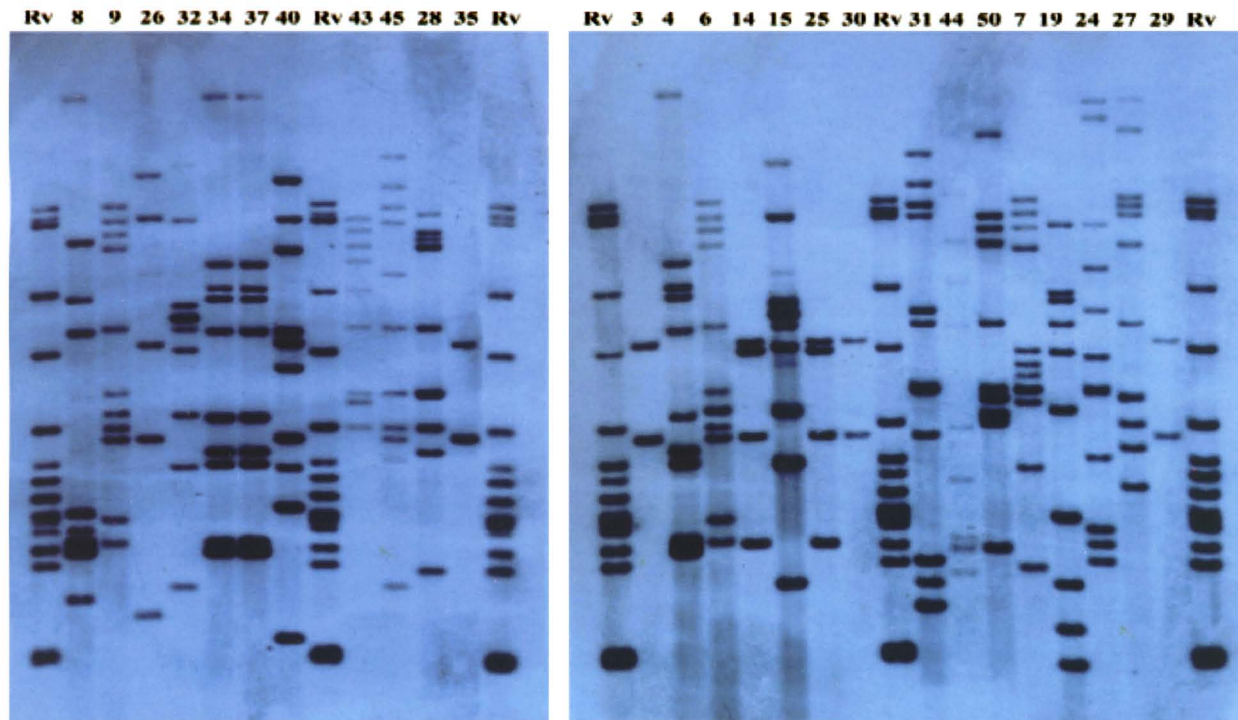


Figure 1. IS6110-RFLP patterns of 26 rifampin-resistant *Mycobacterium tuberculosis* isolates. Isolates 4, 34, and 37; isolates 6 and 9; isolates 14 and 25 share the same banding patterns. They also have the same rifampin-resistance genotype. Isolates 15 and 32 have the same banding pattern but their rifampin genotypes are different. Rv: *Mycobacterium tuberculosis* H37Rv strain

Table 1. The IS6110-RFLP patterns and genotypic and phenotypic characteristics of RIF-resistant *M. tuberculosis* isolates from Aegean region

IS6110-RFLP patterns	Isolate no	IS6110 copy number	Resistance genotype	Resistance phenotype
1	4	10	526 CAC CGC	RIF/INH/STR
	34	10	526 CAC CGC	RIF/INH/STR/EMB
	37	10	526 CAC CGC	RIF/INH/STR/EMB
2	6	11	531 TCG TTG	RIF/INH/STR
	9	11	531 TCG TTG	RIF/INH/STR/EMB
3	14*	4	531 TCG TTG	RIF/INH/STR
	25*	4	531 TCG TTG	RIF/INH/STR
4	15	9	526 CAC GAC	RIF/INH/STR
	32	9	531 TCG TGG	RIF/INH/STR/EMB
5	3*	2	531 TCG TTG	RIF/INH/STR
	29*	2	531 TCG TTG	RIF/INH
	30*	2	522 TCG TGG	RIF/INH/STR
	35*	2	531 TCG TTG	RIF
6	26*	5	526 CAC TAC	RIF/INH/STR/EMB
7	8	9	531 TCG TGG	RIF/EMB
8	28	9	533 CTG CCG	RIF
9	43	9	516 GAC TAC	RIF/INH/STR/EMB
10	44	9	531 TCG TTG	RIF/INH/EMB
11	19	10	513 CAA CCA	RIF/INH
12	40	10	531 TCG TTG	RIF/INH/STR/EMB
13	50	10	531 TCG TTG	RIF/INH/STR
14	7	11	531 TCG TTG	RIF/INH
15	24	11	531 TCG TTG	RIF/INH
16	27	11	526 CAC TGC	RIF/INH
17	31	11	531 TCG TTG	RIF/INH/STR
18	45	11	516 GAC GTC	RIF/INH/STR/EMB

RIF: rifampin, INH: isoniazid, STR: streptomycin, EMB: ethambutol

* Isolates with a copy number fewer than six were also typed by pTBN12-RFLP

same period, the incidence of resistance to both rifampin and isoniazid was 6.8%.¹¹ Although the rate of multiple drug resistance is high, there is no published data based on molecular methods concerning the transmission rate of drug-resistant strains in this region.

In the present study, seven of the rifampin-resistant *M. tuberculosis* isolates (4, 34 and 37; 6 and 9; 14 and 25) that had identical banding patterns also had the same rifampin-resistance genotypes described in our previous report⁷ (Table 1). However two isolates (15 and 32) with the same IS6110-RFLP pattern had different rifampin-resistance genotypes. Isolates 4, 34, and 37 had a mutation CAC to CGC at codon 526. The patient data could not be obtained

for isolate 4. However, it was determined that isolates 34 and 37 were obtained from patients living in the same town. Although isolates 6 and 9 were obtained from patients living in different areas, they had the same rifampin-resistance genotype (TCG to TTG at codon 531). Isolates 15 and 32 had the same banding pattern, but their resistance genotypes were different, and therefore, they may have had a low probability of being directly linked epidemiologically and due to recent transmission. It is more probable that the patients who shared the same fingerprinting pattern without a direct epidemiological link acquired their infection from the previous source case(s) that was (were) not included in the current study. The clustering in these patients may have

resulted from ongoing rather than recent transmission.

When both typing methods were evaluated together the level of clustering was 34.6% and the average number of isolates per pattern was 1.23 (26/21). A relatively low level of DNA polymorphism and high level of clustering were also described in other studies in regions with a high level of TB transmission between patients.¹²⁻¹⁴

In conclusion, although a limited numbers of isolates were evaluated in this study, there were high levels of clustering in drug-resistant *M. tuberculosis* strains in our region. Therefore, we demonstrated that active transmission of drug-resistant

M. tuberculosis strains may be common among patients in our region and urgent measures should be taken to prevent the spread of resistant strains. Molecular typing of *M. tuberculosis* strains may add information about the epidemiological situation and may also be used in surveying pandemics of TB. However, molecular typing results should always be evaluated with conventional epidemiological data.

Acknowledgement

This work is partially supported by the Ege University Medical Science Program (grant 2000/TIP/001).

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