Mutations of *rpoB* associated with rifampin resistance were studied in 37 multidrug-resistant (MDR) clinical strains of *Mycobacterium tuberculosis* isolated in Italy. At least one mutated codon was found in each MDR strain. It was always a single-base substitution leading to an amino acid change. Nine different *rpoB* alleles, three of which had not been reported before, were found. The relative frequencies of specific mutations in this sample were different from those previously reported from different geographical areas, since 22 strains (59.5%) carried the mutated codon TTG in position 531 (Ser → Leu) and 11 (29.7%) had GAC in position 526 (His → Asp).

The emergence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* poses a serious problem in tuberculosis control and stresses the need for the development of rapid and reliable diagnostic methods for drug susceptibility testing in clinical isolates. Recent advances in the understanding of the genetic basis of drug resistance have allowed for the development of DNA-based methods for the detection of resistance to antituberculosis drugs (for a review, see Musser [10]). These methods can be used, in conjunction with molecular typing (18), to elucidate the molecular epidemiology of drug resistance in *M. tuberculosis*. Epidemiological data are essential for the development of diagnostic strategies and, by providing information on the geographical distribution of resistant alleles, can help us understand whether mutated alleles arise independently or are attributable, in certain instances, to the spread of a genotype (6, 15). Mutations in *rpoB*, the gene coding for the β subunit of the RNA polymerase, are responsible for resistance to rifampin (RFM) (10), a fundamental drug for the therapy of tuberculosis (1). In this work we analyzed the mutations occurring in the *rpoB* gene of MDR strains of *M. tuberculosis* isolated in Italy.

**RFM-resistant strains.** MDR isolates of *M. tuberculosis* from hospitals in northern and central Italy were collected from three clinical microbiology laboratories (Ospedale Umberto I, Ancona; Ospedale di Careggi, Florence; and Ospedale Forlanini, Rome). The 37 MDR strains that formed the object of this investigation (Table 1) were from different patients and were typed by DNA fingerprinting (IS6110) (17) in order to avoid the inclusion of identical strains responsible for outbreaks (data not shown). The MICs for RFM and rifabutin (RFB) were determined in 7H11 agar (4). Strains were considered resistant to RFM when MICs were >1 μg/ml and resistant to RFB when MICs were >0.5 μg/ml. All MDR strains were resistant to both RFM and RFB.

**Sequencing of *rpoB*.** To investigate the mutations associated to RFM resistance, a segment of the *rpoB* gene was sequenced. Genomic DNA from heat-inactivated suspensions of *M. tuberculosis* cells was obtained with the QIAamp Tissue kit (Qiagen). A 318-bp fragment of *rpoB*, from nucleotide 1807 to nucleotide 2124 (GenBank accession no. U12205), was amplified by PCR and sequenced by a nonradioactive manual method (Silver Sequence DNA sequencing system; Promega). The same primers (5'-CGA TCA CAC CGC AGA CGT TG-3' and 5'-GGT ACG GCG TTT CGA TGA AC-3') were used for PCR and DNA sequencing. Both DNA strands were sequenced, each using as a template the product of a different PCR. At least one mutated codon was found in each RFM-resistant strain; it was always a single-base substitution leading to an amino acid change. Nine different *rpoB* alleles were found, five with one mutation (33 strains [89.1%]), three with three mutations (3 strains [8.1%]), and one with two mutations (1 strain [2.7%]) (Table 2). The mutated allele carrying the
TTG codon in position 531 was the most common (56.7%), together with the one carrying GAC in position 526 (24.3%). Three new alleles, those with three mutated codons, were recognized in this investigation (Table 2). Mutations already reported in the literature were found in codons 511, 512, 516, 526, and 531. In two strains we also found four new mutations: in codons 523 (TGG) and 525 (ATC) in one strain (GenBank accession no. AF055891) and in codons 541 (GAT) and 553 (CGC) in the other (GenBank accession no. AF055892). Twenty-two strains (59.5%) carried the mutated codon TTG in position 531 (Ser → Leu), and 11 strains (29.7%) had GAC in position 526 (His → Asp). A total of 34 of 37 strains (91.9%) had mutations in position 531 and/or position 526.

In this study on MDR strains of *M. tuberculosis* from north-
eral and central Italy, RFM resistance was always found associated with RFB resistance, and in each strain we could detect the presence of a mutated rpoB allele. By comparing our data with the literature (Fig. 1), we observed that the two mutations occurring most frequently in Italian strains (531TTG and 526GAC) have different relative frequencies in strains from different countries. While 531TTG is absolutely the most common mutation worldwide, 526GAC appears to be prevalent in different countries. Among the possible explanations, transmission of MDR resistant alleles in M. tuberculosis cannot be completely ruled out, even if, due to the current understanding of mycobacterial genetics, they would be more difficult to postulate.

**Nucleotide sequence accession numbers.** The new alleles found in this study have been deposited in GenBank under accession no. AF055891, AF055892, and AF055893.

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