MICROBIAL DRUG RESISTANCE
Volume 11, Number 2, 2005

Molecular Analysis of Isoniazid and Rifampin Resistance in *Mycobacterium tuberculosis* Isolates Recovered from Barcelona

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ABSTRACT

We studied the presence of mutations in the whole *katG* gene and specific regions of the *oxyR-ahpC* and *mabA-inhA* regulatory region in 61 *Mycobacterium tuberculosis* isoniazid-resistant isolates. An 81-bp region of the *rpoB* gene was also sequenced in 17 rifampin-resistant strains. Alterations in the *katG* gene were detected in 55% of the isolates. Mutation in codon 315 was the most prevalent (32%). Strains showed a high level of resistance, and most maintained a substantial catalase-peroxidase activity. Three strains with an isoniazid MIC of ≥32 μg/ml lacked catalase-peroxidase activity. Two of them had deletions in the catalytic domain of the KatG protein. One strain with deletion and three strains with mutations in the C-terminal domain showed low-level resistance and conserved the catalase-peroxidase activity. Mutations in the *mabA-inhA* regulatory region were identified in 32% of the isolates. All had low-level resistance, and the vast majority conserved catalase-peroxidase activity. Seventeen percent of the isoniazid-resistant isolates had no detectable alterations at the studied loci. Resistance to rifampin was associated with mutations in the 81-bp of the *rpoB* gene in all cases. *IS*6110 analysis indicated that recent transmission contributed substantially to the emergence of isoniazid-resistant tuberculosis in Barcelona through short transmission chains. A rapid genotypic assay, including the 315-*katG* codon and the −15 nucleotide of the *mabA-inhA* regulatory region, may cover 62% of isoniazid-resistant strains in Barcelona. In contrast, the targeting of the 81-bp region of *rpoB* would detect all our rifampin-resistant isolates.

INTRODUCTION

TUBERCULOSIS (TB) is presently the second leading cause of adult mortality due to infectious diseases and is responsible for approximately 2 million deaths a year worldwide. WHO estimates that one-third of the world's population is infected with *Mycobacterium tuberculosis*.[56] Multidrug-resistant tuberculosis (MDR-TB) is an emerging public health problem in many regions of the world, particularly in developing nations.[55] Accurate and rapid diagnosis is essential in the management of drug and MDR-TB, not only to optimize treatment but also to prevent transmission. The retrieval of antibiograms for *M. tuberculosis* is severely delayed when drug susceptibility testing is culture based. Understanding the genetic events leading to drug resistance in clinical *M. tuberculosis* isolates, as well as their prevalence in different geographic areas, makes it possible to develop rapid genetic assays for their detection.[10,32,48]

Mutations in an 81-bp "core region" of *rpoB* gene have been found in approximately 95% of rifampin (RIF)–resistant strains.[7,47] In contrast, resistance to isoniazid (INH) is associated with a variety of mutations affecting one or more genes. Isoniazid is a pro-drug that requires activation by the catalase-peroxidase KatG. The resulting active principle, still unknown, inhibits the activity of the InhA enzyme that belongs to a type II fatty acid elongation system together with KasA/KasB and MabA. Several studies have revealed that mutations in the *katG* gene are responsible for 60–70% of INH-resistant strains,[31] with the majority of mutations occurring at codon 315. Isolates that

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carry other less frequently occurring mutations in *katG* have been described,[24,29,34] but few studies have sequenced the complete *katG* gene. Mutations in the *mabA-inhA* regulatory region that exhibit both low-level INH resistance and ethionamide resistance have been found in clinical isolates.[2,46] It has been proposed that over-expression of the protein may explain isoniazid resistance. Less commonly, strains with mutations in the structural gene *inhA* have been described.[30] The role of *mabA* and *kasA* mutations in isoniazid resistance remains unclear. Mutations in the *oxyR-ahpC* region, a gene that encodes an alkyl hydroperoxidase reductase (AhpC), do not appear to play a direct role in INH resistance, although they may be important in maintaining peroxide homeostasis of the organism when KatG activity is low or absent.[14]

A priority of TB control programs is to prevent the transmission of resistant strains by early detection and appropriate treatment. Only cases of drug resistance among new cases are assumed to be due to transmission of drug-resistant strains.[31] Many outbreaks of drug-resistant strains in community settings have been described using *IS*6110–RFLP analysis, the standardized and most widely applied molecular typing method for *M. tuberculosis*.[8]

Our aim was to prospectively analyze the prevalence of molecular genetic mechanisms for *M. tuberculosis* INH and RIF resistance in the urban area of Barcelona. We also compared the genotypic analysis with phenotypic properties such as catalase-peroxidase activity or minimum inhibitory concentration (MIC) of INH. Finally, we assessed the community transmission of drug-resistant tuberculosis.

MATERIALS AND METHODS

Study setting, patients, and isolates

In a previously published project,[23] *Mycobacterium tuberculosis* isolates were collected from 1,749 consecutive tuberculosis patients attending the six tertiary hospitals of the Barcelona area over a 2-year period (from October 1995 to September 1997). Most patients (1,535; 87.8%) had no history of previous treatment. A rate of 5.7% drug resistance among new cases and 20.5% drug resistance among previously treated patients was observed. The number of INH- and RIF-resistant strains was 58 (3.8%) and 15 (1%), respectively. The same figures for INH and RIF resistance among previously treated patients were 37 (17.3%) and 21 (9.8%), respectively.[23] Isoniazid resistance among new cases was associated with foreign people. Drug resistance among previously treated patients was associated with people over 60 years old and women.[23] A sample of 61 INH-resistant strains and 17 RIF-resistant strains was available for study.

Laboratory procedures

Phenotypic drug susceptibility testing was carried out using the BACTEC 460 radiometric method (Becton Dickinson, Towson, MD), which contained critical concentrations of 0.1 μg/ml of isoniazid and 2 μg/ml of rifampin. In strains resistant to INH and RIF, MICs were obtained in Middlebrook 7H10 medium incubated for 3 weeks. Six dilutions of INH and RIF were prepared to give final concentrations of 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 μg/ml in the plate. The catalase and peroxidase activities were semiquantitatively determined with the Bogen reactive following a standard protocol.[5]

Epidemiological characterization

In order to rule out outbreaks that could influence the relative importance of drug resistance mechanisms, isolates were typed by a standard *IS*6110-based restriction fragment length polymorphism (RFLP) method.[51] Banding patterns were analyzed using BioImage whole-band analysis software (Genomic Solutions, Inc., Ann Arbor, MI). Banding patterns were compared by the Dice coefficient to calculate similarities. The unweighted pair group method using arithmetic averages was used for clustering. Patients were grouped in the same cluster if their isolates were identical with respect to the number and size of hybridizing bands when six or more bands are present.[22]

Detection of mutation

The isolates were cultured on Lowenstein-Jensen slants. Colonies were scraped, resuspended in 500 μl of TE (10 mM Tris, 1 mM EDTA [pH 8]), and killed by freezing at −70°C followed by heating at 80°C for 20 min. The DNA was extracted using a previously described protocol.[52] The entire *katG* gene (six fragments; 2405 bp), specific regions of the *oxyR-ahpC* located upstream of the gene (105 bp) and the *mabA-inhA* regulatory region (248 bp) related to isoniazid resistance, and the region of the *rpoB* gene (165 bp) involved in rifampin resistance were PCR amplified. The primers used for *rpoB* gene were designed with the program Primer Express (Applied Biosystems) and the primers for other target regions were derived from published sequences[44,46] (Table 1). DNA sequencing was performed with the *fmol* DNA Cycle Sequencing System (Promega Corporation, Madison, WI) with ALF *Express* II (Amersham Pharmacia Biotech).

RESULTS

Genotypic analysis of M. tuberculosis *isolates*

Of the 61 INH-resistant strains, 46 (75%) had a unique *IS*6110 RFLP pattern and 15 (25%) matched at least one other isolate belonging to one of seven clusters of drug-resistant isolates. All clusters contained RFLP patterns (A–G) with more than six bands (Table 2). Twelve out of 15 clustered patients were in six clusters of two, and the others were in a cluster of three.

Conventional contact tracing showed that the two patients with isolates of pattern A had been living together. All other patients, whose isolates had shared patterns, had no discernible connection with each other.

Isolates belonging to one of seven clusters had different resistance patterns. Thus, pattern B was seen in two isolates, one resistant to INH (*katG* and *inhA* wild-type) and the other resistant to INH and RIF (*katG* and *inhA* wild-type and *rpoB* S531L). A representative of each cluster was chosen for the epidemiological analysis of resistance mechanisms. In the case of cluster with several resistance phenotypes, the most resistant strain was selected.

TABLE 1. PRIMERS USED FOR AMPLIFICATION OF DNA SEGMENTS FROM *rpoB*, *katG*, *mabA-inhA*, AND *oxyR-ahpC*

Drug	*Gene*	*Length of amplicon*	*Sequence 5′–3′*
Rifampin	*rpoB*	165	5′-accgcagacgttgatcaacat-3′
			5′-ggcacgctcacgtgacag-3′
Isoniazid	*katG*	337	5′-gtgcccgagcaacacccacc-3′
			5′-taggtgccggcagcgtgcca-3′
		422	5′-tggcacgctgccggcaccta-3′
			5′-aatgtcgaccgccgcggcca-3′
		418	5′-tggccgcggcggtcgacatt-3′
			5′-ggtcagtggccagcatcgtc-3′
		406	5′-ccgacgatgctggccactga-3′
			5′-tcggggtcgttgacctccca-3′
		408	5′-tgggaggtcaacgaccccga-3′
			5′-ccagcgctaagcgcttgtag-3′
		414	5′-ctacaagcgcttaccgctgg-3′
			5′-caaatcgcgccgggcaaaca-3′
	mabA-inhA	248	5′-cctcgctgcccagaaaggga-3′
			5′-atcccccggtttcctccggt-3′
	oxyR-ahpC	105	5′-gcttgatgtccgagagcatcg-3′
			5′-ggtcgcgtaggcagtgcccc-3′-

Analysis of the rpoB *gene responsible for the RIF resistance phenotype*

Mutations in the core region of *rpoB* gene were detected for all 15 RIF-resistant strains. Eight different *rpoB* mutations were identified involving codons 512, 516, 526, and 531. The most common mutation, which changes serine to leucine (position 531), was detected in seven of the 15 RIF-resistant isolates (Table 3).

Analysis of katG, inhA, *and* oxyR-ahpC *genes involved in the isoniazid resistance phenotype*

As shown in Table 4, 29 of 53 INH-resistant strains (55%) had *katG* alterations consisting of small deletions (3–15 nucleotides; $n = 3$) or nucleotide substitutions ($n = 26$), resulting in either amino acid replacement ($n = 25$) or stop mutation ($n = 1$). All but two strains had a single alteration in the *katG* gene. The genetic alterations were detected all along the gene: codons 94, 152, 172, 189, 204, 234, 315, 463, 560, 592, 640, 678, and 728. However, the most frequent mutation occurred at codon 315 (17 of 53; 32%), where the most common substitution was Ser → Thr (15 of 53; 28%). The second most frequently affected codon was the 463 with an Arg → Leu substitution (3 of 53; 6%).

mabA-inhA regulatory region analysis showed a nucleotide substitution for 17 of the 53 INH-resistant strains (32%) with the following changes: C → T substitution involving nucleotide at the position 15 upstream of the *mabA* initiation codon (–15; 14 of 53; 26%); T → C involving nucleotide –8 (two of 53; 4%) and G → T involving nucleotide –17 (one of 53; 2%).

In the intergenic region *oxyR-ahpC*, 7 (13.2%) of those 53 INH-resistant strains had simple nucleotide substitutions (positions −6, −12, −39), deletions (position –39) or insertions (one or two nucleotides; positions (–38/–39 and –45/–46; designated relative to *ahpC* mRNA start site).

TABLE 2. GENOTYPIC DRUG RESISTANCE ANALYSIS OF 15 CLUSTERED INH-RESISTANT ISOLATES

RFLP pattern (no. of bands)	*No. of isolates*	*INH*		*RIF*	
		MIC (μg/ml)	katG *mutation*	*MIC, μg/ml*	rpoB *mutation*
A (11)	2	8	S315T[a]	<0.06	—
B (13)	1	0.25	—[c]	<0.06	—
	1	0.25	—	32	S531L[a]
C (13)	2	8	S315T	<0.06	—
D (14)[b]	3	0.25	—	<0.06	—
E (10)	2	8	S315T	32	S531L
F (13)	2	8	S315T	4	A516V
G (11)	2	8	S315T	<0.06	—

[a]Codon number affected and amino acid substitution.
[b]-15T nucleotide substitution of the *mabA-inhA* regulatory region.
[c]—, Wild-type. All strains, including in the clusters, displayed the *oxyR-ahpC* region wild type.

TABLE 3. MIC TO RIFAMPIN AND MUTATIONS IN *rpoB* OF CLINICAL *M. tuberculosis* ISOLATES

Strain	MIC (μg/ml)	*rpoB* gene		
		Codon	Amino acid changes	
059/R	1	512	AGC → ACC	Ser → Thr
019/R	1	516	GAC → TTC	Asp → Phe
005/R	4	516	GAC → TAC	Asp → Tyr
063/R	4	516	GAC → GTC	Asp → Val
028/R	16	526	CAC → GAC	His → Asp
047/R	32	526	CAC → TAC	His → Tyr
003/R	32	526	CAC → GAC	His → Asp
018/R	ND	526	CAC → TAC	His → Tyr
016/R	8	531	TCG → TTG	Ser → Leu
007/R	16	531	TCG → TGG	Ser → Trp
052/R	ND	531	TCG → TTG	Ser → Leu
044/R	32	531	TCG → TTG	Ser → Leu
039/R	32	531	TCG → TTG	Ser → Leu
048/R	32	531	TCG → TTG	Ser → Leu
017/R	32	531	TCG → TTG	Ser → Leu

ND, not done.

INH MIC and catalase-peroxidase activity correlation with genotype

MICs of isoniazid were ≤1 μg/ml in 25 strains (47%), 2–16 μg/ml in 18 (34%), and ≥32 μg/ml in three (6%). MICs of the 17 strains with mutation in the regulatory region of *inh*A were 0.25–2 μg/ml, 71% of them having a MIC of 0.25–0.5 μg/ml. MICs of the strains with mutation at codon 315 of *kat*G gene was 4–16 μg/ml. Two of the three strains with MIC of ≥32 μg/ml presented a deletion in the *katG* gene, and all strains lacked catalase and peroxidase activity. The presence of catalase activity was detected in 71% of the strains with mutation at codon 315 of *katG*, and the peroxidase was detected in 35% of these strains. The MICs of the strains with mutations in the *katG* gene, but not in codon 315, was 0.125–2 μg/ml. Catalase activity was detected in 71% of these strains, whereas peroxidase activity was detected in 42.8%. Ninety percent of the strains with MIC of ≤1 μg/ml conserved the catalase and peroxidase activities.

DISCUSSION

In this study, we report the presence of mutations in the core region of *rpoB* gene in all the *M. tuberculosis* strains that exhibited RIF resistance. The most frequent mutation (41%) occurred at codon position 531. Although frequencies of particular mutations in RIF-resistant *M. tuberculosis* isolates may differ in different geographic areas,[4] generally the most frequent codon involved is also 531.[15,37,50]

In contrast, resistance to INH was associated with a variety of mutations. The *katG* gene, which encodes catalase-peroxidase, was the most commonly altered gene, with the majority of mutations occurring at codon 315 (32% of INH-resistant strains). The absence of additional mutations within or outside of the *katG* gene in fifteen of seventeen codon 315 mutated INH-resistant strains confirms that codon 315 plays an important role in the development of INH resistance. This is in agreement with previous studies.[8,38,54] The apparently conservative substitution of serine by threonine is of a maximum benefit to *M. tuberculosis*, as it reduces the activation of INH while maintaining a substantial catalase-peroxidase activity.[25,28,30,38] Indeed, in our series, 71% and 35% of INH-resistant strains with a mutated codon 315 showed some catalase and peroxidase activity, respectively. The catalase-peroxidase negative strains probably reflect the inaccuracy of the routine assays, which is nevertheless useful in clinical laboratories.[6] Like many other authors,[17,25,36,40,43,53] we found a high level of INH resistance (4–16 μg/ml) in the *katG* 315 mutated strains, although one study in the literature reported contrasting figures.[41] On the other hand, it has been reported that isolates with deletions in *katG* gene lost their catalase-peroxidase activities, and the MICs for these isolates are >32 μg/ml.[11,30] In our study, three strains with deletions in the *katG* gene were found. Two of these had deletions affecting the catalytic domain of the protein showed MIC of ≥32 μg/ml and lacked catalase-peroxidase activities. The third strain had a low-level resistance (0.25 μg/ml), conserved its catalase activity, and had a deletion of 15 bp (codons Δ640–644) in the C-terminal domain. Mutations affecting the C-terminal part are either associated with low-level resistance or appear to be without effect.[12,13,36] Moreover, strains 27R, 9R, and 36R with low-level resistance and conserved catalase-peroxidase activity had mutations in this C-terminal domain. We failed to detect a complete *katG* deletion in any of these 53 resistant isolates.

The second most frequently affected codon in the *katG* gene was 463 (5.6%), always in conjunction with additional mutations outside this codon. Unlike mutations at codon 315, mutations at codon 463 have also been detected in isoniazid-susceptible isolates,[9,26,29] which suggests that it is a rather common polymorphism, independent of INH resistance.

inhA operon consists of two genes, *mabA* and *inhA*, both of which encode enzymes involved in mycolic acid biosynthesis.[2,3] Seventeen isolates in our series had mutations in the *mabA-inhA* regulatory region; 14 of these strains (82%) had no other detectable molecular alterations at *katG* gene. All the

TABLE 4. PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF ISONIAZID-RESISTANT STRAINS

Strain	Results of phenotypic analysis for: MIC (μg/ml)	Activity: Catalase	Activity: Peroxidase	Results of genotypic analysis for: katG	inhA	ahpC-oxyR
058/R	0.125	2	+++	—	—	—
027/R	0.125	3	+	592 Asp → Glu	—	—
030/R	0.25	3	++	—	—	—
032/R, 041/R	0.25	3	+++	—	—	—
039/R	0.25	1	+++	—	—	—
040/R	0.25	1	0	94 Asp → Asn	—	—
046/R	0.25	0	+++	463 Art → Leu	–15 C → T	—
054/R	0.25	2	0	640 Del	—	—
009/R	0.25	2	++	728 Trp → Tyr	—	–12G → A
067/R	0.25	0	++	—	–15 C → T	—
052/R	0.25	3	++	—	–15 C → T	—
061/R	0.25	4	+++	—	–15 C → T	—
042/R	0.25	2	+++	—	–8 T → C	—
034/R	0.25	3	+++	—	–8 T → C	—
029/R	0.5	1	+++	—	—	—
033/R	0.5	3	+++	—	—	—
050/R	0.5	0	+++	—	—	—
028/R	0.5	0	0	172 Ala → Thr	—	–6G → C
047/R	0.5	2	+++	—	–15 C → T	—
001/R	0.5	3	+++	—	–15 C → T	—
014/R	0.5	3	+	—	–15 C → T	—
013/R	0.5	4	+	—	–15 C → T	—
011/R	0.5	4	+	—	–15 C → T	–38/–39 ins
026/R	0.5	4	+	—	–17 G → T	—
059/R	1	3	++	—	—	—
036/R	1	2	+	678 Tyr → Cys	–15 C → T	—
003/R, 053/R	1	3	++	—	–15 C → T	—
060/R	1	2	++	—	–15 C → T	—
017/R	2	0	0	560 Gly → Ala	—	—
007/R	2	1	++	204 Trp → stop	—	—
057/R	2	0	++	189 Asp → His	–15 C → T	—
004/R	4	4	0	315 Ser → Thr	—	—
016/R	8	2	0	315 Ser → Arg	—	—
008/R	8	2	0	315 Ser → Asn	—	—
035/R	8	0	+	315 Ser → Thr	—	—
063/R	8	1	+	315 Ser → Thr	—	—
015/R	8	2	0	315 Ser → Thr	—	—
044/R	8	2	++	315 Ser → Thr	—	—
062/R	8	2	0	315 Ser → Thr	—	—
018/R, 045/R, 066/R, 023/R	8	3	0	315 Ser → Thr	—	—
048/R	8	3	+	315 Ser → Thr	—	—
002/R	8	4	+++	315 Ser → Thr	—	—
019/R	8	0	0	315 Ser → Thr	—	–39 Del
012/R	8	3	+	315 Ser → Thr	—	–45/–46 ins
				463 Arg → Leu	—	—
				234 Ala → Gly	—	—
065/R	16	0	0	315 Ser → Thr	—	—
				463 Arg → Leu	—	—
005/R	>32	0	0	234 Del	—	—
				155–158 Del	—	—
022/R	>32	0	0	152 Del	—	–39 A → G
024/R	>32	0	0	—	—	–12 G → A

strains with mutations in the *mabA-inhA* regulatory region had low-level resistance (MIC 0.2–1 μg/ml). The most frequent substitution involved nucleotide –15 upstream of *mabA* (82.3%). Fifteen of the 17 strains (88.2%) conserved catalase activity, and all of them had peroxidase activity. To our knowledge, no mutations have been mapped to the MabA ORF in INH-resistant organisms. In contrast, mutations have been mapped to the *mabA* RBS with high frequency and also in the InhA ORF with

lower, but significant, frequency.[2,33,35] Mutations in the *mabA-inhA* regulatory region result in the overexpression of the InhA protein through transcriptional upregulation,[29] and are associated with both INH and ETH resistance.[19,21] The data presented here for *katG* and *inhA* loci therefore emphasize the fact that a mutation alone is sufficient to confer the resistance phenotype.

The promotor region *oxyR-ahpC* takes part in the cellular answer to oxidative stress.[39] In our study, genetic alterations (mutations, deletions, or insertions) in the promoter region *oxyR-ahpC* were identified in 13.2% of INH-resistant strains, as described similarly in previously published reports.[16,29,39,45] Conversely, taken together our data clearly demonstrate that most strains with *oxyR-ahpC* region mutations have alterations at other loci (*katG* or *inhA*), which may readily explain the INH resistance phenotype.[16,39]

Our results showed, however, that 17% of the INH-resistant isolates had no detectable alterations at the studied loci. The fact that we sequenced the whole *katG* gene suggests that some target regions are still undefined and that other molecular mechanisms may play some role. A similar result was found by Silva et al. in Brazil.[40] The genetic mechanisms of INH resistance are highly complex and affect several genes that are involved in mycolic acid biosynthesis or are overexpressed as a response to the buildup or cellular toxicity of INH.[20,30] At least 18 alternative genes have been implied in the mechanism of resistance to isoniazid. Among these there are the *ndh* (encoding NADH dehydrogenase) *fadE23, fadE24* (involved in fatty acid β-oxidation), *Rv1592c*, *Rv1772* (of unknown function but transcriptionally induced by INH), and *iniBAC* region (*Rv0340* genes *iniA*, *iniB*, and *iniC*) induced by both INH and ethambutol.

There are geographic variations in the molecular mechanism responsible for INH resistance. S315T mutation in the *katG* gene has been detected in 91.7% of INH-resistant strains in Russia,[24] 87% in Brazil,[40] 83.9% in Lithuania,[1] 62.2% in New York,[27] 59.2% in Korea,[18] 34.6% in Madrid,[27] 32% in the present study in Barcelona, and none of the Equatorial Guinea strains.[49] Therefore, the mapping of the frequency and types of mutation in a country or a geographic region is not generally applicable.

RFLP analysis indicates that transmission of INH-resistant strains contributes to the emergence of INH-resistant tuberculosis in Barcelona through short transmission chains. We were unable to identify links among many of the patients in RFLP-defined clusters, probably due to the difficulty of reconstructing the epidemiological links in locations involving casual contact outside the home.[42] The absence of major outbreaks suggests that the TB control program has been sufficiently effective. This finding is consistent with the fact that the incidence of primary resistance to INH in Barcelona is low.[23]

With regard to the correlation between the molecular mechanism of INH resistance and transmission (Table 2), 10 of 15 isolates belonging to five clusters had a mutation at codon 315 of the *katG* gene. Substitution involving nucleotide –15 upstream of the *mabA-inhA* regulatory region was found in three isolates belonging to one cluster. Finally, two strains belonging to one cluster had no detectable alterations at the studied loci.

In summary, the screening of codon 315 of *katG* gene and the *mabA-inhA* upstream region should be included in a rapid genotypic assay for direct detection of INH resistance. This target approach would cover 62% of an INH-resistant sample in Barcelona. In contrast, if a 81-bp region of *rpoB* is selectively targeted, all the RIF-resistant isolates of our series would be rapidly detected. Such a PCR-based method would facilitate the rapid detection and drug susceptibility testing of *M. tuberculosis*, which is necessary for the effective control and prevention of tuberculosis and MDR-TB. In Barcelona, this would be especially suitable to study the drug resistance among immigrants new cases and among previously treated patients.

ACKNOWLEDGMENT

This study was funded by Fondo de Investigación Sanitaria de la Seguridad Social (proyecto: 95/1957).

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