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Systematic Molecular Characterization of Multidrug-Resistant *Mycobacterium tuberculosis* Complex Isolates from Spain

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We used spoligotyping and restriction fragment length polymorphism (RFLP) of the IS*6110*-insertion sequence to study the molecular epidemiology of multidrug-resistant (MDR) tuberculosis in Spain. We analyzed 180 *Mycobacterium tuberculosis* complex isolates collected between January 1998 and December 2000. Consecutive isolates from the same patients ($n = 23$) always had identical genotypes, meaning that no cases of reinfection occurred. A total of 105 isolates (58.3%) had unique RFLP patterns, whereas 75 isolates (41.7%) were in 20 different RFLP clusters. Characterization of the *katG* and *rpoB* genes showed that 14 strains included in the RFLP clusters did not actually cluster. Only 33.8% of the strains isolated were suggestive of MDR transmission, a frequency lower than that for susceptible strains in Spain (46.6%). We found that the Beijing/W genotype, which is prevalent worldwide, was significantly associated with immigrants. The 22 isolates in the largest cluster corresponded to the *Mycobacterium bovis* strain responsible for two nosocomial MDR outbreaks in Spain.

Tuberculosis (TB) remains one of the infectious diseases responsible for the most adult deaths in the world because of the lack of access to effective and rationally delivered therapy for drug-susceptible TB. Spain has one of the highest incidences of TB in Western Europe. The incidences of pulmonary TB were 22.67, 21.05, and 19.64 per 100,000 inhabitants, respectively, in 1998, 1999, and 2000 (29). Standard restriction fragment length polymorphism (RFLP) analysis (30) revealed that 39% of isolates collected in Zaragoza (where the incidence of TB is similar to that in the whole of Spain) in 1993 were clustered (30), suggesting recent transmission. This was recently confirmed by van Deutekom et al. (37). The percentage of clustered isolates increased to 46.6% when the study period was extended to 3 years (13). Multidrug-resistant (MDR) TB is emerging as an increasingly major cause of morbidity and mortality (22). MDR TB outbreaks have been described worldwide (1, 2, 20, 24, 27). Highly resistant strains of TB have caused numerous institutional outbreaks (hospitals, prisons, and shelters), with high case-fatality rates among the immunosuppressed and high rates of transmission to immunocompetent health care workers (44). A study with guinea pigs showed that mutations or deletions within the *katG* gene decrease the pathogenicity of isoniazid (INH)-resistant strains of *Mycobacterium tuberculosis* (16). A recent study in Los Angeles County showed that the transmission of MDR TB can be limited (7.8%) by aggressive surveillance (21). A recent study in The Netherlands showed that INH-resistant isolates harboring a mutation at amino acid 315 and INH-susceptible strains are equally likely to cluster (40). The precise extent and features of drug-resistant TB in Spain are not known. Incidences of primary MDR TB in 2001 were 0.8% in Barcelona and 1.4% in Galicia (43), suggesting a low incidence of MDR TB in Spain. The transmission of a particular MDR *Mycobacterium bovis* "B" strain (28) causing nosocomial outbreaks in Spain has also been described (11, 31). This strain affected both human immunodeficiency virus (HIV)-infected and immunocompetent patients (23). This strain is also characterized by a high reinfection rate (28). The genetic characteristics of this strain have been described in detail (3).

Since 1998, the MDR *M. tuberculosis* strains isolated in Spain have been systematically typed by our group. The preliminary results for 1998 were presented in *Euro Surveillance* (32). Due to the long incubation period of TB, we believed that it was important to expand the study period to 3 years. Here, we extend these data to 31 December 2000. The main purpose of this study was to increase our knowledge of MDR TB in Spain by identifying the strains that are being transmitted.

MATERIALS AND METHODS

Bacterial isolates and the Spanish MDR TB network. The Spanish MDR TB study group includes 118 mycobacteriology laboratories located within different hospitals in Spain (http://www.eurosurveillance.org/em/v05n04/0504-223.asp?langue=02&; http://genmico.unizar.es/anglais/mdr%20group.htm). This study group includes 90% of the microbiology laboratories belonging to the Spanish National Health System, so that the isolates investigated in this study represented at least two-thirds of all Spanish MDR TB cases. In Spain, the susceptibility of isolates to anti-TB drugs is always tested when clinical signs are suggestive of resistant TB. Sixty percent of the isolates studied came from the two national mycobacterial reference laboratories of the Instituto Carlos III in Madrid, where drug susceptibility tests were performed. Nine percent of the isolates

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TABLE 1. Oligonucleotides used to prepare the RIFO assay membrane

Oligonucleotide no.	Type	Sequence (5′–3′)	Position (codon)
1	Wild	AGC CAG CTG AGC CAA TTC AT	
2	Wild	TTC ATG GAC CAG AAC AAC CCG	
3	Wild	G CTG TCG GGG TTG ACC	
4	Wild	TTG ACC CAC CCC AAG CGC CGA	
5	Wild	CTG TCG GCG GCG CTG GGG C	
6	Mutant	C ATG GTG GAC CAG AAC AAC C	516 (GTG)
7	Mutant	TTG ACC GAC AAG CGC CGA	526 (GAC)
8	Mutant	TTG ACC TAC AAG CGC CG	526 (TAC)
9	Mutant	CAC AAG CGC CAA CTG TCG	529 (CAA)
10	Mutant	CTG TTG GCG CTG GGG C	531 (TTG)
11	Mutant	CTG TGG GCG CTG GGG C	531 (TGG)

were subjected to routine susceptibility testing in the reference laboratory located in the autonomous region in which they were collected. The other 31% came directly from mycobacterial laboratories, where their susceptibilities to at least the five first-line drugs for TB treatment were tested. *M. tuberculosis* complex isolates were considered to be MDR if they were resistant to INH and rifampin. The isolates were sent to the University of Zaragoza for molecular typing. Between 1998 and 2000, 271 suspected MDR TB isolates collected in Spain were typed and included in the MDR TB database. Fifty-one of these isolates were excluded because either they were repeatedly isolated from patients in different hospitals in the same year, they turned out not to be MDR, they were laboratory contaminants, or they turned out to have been isolated before 1998. Thus, 220 MDR TB isolates were included. These isolates came from 95 patients in 1998, 65 patients in 1999, and 60 patients in 2000.

RFLP analysis could not be performed for 14 isolates because no DNA was available. Finally, 206 MDR isolates from 180 different patients were studied. More than one isolate was collected from 23 patients in different years. These isolates were typed in order to detect cases of exogenous reinfection in these chronically infected patients. In three cases there was not enough DNA for RFLP, but spoligotyping was considered enough to exclude the possibility of reinfection. Only one strain from each patient was included in the epidemiological study.

The data for the isolates that clustered according to the RFLP method were sent to the Spanish National Epidemiology Center in order to establish a surveillance system for MDR TB. Reports including the pattern of the strain were sent to the laboratory in which the strain was originally identified.

Typing methods. RFLP analysis using Southern blot transfer and DNA hybridization with IS*6110* was performed according to the standard fingerprinting method (39). *M. tuberculosis* strain 14323 was used as an external standard. The direct repeat (DR) region was analyzed by spoligotyping as described by Kamerbeek et al. (14). Spoligotyping is quicker and easier to perform than RFLP but less discriminatory (15).

Analysis of the *katG* gene polymorphism. Point mutations in codons 315 and 463 of the catalase-peroxidase gene (*katG*) were detected as described by Uhl et al. (36). Briefly, a 620-bp portion of the *katG* gene was PCR amplified, and the resulting product was digested with MspI (Boehringer Mannheim). After separation in a 4% agarose gel, the fragments were visualized by ethidium bromide staining and exposure to UV light. Mutations in codons 315 and/or 463 resulted in fragments of different sizes. MspI digestion resulted in four different patterns (see Fig. 3).

Analysis of the *rpoB* gene. Point mutations within an 81-bp fragment of the *rpoB* gene conferring rifampin resistance were detected as described previously (18). Details of this assay can be obtained upon request from the National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands. This rifampin oligonucleotide assay (rifotyping) method is briefly detailed as follows: a 156-bp region of the *rpoB* gene including the 81-bp mutation "hot spot region" was amplified by using primers TR10a (5′-CGC CGC GAT CAA GGA GT-3′) and TR11a (biotin-5′-ACG TCG CGG ACC TCC A-3′). The amplified product was hybridized to a set of 11 oligonucleotides, 5 of which corresponded to the wild-type sequence, and 6 of which corresponded to the mutant sequences, of the *rpoB* gene covalently bound to a membrane (Table 1). The hybridization product was visualized by enhanced chemiluminescence (ECL detection liquid; Amersham, Little Chalfont, Buckinghamshire, United Kingdom) and by using the membrane to expose light-sensitive film (Hyperfilm ECL; Amersham). Mutations in the hot spot region always prevent hybridization to one of the five wild-type oligonucleotides and usually cause hybridization to one of the six mutant oligonucleotides.

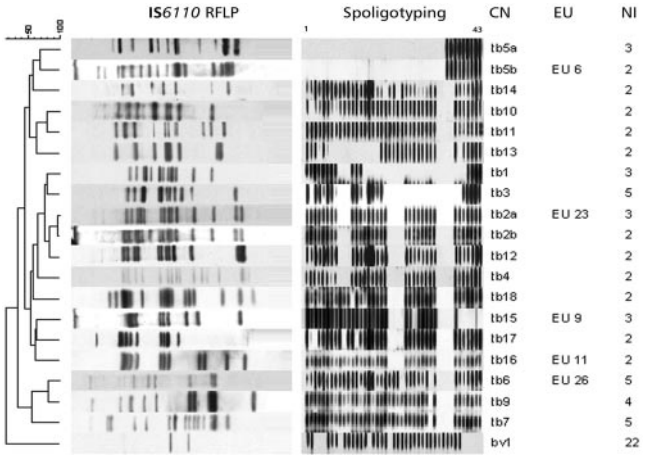

FIG. 1. Dendrogram based on the 20 different IS*6110*-RFLP patterns of the clustered *M. tuberculosis* complex isolates. CN, cluster name assigned by the University of Zaragoza; EU, name of the cluster in the European Database in the RIVM; NI, number of isolates in each RFLP cluster.

Patient characteristics. Data on the patients from whom MDR TB isolates were collected were recorded on a standardized form. The data collected included age, gender, date of isolate recovery, results of the susceptibility test, previous treatment for TB, major risk factors for TB, and demographic and clinical characteristics. It was not always possible to distinguish between primary and acquired drug resistance, because the date of previous anti-TB treatment was not always known.

Computer analysis. The IS*6110* fingerprints, spoligopatterns, and *rpoB* types of the *M. tuberculosis* isolates were analyzed by the Bionumerics program (version 3.0; Applied Maths, Kortrijk, Belgium). The results for codon 315 of the *katG* gene were also introduced into Bionumerics with numerical values from 0 to 3. The patterns obtained by the different methods were compared, and dendrograms were constructed by use of Dice coefficients and UPGMA (unweighted pair-group method using arithmetic means) clustering. A composite experiment was carried out to study the similarity between the strains by using the averages from the experiments and UPGMA clustering.

Statistical analysis. Statistical analyses were carried out with the SPSS program for Windows (version 11.5). We compared patient and isolate variables between the group of patients with supposed recent transmission of MDR and the group with acquired MDR by using the chi-square test or Fisher's exact test (9, 30, 40). Recent transmission of MDR TB was suspected when the isolated strains had identical RFLP patterns and the same *katG* and *rpoB* codons.

RESULTS

RFLP and spoligotyping analysis. We studied 180 *M. tuberculosis* complex isolates from different patients. The IS*6110*-RFLP analysis revealed 125 different patterns: 105 isolates (58.3%) had unique patterns, and 75 were included in 20 clusters (Fig. 1). The number of copies of IS*6110* varied from 2 to 23. The largest cluster included 22 patients. The isolates from these patients corresponded to the MDR *M. bovis* "B" strain, which caused a previously described outbreak (31) and carries 2 copies of IS*6110* in its genome. Seven other *M. tuberculosis* isolates carried 5 or fewer copies of IS*6110*. All seven of these isolates presented different RFLP patterns.

Spoligotyping analysis of the DR region revealed 73 different spoligotype patterns, 53 of which were unique. Twenty-seven (15%) isolates displayed the most common pattern, including the isolates that belonged to RFLP clusters tb6, tb7,

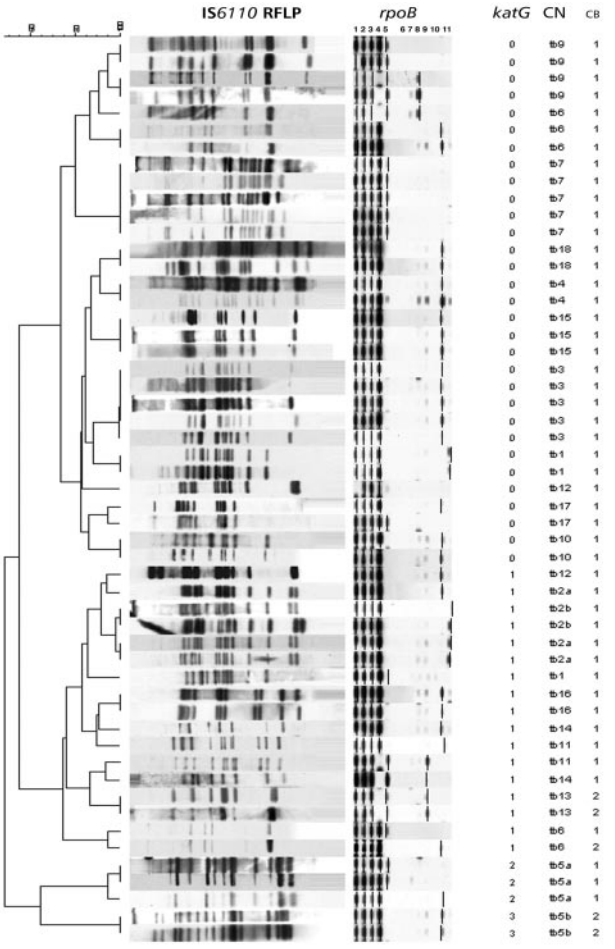

FIG. 2. Dendrogram showing the redefined clusters (excluding the B strain cluster) based on the results of the three genetic techniques: IS*6110*-RFLP, rifotyping, and PCR-RFLP of the *katG* gene. Isolates with identical IS*6110*-RFLP patterns, but different mutations conferring resistance, were transmitted before the acquisition of drug resistance. RFLP clusters tb11, tb12, tb14, and tb17 disappeared, and tb9 was divided into tb9a and tb9b. The methods are shown in the following order: RFLP-IS*6110*, *rpoB* mutations (lanes 1 through 5, wild-type sequence of the gene; lane 6, Asp516Gly mutant; lane 7, 515-to-516 insertion; lane 8, His526Tyr mutant; lane 9, Arg529Gln mutant; lane 10, Ser531Leu mutant; lane 11, Ser531Tyr mutant), and *katG* (mutations are represented by numerical values, as follows: 0, wild-type; 1, Ser315Thr; 2, Arg463Leu; 3, Ser315Thr and Arg463Leu). CN, number of the RFLP cluster. CB, country of birth (1, Spain; 2, foreign country).

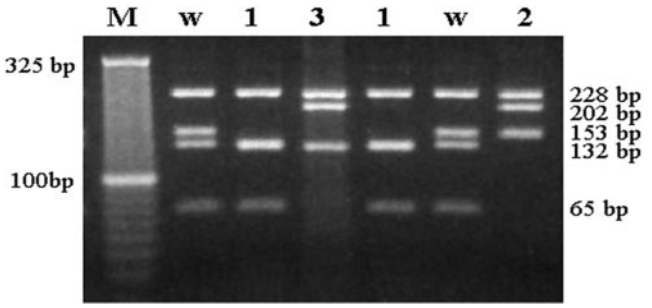

FIG. 3. MspI restriction fragments of the 620-bp portion of the *katG* gene of six MDR *M. tuberculosis* isolates. Lanes: M, 10-bp marker; w, wild type (bands at 228, 153, 137, and 65 bp); 1, codon Ser315Thr (bands at 228, 137, 132, and 65 bp); 2, codon Arg463Leu (bands at 228, 202, and 153 bp); 3, codons 315Thr and 463Leu (bands at 228, 202, and 132 bp).

and tb9 (Fig. 2). This pattern was the ST 50 pattern from the international database constructed by Sola and colleagues (8); it is one of the most common patterns in Europe and belongs to the Haarlem family. The "B" strain pattern was the second most common (12.2%). The Beijing/W family pattern, characterized by reaction with no more than the last 9 of the 43 spacers (10, 15, 41), was found in 11 isolates (including clusters tb5a and tb5b).

Study of *katG* and *rpoB* mutations. We studied mutations at codons 315 and 463 of the *katG* gene in order to improve RFLP discrimination among MDR clustered strains (Fig. 2 and 3). The Ser315Thr mutation was found in 19 of the 75 clustered isolates (25%). None of the strains in the largest clusters—bv1 (22 elements), tb7, and tb3 (both including 5 elements)—contained this mutation. A leucine instead of an arginine was present at codon 463 of the *katG* gene in 27 of the 75 isolates that belonged either to the *M. bovis* "B" strain isolates ($n = 22$) or to the Beijing/W family isolates ($n = 5$). Two isolates from the latter cluster had a double mutation (tb5b).

In the *rpoB* gene, the Ser531Leu mutation was the most frequent among the clustered strains, found in 65.3% of the isolates (49 out of 75) including the "B" strain. The His526Asp mutation was the second most frequent among these isolates ($n = 6$ [8%]). The hot spot regions of the *rpoB* genes of 12 clustered isolates (16%) did not contain any mutations according to the rifotyping method, including the 5 isolates from cluster tb7.

Cluster analysis. The MDR isolates found to be identical by RFLP-IS*6110*, spoligotyping, and *katG* and *rpoB* gene analyses were considered to belong to a redefined MDR TB cluster and were suspected of being transmitted.

The results obtained by the four different methods showed that 14 isolates found to be part of clusters by RFLP-IS*6110* did not actually cluster. Thus, four of the RFLP clusters disappeared (tb11, tb12, tb14, and tb17) and one (tb9) was divided into two clusters (tb9a and tb9b). Isolates with identical IS*6110*-RFLP patterns, but different mutations conferring resistance, were transmitted before the acquisition of drug resistance. Finally, a total of 119 isolates (66.1%) were not in a cluster. These strains were considered to be derived from independent cases of infection. The remaining 61 isolates (33.9%) belonged to 17 clusters, distributed as follows: 13 clusters of 2 isolates, 1 cluster of 3 isolates, 2 clusters of 5 isolates, and 1 cluster of 22 isolates (Fig. 2).

Study of reinfection. We studied the possibility of exogenous reinfection among the 23 MDR TB patients from whom multiple isolates were collected in different years. The RFLP-IS*6110* fingerprints of the isolates from each patient were consistently identical. No significant association was found between patients with multiple isolates and recent transmission of MDR TB ($P > 0.05$).

Patient characteristics. We studied the characteristics of the 180 patients (141 men and 39 women). No association was found between sex and supposed recent transmission ($P >$

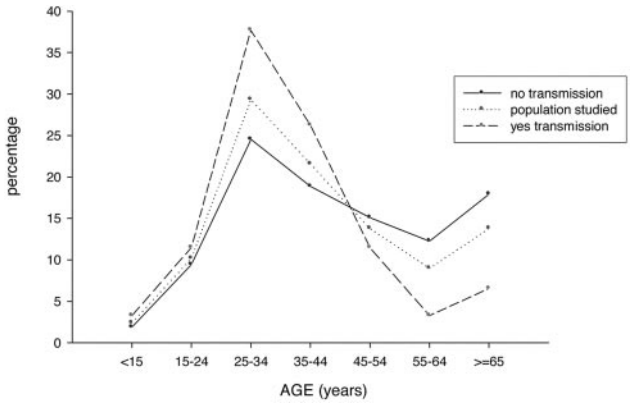

FIG. 4. Age distribution of the 167 MDR TB patients (for whom age was known) according to transmission status. We considered transmission cases to be those with the same RFLP pattern and the same mutations in the *katG* and *rpoB* genes.

0.05). MDR TB was more common in the 25- to 34-year age group (Fig. 4). Older people were less likely to be clustered than younger people; a significant association was found between transmission and youth ($P < 0.05$). Sputum smears was positive in 109 cases and were not significantly associated with transmission ($P = 0.16$). Twenty-two (12.2%) of the patients were immigrants, and 158 were native Spaniards. We found an association between being an immigrant and being infected by a Beijing/W family isolate ($P < 0.05$) (Table 2).

Epidemiological investigation of the fingerprint clusters. The largest cluster (bv1) included 22 cases caused by the "B" strain. These isolates came from 14 hospitals in six different cities. In 1998, 11 "B" strain isolates were collected, compared to 10 in 1999 and 1 in 2000. Ninety-five percent of the patients carrying isolates in this cluster had been in contact with patients infected by this strain. Eight of these patients were HIV negative (in one case, HIV status was unknown).

TABLE 2. Association between some of the characteristics of the patients in this study and suggested recent transmission of MDR TB[a]

Characteristic	No. (proportion) of patients			*P*	Test
	No transmission (n = 119)	Transmission (n = 61)	Total (n = 180)		
Sex					
Male	95 (0.798)	46 (0.754)	141 (0.783)	0.495	χ^2
Female	24 (0.202)	15 (0.246)	39 (0.217)		
Geographic origin					
Spanish-born	101 (0.848)	56 (0.918)	157 (0.872)	0.187	χ^2
Foreign-born	18 (0.152)	5 (0.082)	23 (0.128)		
Site of tuberculosis					
Pulmonary	106 (0.946)	57 (0.934)	163 (0.942)	0.495	Fisher
Extrapulmonary only	6 (0.0534)	4 (0.066)	10 (0.058)		
Sputum smear					
Positive	67 (0.656)	42 (0.763)	109 (0.694)	0.166	χ^2
Negative	35 (0.344)	13 (0.237)	48 (0.306)		
History of tuberculosis					
Yes	89 (0.816)	32 (0.571)	121 (0.733)	0.001	χ^2
No	20 (0.184)	24 (0.429)	44 (0.267)		
HIV serology					
Positive	22 (0.209)	20 (0.344)	42 (0.257)	0.059	χ^2
Negative	83 (0.791)	38 (0.656)	121 (0.743)		
Intravenous drug use					
Yes	18 (18.0)	17 (29.8)	35 (22.3)	0.087	χ^2
No	82 (82.0)	40 (70.2)	122 (77.7)		
Alcohol abuse					
Yes	14 (14.3)	9 (15.5)	23 (14.7)	0.834	χ^2
No	84 (85.7)	49 (84.5)	133 (85.3)		
History of contact with TB case					
Yes	9 (0.083)	21 (0.355)	30 (0.179)	0.000	χ^2
No	99 (0.917)	38 (0.645)	137 (0.821)		
Combination of factors					
Yes	37 (0.345)	31 (0.516)	68 (0.407)	0.031	χ^2
No	70 (0.655)	29 (0.484)	99 (0.593)		

[a] One hundred twenty-one patients had received previous treatment, and this factor was associated with an absence of recent transmission ($P = 0.001$). No accurate history of prior treatment could be obtained for 15 patients. Forty-two of the patients for whom information was available were HIV positive. HIV infection was not a risk factor associated with transmission ($P = 0.059$). However, a combination of intravenous drug use, HIV infection, and alcohol abuse was significantly associated with recent transmission ($P = 0.031$).

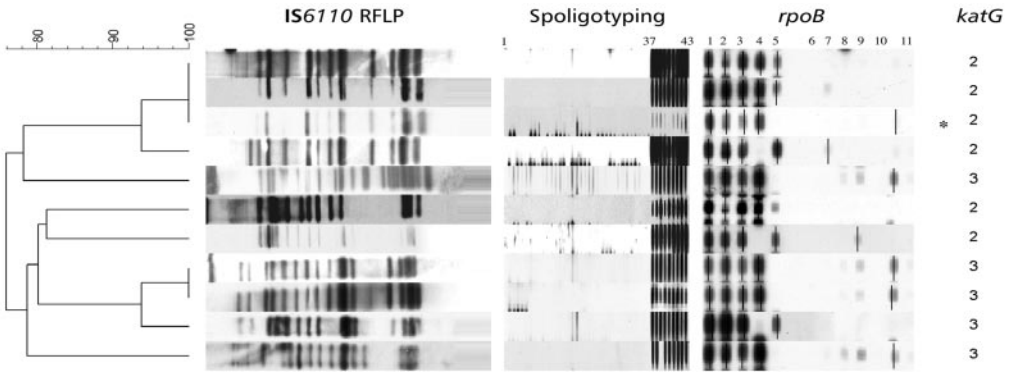

FIG. 5. Genotyping analysis of strains with the Beijing/W genotype. The dendrogram based on RFLP-IS*6110* patterns shared 65% similarity. The 11 strains harbored leucine at codon 463 of the *katG* gene (2, codon 463Leu; 3, codons 315Thr and 463Leu). Three isolates had identical RFLP patterns, but one of them (asterisked) had different mutations conferring resistance to rifampin.

Eleven isolates with the Beijing/W genotype were detected (Fig. 5). Six of them were detected in patients born abroad. The isolates from two of these patients, who were Russian immigrants, were clustered (tb5b). Four other isolates were from patients from the Canary Islands (4). The RFLP fingerprints of three of these isolates were identical, and a fourth isolate contained only one more copy of IS*6110*. In addition, the mutation in the *rpoB* gene was different for one isolate, indicating that only two of these isolates could be considered to represent cases of MDR transmission (tb5a).

Specific epidemiological links could be established among the patients in 10 clusters who came from the same hospitals or the same province. There was a familial link in one cluster.

Two other clusters included immigrants. The first affected two Romanian immigrants who lived together. The second cluster, "tb15," affected two patients from a region near Portugal and a Portuguese immigrant. This isolate was identical to a strain responsible for a nosocomial outbreak in Lisbon (24).

No links were found between the patients in two other different clusters.

We compared the patterns of clustered isolates with the RFLP types included in the European MDR TB genotype data set (www.CAonTB.nl). Patterns identical to those of clusters tb2a, tb5b, tb6, tb15, and tb16 were found (Fig. 1). No clear epidemiological links were found between these isolates.

DISCUSSION

Drug resistance is a worldwide problem that is threatening to undermine effective control of TB. The aim of this study was to address the problem of MDR TB in Spain at a molecular epidemiological level. We genotyped all the available MDR strains isolated in the participating laboratories during the 3-year study period. We estimated that these strains represented more than two-thirds of all Spanish MDR TB cases identified during this period. As found previously, spoligotyping was less discriminatory than RFLP-IS*6110* (15). To increase the discriminatory power of our study, we also studied mutations in the *katG* and *rpoB* genes. The diversity of IS*6110* fingerprints (58%) combined with characterization of the *rpoB* and *katG* genes showed that 66.1% (119 of 180) of the isolates had evolved drug resistance independently in Spain. On this basis, it was shown that active transmission of MDR TB (33.8%) was lower than that of susceptible TB (46.6%) (13). This result is in agreement with previous studies that found that MDR strains are disseminated less than susceptible strains but that specific strains (such as the B strain or the Beijing/W family strains) are able to cause MDR TB outbreaks (2, 31). Rifotyping (18), which can analyze 43 strains in the same experiment, and PCR-restriction analysis of the *katG* gene are both easy to perform and should be included in epidemiological studies of MDR TB.

A high rate of reinfection has been described among HIV patients affected by an MDR "B" strain (28). No cases of exogenous reinfection were observed in our study based on the multiple isolates collected from 23 patients. This could be because we lost the isolates from patients reinfected during a primary episode of susceptible TB. No changes in the IS*6110*-RFLP patterns of the isolates were found, in contrast to the studies carried out in The Netherlands (4.6%) (6) and San Francisco (29%) (45).

It has been reported that more than 40% of all INH-resistant isolates of *M. tuberculosis* present a threonine at codon 315 of the *katG* gene (25). In The Netherlands, 53% of INH-resistant isolates were found to harbor this mutation (Ser531Leu) (42). This seems to contradict our findings, since we detected this mutation in only 25.3% of the isolates that clustered based on RFLP, and this percentage decreased to 16% in the redefined clustered strains.

The presence of a leucine at codon 463 of the *katG* gene does not confer resistance to INH (38). Remarkably, in our study, this mutation was present only in the *M. bovis* "B" isolates (22 cases) and the Beijing/W family isolates.

More than 90% of the *M. tuberculosis* isolates resistant to rifampin have been reported to contain single-nucleotide substitutions or deletions in the so-called hot spot region of the *rpoB* gene (12, 34, 35). In our study, Ser531Leu was the most common mutation, found in 49 isolates (65.3%), which is in agreement with other studies (17). However, we found no mutations in 12 of the 75 clustered isolates (16%), possibly due to the failure of this method to detect other, less frequent point mutations.

The size of clusters appears to be an important characteristic in our study. Most clusters (13 of 17) in our study included two

isolates. This is different from the findings of population studies of TB in Spain, in which only 50% of clusters were formed by two susceptible isolates (13).

The results of the three techniques were consistent for the 22 MDR "B" strain isolates. The "B" strain was the most frequently isolated MDR strain in our country. The recent nosocomial outbreak affected HIV-positive patients first, but transmission to an immunocompetent patient has been described (23). Interestingly, some of the patients in our study were HIV negative. More than 120 such cases were detected between 1994 and 2003 (unpublished data). This outbreak was associated with a high mortality rate. The virulence or transmissibility of this strain is enhanced, and at least some of the MDR strains do not have attenuated pathogenicity. For this strain, this could be due to up-regulation of the *phoP* virulence gene following the insertion of a copy of IS*6110* (33).

The strains of the Beijing/W genotype family are genetically highly conserved and are predominant in some geographic areas. The ubiquitous nature of the Beijing/W strains stresses their importance, as does their frequent association with outbreaks and drug resistance. Previous studies have shown that this family is not endemic in Spain (unpublished data). This was also shown in our study, in which only 5 of the 11 patients affected with *M. tuberculosis* isolates from this family were Spanish; 4 of the patients came from Gran Canaria Island, where an *M. tuberculosis* strain of the Beijing/W genotype caused an outbreak (4). The fifth Spanish patient affected had previously traveled to Russia. The other six patients came from abroad. The RFLP pattern of the strains in cluster tb5b had previously been described in the northwest of Russia, where these strains are highly prevalent (19). Immigrants play an important role in the spread of TB in some countries (5, 9, 21, 40). However, relatively few of our patients (13.3%) were immigrants. Nevertheless, we found a significant association between the Beijing/W strains and immigrants. This suggests that both susceptible (4) and MDR strains are being imported into Spain.

HIV infection promotes the progression to active TB both in people with recently acquired *M. tuberculosis* infections and in those with latent *M. tuberculosis* infections (7, 26). Other studies have also found that HIV infection is associated with recent transmission. However, we did not found a significant association between HIV infection and recent transmission. This could be due to the high TB incidence in our country.

MDR TB is a major public health problem, and our main priorities should be its prevention and breaking certain transmission chains. Transmission occurs even in countries such as Norway that have good national TB surveillance programs, effective treatment, and contact tracing (5). In Spain, it is not possible to type all isolates of *M. tuberculosis* due to the high prevalence of the disease. Nevertheless, in view of the exceptional importance of MDR TB, our study shows that molecular typing and the additional characterization of resistance genes are necessary to identify the origin and transmission routes of MDR TB. Centralized DNA fingerprint databases of these strains must also be maintained at the international level. Special attention must be paid to the isolates belonging to the Beijing/W family, which have caused MDR outbreaks worldwide, and to the new B strain isolates, which are being transmitted in our country.

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