Single-Nucleotide Polymorphism in Two Representative Multidrug-Resistant 
Mycobacterium bovis Isolates Collected from Patients in a Spanish Hospital 
Harboring a Human Infection Outbreak

*Mycobacterium bovis* is the etiological agent of tuberculosis in domestic and wild animals. Its involvement as a human pathogen has been highlighted again with the recent descriptions of transmission through dairy products (18), reactivation or primary infection in human immunodeficiency virus-infected patients (5), and association with meat industry workers, animal keepers, or hunters (3). Strains resistant to antituberculous drugs (*M. bovis* is naturally resistant to pyrazinamide) pose an additional risk (2). Several studies have demonstrated that mutations in target genes are associated with resistance to antituberculous drugs (4, 7, 10, 11, 16). However, most of them have been developed in *Mycobacterium tuberculosis* strains and limited data are available regarding *M. bovis* isolates.

The aim of this study was to characterize by sequencing the main genes involved in antibiotic resistance in two multidrug-resistant (MDR) *M. bovis* isolates in a human outbreak detected in a hospital in Madrid that subsequently spread to several countries (5, 6, 15). The isolates were resistant to 11 drugs, but only their *rpoB* and *katG* genes have been analyzed so far (1, 14). We studied the first (93/R1) and last (95/R4) *M. bovis* isolates of this nosocomial outbreak, characterized by spolityping as SB0426 (hexacode 63-5F-5E-7F-FF-60 in the database at www.mbovis.org) (1, 13).

Several genes involved in resistance to isoniazid (*katG*, *ahpC*, *inhA*), and the oxyR-ahpC intergenic region), rifampin (*rpoB*), streptomycin (*rs*, *rpsL*), ethambutol (*embB*), and quinolones (*gyrA*) were studied. These genes, or fragments of genes, were amplified and sequenced as previously described (12).

The sequence analysis revealed polymorphisms in five (*ahpC*, *rpoB*, *rpsL*, *embB*, and *gyrA*) out of nine analyzed genes (Table 1). Nucleotide substitutions in four genes cause a change in the encoded amino acid. Two additional synonymous mutations in *ahpC* and *rpsL* differentiated the first and last isolates from the outbreak.

The analysis of the main genes involved in isoniazid resistance did not show polymorphism involved in resistance. Both *M. bovis* isolates displayed a CTG codon at position 463 of the *katG* gene. It was initially hypothesized that this Arg463Leu change might be involved in resistance to isoniazid according to reports on *M. tuberculosis* isolates (9), but it was shown later that they are not associated (17). Sequencing of the *ahpC* gene showed that the last MDR isolate from the outbreak (95/R4) had a silent mutation at codon 70 (AAG→AAA, Lys→Lys).

The sequencing of the *rpoB* gene revealed a substitution mutation in codon 531 (TCG→TTG, Ser→Leu) in the two human MDR isolates (1, 14). Greater than 95% of the rifampin-resistant *M. tuberculosis* complex strains have mutations between codons 507 and 533 of the *rpoB* gene (8).

The study of two genes involved in streptomycin resistance showed no polymorphisms in *rs* and two mutations in *rpsL*. Both human isolates had a substitution mutation with a replacement in codon 43 (AAG→AGG, Lys→Arg) that has been found in *M. tuberculosis* strains with resistance to this drug, predominantly associated with a high-level resistance phenotype (7). Also, the last MDR isolate (95/R4) accumulated a silent mutation at codon 38 (TAC→TAT, Tyr→Try) that has not been described. In relation to ethambutol resistance, both human isolates also showed a mutation located at codon 306 (ATG→GTG, Met→Val) in the *embB* gene. This mutation has been described in *M. tuberculosis* isolates (10), but it has not been reported in *M. bovis* so far. The *gyrA* gene showed a nucleotide replacement at codon 91 (TCG→CCG, Ser→Pro), which has been described in resistant *M. tuberculosis* strains (4), that was associated with resistance to fluoroquinolones.

In summary, the MDR *M. bovis* isolates harbored nucleotide substitutions in the *rpoB*, *rpsL*, *embB*, and *gyrA* genes previously described in *M. tuberculosis* strains but the genetic mechanism of isoniazid resistance remains unknown. Knowledge of frequent mutations would help in the development of precise molecular tests to quickly detect resistance. However, more studies of the mutations underlying drug resistance in *M. bovis* strains are needed before reliable use of the rapid commercial systems available for the detection of gene mutations is possible.

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### Table 1. Mutations in genes associated to drug resistance found in the first (93/R1) and last (95/R4) human MDR *M. bovis* isolates

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide position</th>
<th>Codon no.</th>
<th>AF2122/97</th>
<th>93/R1*</th>
<th>95/R4*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ahpC</em></td>
<td>210</td>
<td>Lys 70 Lys</td>
<td>AAG</td>
<td>AAG</td>
<td>AAA</td>
</tr>
<tr>
<td><em>rpoB</em></td>
<td>1367</td>
<td>Ser 531 Leu</td>
<td>TCG</td>
<td>TGT†</td>
<td>TGT†</td>
</tr>
<tr>
<td><em>rpsL</em></td>
<td>114</td>
<td>Tyr 38 Tyr</td>
<td>TAC</td>
<td>TAT</td>
<td>TAT</td>
</tr>
<tr>
<td><em>rpsL</em></td>
<td>128</td>
<td>Lys 43 Arg</td>
<td>AAG</td>
<td>AGG</td>
<td>AGG</td>
</tr>
<tr>
<td><em>embB</em></td>
<td>916</td>
<td>Met 306 Val</td>
<td>ATG</td>
<td>GTG</td>
<td>GTG</td>
</tr>
<tr>
<td><em>gyrA</em></td>
<td>271</td>
<td>Ser 91 Pro</td>
<td>TCG</td>
<td>CCG</td>
<td>CCG</td>
</tr>
</tbody>
</table>

* The sequences were compared with the application software at http://www.ebi.ac.uk/clustalw/index.html by using the *M. bovis* AF2122/97 strain as a reference (GenBank accession no. BX248333). Nucleotide substitutions involved in drug resistance are in bold.

* The results for the *rpoB* sequence of MDR isolates were described previously (1).


