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 spoligotyping 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, rpsL, 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 isolates 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 → Tyr) 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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**REFERENCES**


**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>93/R1</th>
<th>95/R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ahpC</td>
<td>210</td>
<td>Lys 70 Lys</td>
<td>AAG</td>
<td>AAG</td>
</tr>
<tr>
<td>rpoB</td>
<td>1367</td>
<td>Ser 531 Leu</td>
<td>TCG</td>
<td>TG</td>
</tr>
<tr>
<td>rpsL</td>
<td>114</td>
<td>Tyr 38 Tyr</td>
<td>TAC</td>
<td>TAT</td>
</tr>
<tr>
<td>rpsL</td>
<td>128</td>
<td>Lys 43 Arg</td>
<td>AAG</td>
<td>AGG</td>
</tr>
<tr>
<td>embB</td>
<td>916</td>
<td>Met 306 Val</td>
<td>ATG</td>
<td>GTG</td>
</tr>
<tr>
<td>gyrA</td>
<td>271</td>
<td>Ser 91 Pro</td>
<td>TCG</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. BX48333).

* Nucleotide substitutions involved in drug resistance are in bold.

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

Beatriz Romero
Alicia Aranaz*
Javier Bezos
Lucia de Juan
Ana Mateos
Departamento de Sanidad Animal
Facultad de Veterinaria
Universidad Complutense de Madrid
28040 Madrid, Spain

Enrique Gómez-Mampaso
Servicio de Microbiología
Hospital Ramón y Cajal
IMSALUD
28034 Madrid, Spain

Lucas Domínguez
Departamento de Sanidad Animal
Facultad de Veterinaria
Universidad Complutense de Madrid
28040 Madrid, Spain

*Phone: 34 91 3943721
Fax: 34 91 3943795
E-mail: alaranaz@vet.ucm.es