Update on Rifampin Resistance in the *Legionellaceae*

A recent article described *rpoB* mutations in 18 rifampin-resistant variants of *Legionella* spp. (4). One of these 18 strains, isolate 8749, an in vitro-generated high-level rifampin-resistant (MIC ≥ 256 μg/ml) variant of K26, a susceptible clinical *Legionella bozemanii* isolate, was shown to harbor no codon exchange in the cluster I *rpoB* 69-bp hot spot, the DNA region investigated.

Besides codon exchanges in cluster I (e.g., codon 507 to 533 in *Escherichia coli*), several mutations in at least three *rpoB* regions outside cluster I have been described to be associated with resistance to rifampin (1). Mutations either are located 768 bp (in *Mycobacterium tuberculosis*) to 1,128 bp (in *Helicobacter pylori*) upstream of cluster I at the beginning of *rpoB* (5′ region; codon V146F in *E. coli*), or can be found in two regions 123 bp (cluster II) and 468 to 471 bp (cluster III) downstream of cluster I (Fig. 1). Mutations in cluster II are frequently associated with rifampin resistance; cluster III (R687H in *E. coli* and R701H in *H. pylori*) is suspected to confer very low levels of resistance.

Both the parent *L. bozemanii* strain K26 and the resistant variant 8749 were reinvestigated. PCR primers were designed for amplification of sections encompassing the 5′ region of *rpoB* (bp −124 to 591) (RPlL-F [forward 5′-GAAGARGCNG GYGGCNARGTDGA-3′] and L4 [reverse 5′-CAACCATGA NCCNGYTANGG-3′]) and clusters I, II, and III (bp 1396 to 2174) (Rif U1 [forward 5′-GICIGITTCGTCVGTWGG HGA-3′] and Rif D3 [reverse 5′-ATCAGATGCAACAATTCT TTCC-3′]). Base pair numbering is based on the published sequence of *Legionella pneumophila* (4) and the unfinished Legionella Genome Project of the Columbia Genome Center (contig LP.WG.044.06.111099). IUB code is used for mixed base sites. The PCR products were sequenced on both strands. The 5′ region of *rpoB* (2), especially codon 159 (GTT, valine), was not changed. The only mutation detected (ATT to TAT) was located in cluster II, downstream of the cluster I hot spot. This mutation induces an isoleucine-to-tyrosine amino acid substitution at position 587 of the protein sequence. In the great majority of cluster II mutations described to be associated with rifampin resistance in other species, the corresponding codon is changed. Amino acid exchanges in different species observed in our laboratory or found in the literature are summarized in Fig. 1. Also included is a cluster II isoleucine-to-threonine exchange (ATC to ACC, I586T) newly observed in two (802-1 and 802-2) of three *H. pylori* DNA samples provided by Wang et al., formerly described as rifampin resistant but harboring wild-type sequence in clusters I and II (6). In the third *H. pylori* DNA sample (802-3) from that study, amino acid exchange V149F, corresponding to the V146F exchange in *E. coli* (2) and the V176F exchange in *M. tuberculosis*, could be observed.

In conclusion, no cluster I mutation was found in strain 8749 that could explain the high level rifamycin resistance (4). Here we show that the isolate harbors a mutation in cluster II (I587Y), inducing an amino acid substitution that is known to be associated with rifamycin resistance in other species. In all comparable cases, complete sequence analysis of the respective *rpoB* regions (Fig. 1) revealed mutations corresponding to the Rif-resistant phenotype of *H. pylori* and *Legionella* spp. and also *M. tuberculosis* in our laboratory. To our knowledge, mutations outside the cluster I *rpoB* hot spot in *Legionellaceae* have not been described so far. This is also the first description of I-to-Y and I-to-T substitutions in cluster II.

We thank Ge Wang and Diane Taylor for providing the DNA samples of the rifampin-resistant *H. pylori* variants.

**REFERENCES**


![FIG. 1. Rifampin resistance-determining regions in *rpoB*. Isoleucine codon positions and substitutions in *rpoB* cluster II and the newly described tyrosine (Y) substitution in *L. bozemanii* and threonine (T) substitution in *H. pylori* are indicated.](http://aacid.asm.org/)

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Annerose Serr
Barbara F. Koenig
Markus Heep*
Institut für Medizinische Mikrobiologie und Hygiene
Freiburg University
Herman-Herder-Strasse 11
D-79104 Freiburg, Germany

Kim Nielsen
Aarhus University Hospital
Aarhus, Denmark

Jette Marie Bangsborg
Herlev Hospital
Herlev, Denmark

*Phone: 49 761 2036546
Fax: 49 761 2036562
E-mail: Markus-heep@web.de