Characterisation of *rpsL* and *rrs* mutations associated with streptomycin resistance in multidrug-resistant *Mycobacterium tuberculosis* clinical isolates

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The study was financed by the Ministry of Science and Higher Education: «Iuventus Plus» grant (Project No. IP2011018771) and the Faculty of Biology, University of Warsaw intramural grant DSM.

BACKGROUND

Streptomycin (SM) is a component of the first-line regimens for the treatment of tuberculosis that acts by binding to the 30S subunit of the ribosome, and thus inhibiting protein synthesis [1]. Mutations in genes *rpsL* and *rrs* encoding the ribosomal protein S12, and the 16S rRNA, respectively, have been associated with resistance to SM in *Mycobacterium tuberculosis* clinical isolates [2, 3]. Analysis of mutations in the *rpsL* and *rrs* genes in *M. tuberculosis* may contribute to the development of new diagnostic tests allowing for rapid detection of SM resistance.

OBJECTIVE

The aim of this study was to investigate the presence of mutations in two genetic loci, namely the *rpsL* and *rrs* genes, most frequently associated with SM resistance in *M. tuberculosis*.

METHODS

A total of 50 *M. tuberculosis* (32 SM-resistant and 18 SM-susceptible) strains isolated from as many MDR-TB patients in Poland throughout 2004 were included in the study. Mutations in the *rpsL* and *rrs* genes were detected through PCR amplification of both loci, followed by their direct sequencing in forward and reverse directions. The obtained nucleotide sequences were aligned against the reference sequences of the respective genes of *M. tuberculosis* reference strain H37Rv. The obtained results were interpreted in the context of SM susceptibility profile of the strains tested. Primary isolation, species identification, and drug susceptibility testing had been done beforehand, as described elsewhere [4].

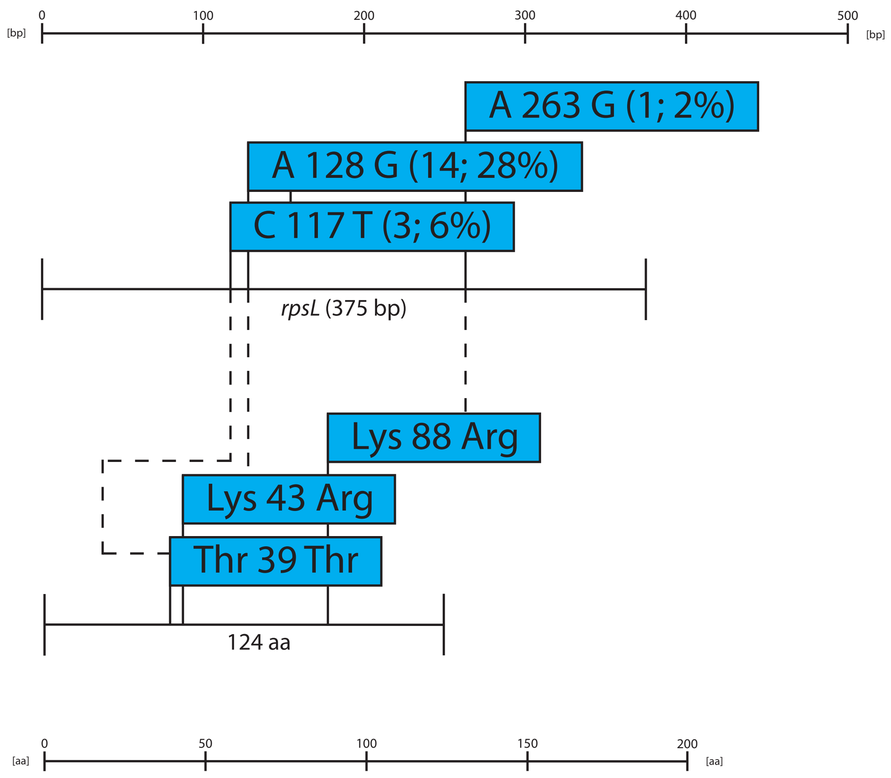

Fig. 1. Schematic representation of mutations in the *rpsL* gene identified among 50 *M. tuberlculosis* strains tested. Nucleotide positions at codons, and their corresponding amino acid residues affected by mutations are given in frames. Number and percentage of strains presenting with a given type of mutation are given in brackets.

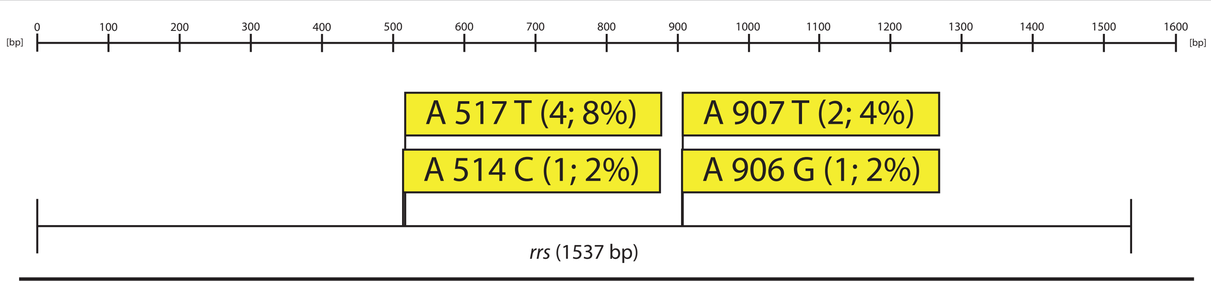

Fig. 2. Schematic representation of mutations in the *rrs* gene identified among 50 *M. tuberlculosis* strains tested. Nucleotide positions at codons are given in frames. Number and percentage of strains presenting with a given type of mutation are given in brackets.

RESULTS

Among the 32 SM-resistant isolates, 24 (75%) contained mutations in either the *rpsL* (16 isolates) or the *rrs* (8) gene. None of the isolates had mutations in both loci concurrently. Of the 16 SM-resistant isolates with *rpsL* gene mutations, 14 (87.5%) isolates had a substitution in codon 43 from lysine to arginine, whereas one isolate had the same amino acid substitution in codon 88. These two mutation types were found exclusively in SM-resistant strains. One SM-resistant isolate had a silent mutation in codon 39. This mutation was also found in two SM-susceptible isolates. Four *rrs* mutation types were identified. They occurred at positions 517 in 4 isolates, 906 in two isolates, 514 in one isolate, and 907 in another isolate. All isolates with mutations in the *rrs* gene were SM-resistant. Eight (25%) SM-resistant isolates were wild-type for both loci analysed.

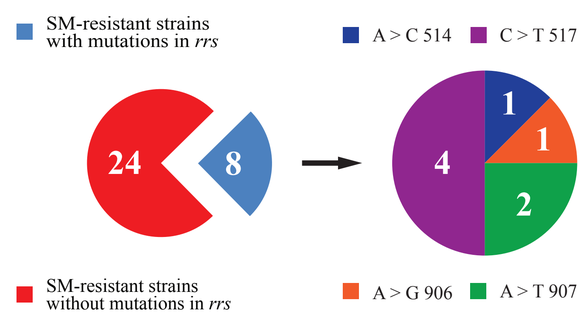

Fig. 3. Number of SM-resistant strains with confirmed mutational changes in *rrs* gene; type and number of mutations in *rrs* gene detected among 32 SM-resistant strains tested.

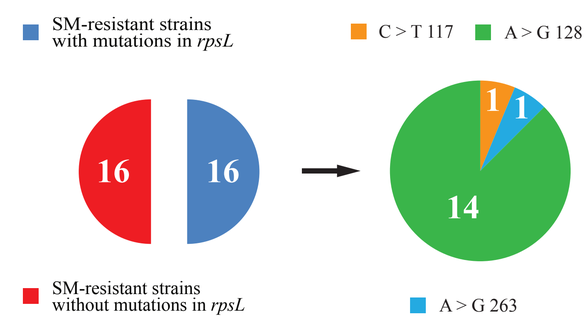

Fig. 4. Number of SM-resistant strains with confirmed mutational changes in *rpsL* gene; type and number of mutations in *rpsL* gene detected among 32 SM-resistant strains tested.

CONCLUSIONS

The study revealed a relatively high frequency of mutations in SM resistance-related genes. Every nonsynonymous mutation detected in the *rpsL* or *rrs* gene was found exclusively in SM-resistant isolates. This underlines the usefulness of *rpsL* and *rrs* mutations as molecular markers predictive of resistance of tubercle bacilli to SM.

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