Gene markers for predicting second-line anti-tuberculosis drug resistance

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BACKGROUND

The emergence and spread of drug-resistant tuberculosis (DR-TB), including multidrug-resistant (MDR)-TB (defined as resistance to at least two most potent anti-TB drugs: isoniazid [INH] and rifampicin [RMP]) and extensively drug-resistant (XDR)-TB (defined as MDR-TB with additional resistance to any fluoroquinolone [FQ] and one of the three injectable drugs: amikacin [AMK], kanamycin [KAN] or capromycin [CAP]) have further aggravated the existing health threat of TB worldwide. Effective DR-TB prevention and control require rapid and reliable methods to identify resistant drug-resistant *Mycobacterium tuberculosis* strains. Mutations in several genetic loci have been implicated in the development of resistance of tubercle bacilli to second line anti-TB drugs (SLDs).

OBJECTIVE

The purpose of this study was to investigate the prevalence of resistance to SLDs and its association with resistance-related mutations in MDR *M. tuberculosis* clinical isolates.

RESULTS

Of the 46 MDR *M. tuberculosis* isolates, 14 (30.4%) showed resistance to at least one of the four SLDs tested. Nine (19.5%) isolates were resistant to either OFX (7; 15.2%) or AMK (2; 4.3%), and thus met the definition of pre-XDR-TB, whereas 3 (6.5%) isolates were resistant to OFX and AMK (2; 4.3%) or CAP (1; 2.2%) simultaneously and were categorized as XDR-TB isolates. Four (8.7%) isolates, including two pre-XDR isolates were resistant to ETH.

None of the AMK-resistant isolates carried a mutation in the *rrs* gene. All isolates were wild-type at the *tlyA* locus. Mutations in the *gyrA* gene occurred in 34 (73.9%) isolates, with the most common amino acid change being Ser95Thr, found in 5 OFX-resistant and 25 OFX-susceptible isolates. The Asp94Asn and Ala90Val substitutions were present exclusively in OFX-resistant strains, yet represented only 40% of all OFX-resistant isolates. The only mutation in the *gyrB* gene was substitution Ser477Phe, detected in one OFX-resistant isolate. Mutations in the *ethA* gene were found in one ETH-resistant and 11 ETH-susceptible isolates.

CONTACT INFORMATION

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METHODS

A total of 46 MDR *M. tuberculosis* isolates, recovered from 46 pulmonary TB patients from Poland were included in the study. Drug susceptibility testing was performed using the 1% proportion method on Löwenstein-Jensen medium, with the following critical drug concentrations: INH, 0.2 µg/mL; RMP, 40 µg/mL; AMK, 16 µg/mL; CAP, 40 µg/mL; ofloxacin (OFX), 2.5 µg/mL, and ethionamide (ETH), 40 µg/mL. Mutation profiling was performed by amplifying and sequencing the following six resistance-associated genes: *rrs* (AMK), *tlyA* (CAP), *gyrA/gyrB* (FQ), and *ethA/ethR* (ETH).

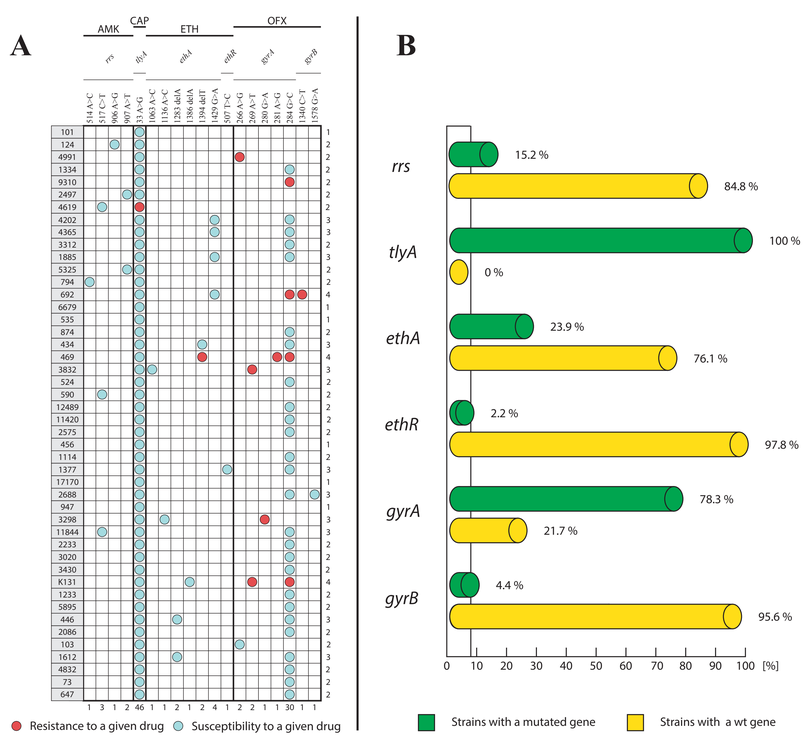

Fig. 1.
A - Distribution of mutations in the investigated genes among 46 *M. tuberculosis* strains tested. Total number of strains with a specific mutation is given below the chart; number of gene mutations in particular strains is given on the right side of the chart.

B - A graph illustrating the percentage of strains carrying mutations in the investigated genes versus the percentage of strains with wt alleles of these genes.

CONCLUSIONS

Apart from two *gyrA* mutations (at codons 90 and 94), none of the amino acid changes detected in all six loci tested were associated with resistance to SLDs. This casts doubt over the usefulness of sequence analyses of these genes for the prediction of SLD resistance pattern. Other genetic loci need to be considered for detection of mutations conferring resistance to SLDs.