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Molecular Detection and Characterization of Drug-Resistant *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis (TB) is an ancient disease, but not a disease of the past. Despite the declaration of TB as a global emergency by the World Health Organization in 1993 the worldwide problem of TB has worsened. The increasing prevalence of drug-resistant strains of *Mycobacterium tuberculosis*, the causative agent of TB, demands new measures to combat the situation. Rapid and accurate diagnosis of the pathogen, and its drug susceptibility pattern, is essential for timely initiation of treatment, and ultimately, control of the disease. Furthermore, knowledge about which precise mutations confer drug resistance in *M. tuberculosis* does not only lead to a basic understanding of drug resistance mechanisms and drug actions, but is also important for the design and development of clinically sensitive and specific molecular methods aiming at detecting drug-resistant *M. tuberculosis*.

Paper I in this thesis investigated cross-resistance between the aminoglycosides amikacin (AMK) and kanamycin (KAN), and the cyclic peptide capreomycin (CAP). The results show that *tlyA* is neither a sensitive nor a specific genetic marker for detection of CAP resistance in *M. tuberculosis*, and that it is advisable to include *rrs* nucleotide position 1401 in a molecular-based assay for the detection of AMK-, KAN- and CAP-resistant *M. tuberculosis* clinical isolates. **Paper II** aimed at developing a pyrosequencing method for detection of first- and second-line resistance in *M. tuberculosis*. Pyrosequencing assays were developed for the genes *rpoB*, *katG*, *embB*, *rrs*, *gyrA* and the promoter regions of *inhA* and *eis*, which are associated with resistance to rifampicin (RIF), isoniazid, ethambutol, AMK, KAN, CAP and fluoroquinolones, respectively. Pyrosequencing is a highly throughput and robust method for detection of novel and a priori known mutations. The method can be used to screen a large sample volume, which is desired if aiming at investigating the prevalence of mutations in large sample collections. In **Paper III**, the utility of padlock probes for detecting drug resistance in *M. tuberculosis* was evaluated. The assay was developed for RIF resistance due to the importance of RIF in the standard TB treatment and its potential role as a surrogate marker for multidrug-resistant TB. The method proved to be robust for detection of specific mutations in the gene *rpoB*, and confirmation of loss of wild type as well as detection of *M. tuberculosis* complex DNA. The padlock probe assay was further extended in **Paper IV** to detection of extensively drug-resistant TB in a multiplexed fashion. Padlock probes were designed to target the most common mutations occurring in *rpoB*, *katG*, *rrs*, *gyrA* and in the promoter region of *inhA*. The analytical sensitivity achieved in **Paper IV** is comparable to that of PCR. The readout format employed in **Paper IV** eliminates the use of extensive equipment, but rather, signal can be detected by the naked eye.

This thesis has contributed to increased knowledge of drug resistance in TB, and has successfully developed new methods for rapid detection of drug-resistant *M. tuberculosis*. The results can guide future research and development of molecular methods aiming at detecting drug-resistant *M. tuberculosis*.

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