Rapid diagnosis and drug resistance of Mycobacterium tuberculosis
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It is estimated that tuberculosis, a disease caused by *Mycobacterium tuberculosis*, has caused more deaths than any other infectious disease ever in the history of mankind. With the discovery of various antibiotics with an antimycobacterial effect came the hope that eradication of tuberculosis would soon be a possibility. However, the complexity of treatment that is required to cure tuberculosis together with the inadequate health care systems in many countries made this an unreachable goal.

The prevalence of tuberculosis was on a steady decline throughout the world due to improvement of sanitation, higher living standards, and antibiotic treatment when in the early 1990s the shocking conclusion had to be drawn that tuberculosis was coming back. For the first time in the 20th century an increase of tuberculosis was observed, in developing countries, but also in the Western world. The emergence of (multi-drug) resistant strains, migratory movements of people from high-incidence countries and the pandemic of AIDS were to blame.

From then on, tuberculosis was 'hot' again. In various countries research projects, which were neglected for decennia due to lack of funding, were brought back to life. This thesis is a small result of this renewed interest in tuberculosis.

Rapid diagnosis of tuberculosis and of resistance of the bacterium is of utmost importance to adequately treat patients and to prevent transmission. The goals of this project were, firstly, to evaluate the available methods for PCR-based diagnosis of tuberculosis. Secondly, to inventory the prevalence of resistance and the causative mechanisms behind it in The Netherlands, and to develop a PCR-based tool for rapid detection of resistance (based on the inventory that was made).

In Chapter 2 we describe the evaluation of two commercial PCR-based kits for the detection of *M. tuberculosis* in clinical samples. We collected 225 samples from patients in the Academic Medical Center in Amsterdam, which were suspected to have tuberculosis. We tried to improve the performance of these assays by replacing the protocol for DNA extraction of the kits by a protocol (Boom-extraction), which is reported to have a very high yield of pure DNA for many different clinical samples (including blood and feces). In this study, the addition of this protocol proved of no additional value. One of the kits, when performed according to its standard protocol, detected all the samples that were positive according to the gold standard, and had a specificity of 100%.
For the second goal we focused on isoniazid resistance because the prevalence of resistance against this drug is by far the highest in The Netherlands (7%). PCR restriction endonuclease assays were developed to screen isoniazid-resistant isolates for the presence of two mutations in the KatG gene that were most frequently reported to be associated with resistance. We found that one mutation (Δ463) was present in 32% of resistant isolates. However, we also found this mutation in 29% of susceptible isolates. Therefore, this mutation would not be a useful target for a diagnostic assay (Chapter 3). Another mutation (Δ315) was found in 55% of resistant isolates, but never in susceptible ones. Furthermore, this mutation was found to be associated with high-level resistance (95% of Δ315 isolates had an MIC of > 2 μg/ml and 89% of isoniazid resistant isolates with an MIC of > 2μg/ml had this mutation), increased transmissibility when compared to resistant isolates without this mutation, and co-resistance to streptomycin and rifampicin. This mutation would make an excellent target for fast diagnosis of (high-level) isoniazid resistance (Chapter 4 and 5).

Finally, in Chapter 6 we describe the development and evaluation of a real-time PCR assay, which detects the presence of the Δ315 mutation in multidrug-resistant M. tuberculosis isolates in respiratory clinical samples with a sensitivity of 76% and a specificity of 100%.

We conclude that 89% of high-level INH-resistant isolates can be detected with rapid PCR-based diagnostic tests with a sensitivity of 76%. When combined with a similar test for detection of rifampicin resistance (e.g. the Inno-LiPA from Innogenetics, or in-house PCRs described elsewhere) tuberculosis patients from high-incidence countries or patients unresponsive to therapy can be screened for the presence of multidrug-resistant isolates. We recommend using these tests only on ZN- or PCR-positive clinical samples.