Summary
The ambition to curb the persistent worldwide tuberculosis (TB) epidemic is challenged by two important problems: the development of acquired drug resistance and the low treatment success rates for multi-drug resistant tuberculosis (MDR-TB). An underlying cause is the combination of large inter-individual variability in drug exposure and decreasing bacterial susceptibility. Individualised dosing of anti-TB drugs using therapeutic drug monitoring (TDM) may be an important step to improve MDR-TB treatment outcomes and minimize the development of acquired drug resistance. However, TDM is considered laborious, expensive, and consequently has not been widely implemented yet. This thesis aimed to study the feasibility of salivary TDM, limited sampling strategies (LSS), and centralized TDM as alternative methods to reduce the burden of TDM as well as to stimulate the programmatic implementation of TDM.

In Chapter 2, we reviewed the literature and identified pharmacokinetic studies which reported anti-TB drug concentrations in both saliva and blood of humans. The aim of this study was to provide an overview of the available data on saliva-blood ratios of anti-TB drugs and to detect knowledge gaps to be filled in by future studies. In total, we included 42 studies with data on rifampicin, isoniazid, moxifloxacin, ofloxacin, gatifloxacin, amikacin, linezolid, amoxicillin/clavulanate, doripenem, and clarithromycin. Large variation in study population, sampling procedure, and saliva-plasma or saliva-serum ratios was observed between studies. The conclusion of this chapter was that, based on the available literature, salivary TDM likely is not possible for all anti-TB drugs due to highly variable saliva-plasma or saliva-serum ratios, but it is worthwhile to further investigate salivary TDM for each individual TB drug especially those that have not been studied yet.

Because many studies included in our review (Chapter 2) did not include patients with TB nor did they evaluate the feasibility of salivary TDM, we decided to perform a prospective observational cohort study in patients with TB (Chapters 3a, 3b, 3c). This study included all TB drugs being part of the treatment regimen. Patients consecutively enrolled in this observational study received traditional blood-based TDM as part of standard of care. Saliva was simultaneously sampled with blood and the measured paired drug concentrations were used to calculate saliva-plasma or saliva-serum concentration ratios. Additionally, non-compartmental AUC\textsubscript{0-24} saliva-plasma or saliva-serum ratios were assessed. To minimize the infection hazard of processing saliva samples of sputum culture positive patients, we developed and tested a secure sampling method (Chapter 3d). Culture fluids containing at least $10^5$ to $10^6$ CFU/mL of different Mycobacterium tuberculosis strains were successfully sterilized using membrane filtration with a pore size of 0.22 µm. This experiment provided evidence for the conclusion that membrane filtration is suitable for safe collection of saliva samples.
Chapter 3a studied the feasibility of salivary TDM of the first-line TB drugs rifampicin and isoniazid. Rifampicin showed very low saliva-serum ratios which can be explained by a high protein binding. We concluded that rifampicin serum AUC$_{0-24}$ could be adequately estimated by applying a correction factor of 6.5 to the AUC$_{0-24}$ in saliva. In contrast, isoniazid saliva-serum ratios were highly variable, especially between patients, but the exact cause remains unclear. For that reason, isoniazid TDM using saliva samples was assumed to be unfeasible using the described methods. It might still be interesting to explore the option of determining acetylator phenotype using salivary isoniazid concentrations.

Chapter 3b focused on moxifloxacin and linezolid; both are preferred (group A) drugs for the treatment of MDR-TB. We felt that salivary TDM of linezolid has great potential due to constant saliva-serum ratios. To adequately predict the serum AUC$_{0-24}$ of linezolid, a correction factor of 1.2 must be applied to the AUC$_{0-24}$ determined in saliva. Moxifloxacin saliva-plasma ratios were noticeably high, but very variable and therefore unpredictable. Based on this data, we concluded that saliva is not a suitable alternative matrix for TDM of moxifloxacin.

Chapter 3c aimed to determine the potential for C$_{max}$/MIC guided TDM of amikacin using saliva samples. However, amikacin could not be quantified in any of the saliva samples, not even in the saliva samples collected at serum T$_{max}$ which were expected to represent salivary C$_{max}$. This low penetration into saliva could be explained by the fact that amikacin is a highly polar compound and as a result does not easily pass through membranes. As the salivary C$_{max}$ could not be determined in any of the patients, we concluded that salivary TDM using C$_{max}$/MIC is unfeasible for amikacin.

Another alternative sampling method studied in this thesis is the use of a limited sampling strategy (LSS) that requires only a small number of ideally timed samples to estimate AUC$_{0-24}$. In Chapters 4a and 4b, we aimed to develop and validate population pharmacokinetic (popPK) models as well as LSS using the Bayesian approach and multiple linear regression.

Chapter 4a included moxifloxacin pharmacokinetic data of 101 patients with TB. Separate popPK models and LSS were created for moxifloxacin alone (77 patients) and in combination with rifampicin (24 patients), because rifampicin is known to significantly increase moxifloxacin clearance and subsequently decrease moxifloxacin exposure. One-compartment popPK models with lag time were developed as they best described the data. Well-performing Bayesian LSS for both moxifloxacin alone and in combination with rifampicin included two samples; one collected before drug intake (t=0 h) and a second sample 6 h after drug intake. Using multiple linear regression, AUC$_{0-24}$ can be adequately estimated with t=0 h and t=4 h samples for moxifloxacin alone, whereas with t=1 h and t=6 h samples when in combination with rifampicin. The popPK model and LSS were all successfully validated using jackknife analysis.
Chapter 4b focused on another important fluoroquinolone in MDR-TB treatment: levofloxacin. Pharmacokinetic data of 30 patients with MDR-TB was used to develop the popPK model and corresponding LSS. The model and Bayesian LSS were externally validated in 20 other MDR-TB patients. The multiple linear regression LSS was internally validated using jackknife analysis. The data was best described by a one-compartment model with lag time. We found that levofloxacin AUC$_{0-24}$ could be adequately estimated using the Bayesian approach using a sample collected before drug intake (t=0 h) and one collected 5 h thereafter (t=5 h). The LSS using multiple linear regression required t=0 h and t=4 h post dose samples to provide a reliable estimation of levofloxacin AUC$_{0-24}$. The LSS for moxifloxacin and levofloxacin described in Chapters 4a and 4b are ready for implementation in clinical practice and were already applied in one of our studies (Chapter 5).

We proposed a study design for a prospective multicentre study in Chapter 5 with the primary aim to evaluate the feasibility of centralized TDM of moxifloxacin and levofloxacin. Sample analysis and clinical dose decisions are performed in a central facility to reduce the costs and to increase the quality of TDM. The turn-around-time between moment of sampling at the local hospital and the clinician receiving dosing advice was chosen as parameter for feasibility. Secondary, this study intended to determine the impact of TDM by comparing sputum culture conversion after two months of treatment between patients who received TDM and historical controls without TDM. A strength of this study is that the burden of TDM for patients was decreased by using LSS. Moreover, this is the first prospective study to investigate the effect of TDM on treatment results of patients with MDR-TB.

All chapters were discussed and put into perspective in Chapter 6. The place for TDM in TB treatment and its efficacy was debated. The need for drug susceptibility testing at start of TB treatment as well as MIC determination for TDM was highlighted as we feel this is an important issue that must be solved by developing rapid and cost-effective tests. We discussed numerous causes for the observed high variability in saliva-blood ratios in general. Based on the results in Chapters 2, 3a, 3b, and 3c, we concluded that TDM using saliva samples can be an attractive alternative for some anti-TB drugs such as linezolid and rifampicin, but is not feasible for others (e.g. moxifloxacin, isoniazid, amikacin), and therefore is not equivalent to regular TDM. We proposed to use saliva in semi-quantitative screening methods to identify the patients who could benefit from blood-based TDM. Furthermore, this chapter included the advantages and limitations of the different types of LSS and the need for proper validation of the LSS. Moreover, we discussed the additional value of developing LSS for a combination of second-line anti-TB drugs, since it was not available yet and would significantly reduce the burden of TDM. Additionally, we briefly reviewed the value of centralized TDM and the necessity for determination of its feasibility. Lastly, we provided our overall conclusion of this thesis on the feasibility of salivary TDM, LSS, and centralized TDM.