Evaluation of rapid molecular drug susceptibility testing for Mycobacterium tuberculosis

Thesis

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LIST OF ABBREVIATIONS	
AFB	**Acid-fast bacillus**
AAM	**Alternatively activated macrophage**
AM	**Amikacin**
ACP	**Acyl carrier protein**
AIDS	**Acquired immunodeficiency syndrome**
ATP	**Adenosine triphosphate**
ATS	**American Thoracic Society**
CAM	**Classically activated macrophage**
CLSI	**Clinical and Laboratory Standards Institute**
CM	**Capreomycin**
DNA	**Deoxyribonucleic acid**
DST	**Drug susceptibility testing**
DW	**Distilled water**
EMB	**Ethambutol**
ETH	**Ethionamide**
Fig	**Figure**
FLQS	**Floroquinolones**
G-CSF	**Granulocyte colony stimulating factor**
GenoType MTBDRplus	**GenoType *Mycobacterium tuberculosis* drug resistance first line**
GenoType MTBDR*sl*	**GenoType *Mycobacterium tuberculosis* drug resistance second line**
HIV	**Human immunodeficiency virus**
HPLC	**high performance liquid chromatographic**

LIST OF ABBREVIATIONS	
HRM	**High-resolution melting**
IFN-γ	**gamma-interferon**
IL	**Interleukin**
INH	**Isoniazid**
IS	**Insertion sequence**
INOS	**Inducible nitric oxide synthetase**
KAN	**Kanamycin**
KH2PO4	**Potassium dihydrogen phosphate**
LAM	**Lipoarabinomannan**
LJ	**Lowenstein-Jensen**
MAS-PCR	**Multiplex allele specific PCR**
MDR	**Multi drug resistance**
MDR TB	**Multidrug resistance tuberculosis**
MGIT	**Mycobacteria growth indicator tube**
MHC	**Major histocompatibility complex**
MIC	**Minimum inhibitory concentration**
MTB	**Mycobacterium Tuberculosis**
MTBC	**Mycobacterium Tuberculosis complex**
MTD	**Mycobacterium Tuberculosis Direct Test**
MODS	**Microscopic observation broth-drug susceptibility**
MUT	**Mutation**
NAD	**Nicotinamide adenine dinucleotide**
NALC	**N-acetyl-L-cysteine**
NAAT	**Nucleic acid amplification technology**
NaOH	**Sodium hydroxide**

LIST OF ABBREVIATIONS	
NAP	**Beta nitro alpha acetylamine beta hydroxy propiophenone**
NO	**Nitric oxide**
NPV	**Negative predictive value**
OFX	**Ofloxacin**
PNB	**Para-nitro benzoic acid**
PCR	**Polymerase chain reaction**
PPV	**Positive predictive value**
PZA	**Pyrazinamide**
QRDR	**Quinolone Resistance- Determining Region**
R	**Resistant**
RIF	**Rifampicin**
RNIs	**Reactive nitrogen intermediates**
ROIs	**Reactive oxygen intermediates**
ROS	**Reactive oxygen species**
RRDR	**Rifampicin resistance determining region**
ST	**Streptomycin**
SOP	**Standard Operation Procedures**
TB	**Tuberculosis**
TCH	**Thiophene-2 carboxylic acid hydrazide**
TDM	**Trehalose 6,6'-dimycolate**
TGF-β	**Transforming growth factor-beta**
Th0	**T-helper type 0 cells**
Th1	**T-helper cell type 1**
Th2	**T-helper cell type 2**
TL7H11	**Thin layer 7H11 agar**
TNF-α	**Tumor necrosis factor alpha**

LIST OF ABBREVIATIONS	
TLR	**Toll-like receptor**
VIO	**Viomycin**
VOCs	**Volatile organic compounds**
WHO	**World Health Organization**
WT	**Wild type**
XDR TB	**Extensively drug resistance TB**
ZN	**Ziehl Neelsen**

Introduction

Tuberculosis (TB) remains one of the leading causes of morbidity and mortality globally, focused principally, but not exclusively, in the non-industrialized world **(Migliori, et al. 2008).**

The World Health Organization (WHO) and Stop TB Partnership called urgently for expanded access to culture and drug susceptibility testing (DST) in response to the spreading out of multidrug resistance TB (MDR TB) and extensively drug resistance TB (XDR TB) which are declared by the WHO as serious emerging threats to public health. This poses significant challenges for TB laboratory capacity and the need for faster DST methods **(WHO, 2007).**

MDR TB, means resistance to at least isoniazid (INH) and rifampin (Rif), and XDR, means MDR plus resistance to amikacin (AM), kanamycin (KAN) or capreomycin (CM) and a fluoroquinolone (FLQs),and it is the most problematic form of resistance because treatment options are limited and the second-line drugs used for therapy are more toxic, less effective, more expensive, and must be administered for a longer period of time than standard first-line drug therapy **(Migliori, et al. 2008).**

Conventional culture methods using egg- or agar-based media are still the most commonly used approach in many countries. To test for drug resistance, the standard methods using Lowenstein–Jensen (LJ) medium include the proportion method, the absolute concentration method and the resistant ratio method, which are well standardized with clinical isolates, at least for the major antituberculosis drugs **(Canetti, et al. 1969)**.

Conventional culture and DST on solid media is a slow process. In high income, low-incidence countries these systems have been supplemented (or replaced) by more rapid automated liquid culture systems such as BACTECT 960 system **(Somoskovi, et al. 2003)**.

Molecular assay techniques based on information about the genes involved in drug resistance represent a great step forward, as they provide results even more rapidly. Rapid molecular methods, including commercial or in-house DNA hybridization or amplification methods **(Somoskovi, et al. 2003)** which allow detection of TB and rifampin resistance (and for some assays, isoniazid resistance as well) in clinical samples within 1–2 days **(Bardarov, et al. 2003 and Hillemann, et al. 2007).**

Conventional DST for XDR strains is performed sequentially in a two-step procedure beginning with a culture and first-line drug testing, proceeding to further drug testing in the case of multidrug resistance. The time needed for testing, even with the most rapid

liquid methods, is still around 1 week per test, constrained by the relatively slow growth of *M. tuberculosis* (**Antonova, et al. 2008**). The required time can be shortened by fast molecular methods to 1 day per test (**Bang, et al. 2006** and **Hillemann, et al. 2006**). Resistance to FLQs, AM-CM, and ethambutol (EMB) in *M. tuberculosis* is most frequently attributed to mutations in the *gyrA*, *rrs*, and *embB* genes, respectively. First investigations have shown that by targeting mutations in codons 90, 91, and 94 in the *gyrA* gene, approximately 70 to 90% of all FLQ-resistant strains can be correctly detected **(Maus, et al. 2005 and Ling, et al. 2008)**. Previous reports have linked mutations A1401G, C1402T, and G1484T in the *rrs* gene to AM, CM, and KAN resistance **(Alangaden , et al. 1998 and Maus, et al. 2005)**, each of them being responsible for a specific resistance pattern. Mutations G1484T and A1401G were found to cause high-level resistance to all drugs, whereas C1402T causes resistance to only CM and KAN. Furthermore, mutations at *embB* codon 306 are found in 30 to 68% of EMB-resistant clinical strains **(Plinke, et al. 2006 and Safi, et al. 2008)**.

PCR-based techniques provide new possibilities for the rapid diagnosis of first- and second-line drug resistance; however, not all mycobacterial laboratories have access to DNA-sequencing facilities. As an alternative, DNA strip assays for the detection of