CHAPTER ONE

1. INTRODUCTION

Although effective anti-tuberculosis (TB) drugs have been available for decades in TB is still a major problem. Drug resistance, an outcome of non-compliance with drug intake, has been known to be the most significant limitation for the success of TB treatment \([1]\). The recent emergence of extensive drug resistance has greatly increased the significance of rapid susceptibility testing of *Mycobacterium tuberculosis* strains \([2]\). Current methods used in susceptibility testing are slow, resulting in delays, which make it difficult to administer appropriate treatment timely. This is one of the most important aspect contributing to the transmission of TB, including multi-drug resistant (MDR) outbreaks and more specifically, extensively drug resistant (XDR) TB.

During the 1990’s, MDR-TB defined as resistance to Isoniazid (INH) and Rifampicin (RIF) (WHO, 1997), was considered to be a threat to TB control \([3]\). However, emergence of the XDR TB cases has aggravated the situation, raising fear of a future epidemic of untreatable TB \([4]\). New anti-TB drug regimens, improved diagnostic tests, and standardised second line drug susceptibility testing, are needed for rapid detection and effective treatment of drug resistant \([4]\). However, with the slow growth rate of *M. tuberculosis*, culture requires weeks or even months to obtain results \([5]\). Patients that harbour infection for weeks or months while they receive ineffective treatment while awaiting susceptibility test results, transmit TB. Such delays in susceptibility tests increase the risk of transmission both in the community and in hospitals. It is crucial to detect drug resistance timeously in clinical isolates of *M. tuberculosis* for
administering of appropriate treatment to avert the development of further resistance and the spread of resistant strains. Identification of these strains is of paramount importance as it can allow initiation of modified treatment regimens. This will impact positively on both public health and patient outcome by reducing the spread of drug resistant strains.

RIF is one of the most potent first line anti-TB drugs [3]. Its resistance heralds a more prolonged treatment for the patient and poor results if the isolate is also resistant to INH (Mitchison and Nunn, 1986) [6]. The presence of RIF resistance increases the likelihood of MDR-TB because *M. tuberculosis* strains resistant to RIF are more likely to be resistant to several other anti-TB drugs [7, 8]. The mechanism of RIF resistance is well characterised and has led to the development of molecular assays for rapid detection of RIF resistance [7, 9, 10, 11, 12, 13, 14].

The occurrence of RIF monoresistance is rare and more than 90% of RIF-resistant isolates are also resistant to INH [15]. Therefore RIF is a good marker of MDR-TB, and thus can be used as a predictor of MDR-TB [16 - 18]. Ninety-five percent of RIF resistant isolates of *M. tuberculosis* harbour specific mutations within the 81-bp region of the RNA polymerase [19, 7]. The presence of these mutations can be detected by a genetic assay and could predict clinical drug resistance in the majority of patients.

While RIF is associated mainly with single point mutations in one region of the RNA polymerase gene (*rpoB*) that can easily be amplified by PCR methods, in contrast INH drug resistance is more complex. It involves mutations in at least four gene complexes and not all mutations result in an alteration of the wild type phenotype [20, 21, 22, 17]. Therefore, development
of an assay for the detection of mutations involved in INH resistance is more complicated and makes detection of RIF resistance more convenient compared to INH resistance.

Detection of mutations is more rapid than conventional RIF susceptibility testing, which depends on culture and therefore, requires an additional 3 to 6 weeks after the primary isolation to obtain the results. Early detection of MDR-TB can improve treatment outcome for the individual patient and reduce the opportunity for spread of the infection. Since RIF resistance is considered the surrogate marker for the identification of MDR-TB \(^{23, 24}\), it would be useful for poorly resourced countries to have simple and inexpensive tests that can rapidly detect resistance to RIF.