CHAPTER I

INTRODUCTION

The genus *Mycobacterium* consists of nonmotile, nonspore-forming aerobic bacilli, 0.2-0.6x1-10 μm in size. The bacilli occasionally form branched filaments, but these can be readily disrupted. The cell wall is rich in lipids, making the surface hydrophobic and the mycobacteria resistant to many disinfectants and chemotherapeutic agents, as well as to such common laboratory stains as the Gram and Giemsa. Once stained, the bacilli are also refractory to decolorization with acid solutions, hence the name acid-fast bacilli (1). Because the mycobacterial cell wall is complex and this group of organisms is fastidious, most mycobacteria grow slowly, dividing every 12 to 24 hours. Isolation of the "rapidly growing" mycobacteria requires incubation for 3 days or more; the slow growing organisms (e.g., *Mycobacterium tuberculosis*, *M. avium-intracellulare*) require 3 to 8 weeks. *M. leprae*, the etiologic agent of leprosy, cannot be grown in cell-free cultures. Although at least 37 species of mycobacteria have been described, more than 95% of all human infection are caused by six species(1): *M. tuberculosis, M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, and M. leprae. M. tuberculosis* is the common species that infected in human, because of its high virulence.

In the last decade, tuberculosis (TB) has reemerged as one of the leading causes of death (nearly 3 million deaths annually) (2). The estimated 8.8 million new cases every year correspond to 52,000 deaths per week or more than 7,000 each day (3-4). These death rates, however, only partially depict the global TB threat; more than 80% of TB patients are in the economically productive age of 15 to 49 years. The emergence of AIDS and decline of socioeconomic standards contribute to the disease's resurgence in industrialized countries (5). In most developing countries, although the disease has always been endemic, its severity has increased because of the global HIV pandemic and extensive social restructuring due to rapid industrialization and conflicts. A major public health problem worldwide, TB is now a global emergency.

Further contributing to the increased death rate is the emergence of new strains of *M. tuberculosis* resistant to some or all current antitubercular drugs. The resistance is attributed primarily to improper prescriptions or patient noncompliance and is often a corollary to HIV infection (6-8). Multidrug-resistant TB (MDRTB), associated with high death rates of 50% to 80%, spans a relatively short time (4 to 16 weeks) from diagnosis to death (9). Delayed recognition of drug resistance, which results in delayed initiation of effective therapy, is one of the major factors contributing to MDRTB outbreaks, especially in health-care facilities (10-11).

The resurgence of tuberculosis and its incidence in human immunodeficiency virus-positive populations in both developing countries and the industrialized world have been accompanied by the

alarming emergence of virulent multidrug-resistant tuberculosis (MDR-TB) strains. Many of these strains have acquired resistance to almost all first- and second-line antituberculosis agents. For this reason, there is an increasing interest in the antimycobacterial actions of the fluoroquinolones (FQs) (12).

The discovery of the new quinolones has broadened rather unexpectedly the therapeutic armamentarium against infections with mycobacteria. It has indeed been several years since new compounds with such an antimycobacterial potential were developed. The lead compound of these DNA gyrase-inhibiting antibacterial agents (13-14), norfloxacin, is not very active, but the N- cyclopropyl analog ciprofloxacin and the tricyclic ofloxacin deserve full attention. The combination fluoroquinolone-first-choice antitubercular drug (for example, ciprofloxacin-isoniazid) appeared to be very effective (15-16). Moreover, these compounds appear to penetrate readily into mammalian cells. This property is important for the treatment of intracellular pathogens, such as *M. tuberculosis* (17-18).

Fluoroquinolones are new effective drugs for the treatment of tuberculosis, but resistant mutants can be rapidly selected during treatment(19-21) and result in treatment failure, as observed in the past with streptomycin, isoniazid, and rifampin (22). Because the proportion of mutants resistant to fluoroquinolones among a population of susceptible *M. tuberculosis.* is about 10^{-6} , and the size of the microbial population is higer (10^{8}) in cavitary pulmonary tuberculosis (22), it is

difficult to prevent the selection of fluoroquinolone-resistant mutants in patients whose organisms remain susceptible to only a few active drugs.

In the past few years, genetic and molecular insights have unraveled the mechanisms involved in the acquisition of drug resistance by *M. tuberculosis*, concomitant with the development of various molecular strategies to rapidly detect MDRTB.

There are many studies on developing methods for detection of several antimycobacterial drug-resistant *M. tuberculosis.*, such as, rifampin, isoniazid (23), and streptomycin (24). For fluoroquinolone, there are many tries to find the association between gene mutation and drug resistance. High-level resistance to fluoroquinolones in various bacteria has been related to mutations in *gyr*A, the structural gene of the gyrase A subunit. These mutations are virtually always clusterd within a conserved region of the *gyr*A gene referred to as the quinolone resistance-determining region(QRDR) (25-31).

Genetic methods for detecting antibacterial resistance genes, including DNA probes and PCR-based assays, are now used in many clinical laboratories (32) because multidrug-resistant strains of *M. tuberculosis* have been recognized in many hospitals and have become a major public health problem (33).

There are four reasons to pursue the identification of antimicrobial resistance genes by genetic methods as described by Tenover (32). **First**, DNA probes or nucleic acid amplifications techniques are helpful for arbitrating MIC results that are at or near the breakpoint for resistance. For example, oxacillin-resistant isolates of *Staphylococcus aureus* with MICs between 2 and 8 μg/ml may contain the *mec* (methicillin) resistance gene determinant or may produce high levels of β-lactamase that slowly hydrolyze oxacillin. While vancomycin would be the drug of choice for the former cases, β-lactamase hyperproducers can be effectively treated with more effective penicillinase-stable beta-lactams or beta-lactam-β-lactamase inhibitor compound (34-35). A test showing the absence of *mec* gene suggests that a physician could use an antimicrobial agent other than vancomycin to treat the infection.

Second, genetic methods can be used to directly detect resistance genes or mutations that result in resistance in organisms from clinical specimens in order to guide therapy early in the course of a patient's disease, long before cultures are positives. For example, PCR assays can detect mutations in the *rpo*B locus associated with rifampin resistance in *M. tuberculosis* (36). Such mutations indicate that the strain is at least resistant to rifampin and may be resistant to several drugs. A positive PCR result for mutations in the *rpo*B locus directs the physician to avoid rifampin and use alternative antimycobacterial agents.

Third, genetics-based tests are more accurate than antibiograms for monitoring the epidemiologic spread of a particular resistance gene in a hospital or a patient population. For example, tracking the spread of the *van*A vancomycin resistance gene in enterococci with PCR assays has helped document the spread of multiresistant enterococci along the eastern seaboard of the United States (37). Antibiograms cannot differentiate between organisms containing the *van*A and those with derepressed *van*B genes (38).

Fourth, genetics-based tests can be used as the "**gold standard**" for resistance when the accuracy of new susceptibility testing methods that use clinical isolates or stock cultures with borderline MICs is being evaluated (39).

PCR assays have been developed to detect mutations of the *M. tuberculosis* genome. PCR and a single-strand conformation polymorphism(SSCP) assay (a technique that examines the mobility of PCR products in a polyacrylamide gel) can differentiate rifampin-resistant strains of *M. tuberculosis* from susceptible strains with high confidence (36). PCR-SSCP is a rapid detection of mutation technique but there are many environmental factors that effect test sensitivity, including radioisotope, that is not safe for technicians. So one interesting technique is heteroduplex formation(HDF) that is simple and not require for radioisotope.