CHAPTER I

INTRODUCTION

Mycobacterium avium complex (MAC), which includes *M. avium* and *M. intracellulare*, is an important opportunistic pathogen. They are the most frequent isolates among the nontuberculous mycobacteria found in specimens taken from patients. MAC causes a wide range of infection (1-5). Among the most are disseminated disease in persons with acquired immunodeficiency syndrome (AIDS) and invasive pulmonary disease including upper lobe cavitary disease in males who are smoker and often abuse alcohol and nodular interstitial disease in older woman with associated cylindrical bronchiectasis and adolescents with cystic fibrosis. Infections caused by these organisms are not transmissible from person to person (6).

Prior to the emerge of the AIDS epidemic, disseminated MAC disease was extremely rare. MAC infection was recognized in AIDS patients in 1982, after that the number of case increases dramatically because of the high incidences of AIDS epidemic (1). Among HIV-infected individuals, disseminated MAC has historically occurred almost exclusively in patients with very advanced HIV disease and peripheral-blood CD4 T-lymphocyte counts below 50 cells per cubic millimeter (7, 8). Disseminated MAC is the most common systemic bacterial infection in patients with AIDS in developed countries (9, 10). For developing countries in Africa, the report of disseminated MAC is uncommon or absent (9). In Thailand which is epidemic area of HIV infection, the prevalence of disseminated MAC infection is very high and comparable to that in the western countries. Suwanagool et al. (11) studied in AIDS patients who had prolonged fever, most of them (24%) were caused by MAC infection. In 1999, Chuchottaworn et al. (12) reported that disseminated MAC infection was found in 17.4 percent of HIV-infected adults with prolonged fever of unknown cause.

The current data indicate that disseminated MAC infection in the late stage of AIDS patients decreases the quality of life and the patient has much lower survival rate than AIDS

patient without MAC infection (13, 14). Effective prevention and therapy of MAC infection would probably improve the quality of life and survival period of patients. Treating MAC infection requires several drugs because no one drug by itself is effective. MAC bacteria can quickly become resistant to a drug and other drug in the same family. Combination therapy, clarithromycin or azithromycin together with ethambutol and rifabutin is effective and may slow the development for MAC infection (15, 16).

Clarithromycin is a potent semi-synthetic macrolide antibiotic used to treat a variety of bacterial infection especially treatment of MAC infection and prevention of MAC infection in AIDS patients with CD4 cell count below 50 cells per cubic millimeter (17, 18). Clarithromycin inhibits protein synthesis by stimulating dissociation of peptidyltransferase region from the ribosome during the elongation reaction (19). However, clarithromycin resistant strain has been also reported in patients treated with clarithromycin in combination with one or more antimycobacterial drugs (16) and cross-resistance between clarithromycin and azithromycin was confirmed with laboratory mutant and clinical isolates (20, 24).

The major cause of clarithromycin resistance in MAC is due to mutation changes in the central loop of domain V in the 23S rRNA gene. Resistance to clarithromycin in *M. intracellulare* and *M. avium* has been identified as being associated with a mutation in the 23S rRNA gene position adenine 2058 (21-25).

Susceptibility testing of clinically significant isolates of MAC against clarithromycin is useful in certain situations, especially blood isolates from patients with AIDS who are receiving clarithromycin prophylaxis and become bacteremia and isolates from patients with disseminated or invasive pulmonary disease who relapse while on clarithromycin therapy (26). Recently, published guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) for susceptibility testing of the nontuberculous mycobacteria recommended broth microdilution or radiometric broth macrodilution (BACTEC), a method that involves measurement of the production of radiolabeled CO_2 by mycobacteria growing in broth containing a radiolabeled fatty acid as a substrate (26). However, the broth microdilution method is laborious and time-consuming to perform and the radiometric broth

macrodilution (BACTEC) method is relatively expensive and generates radioactive waste which must be disposed of.

The Mycobacteria Growth Indicator Tube (MGIT) method and the Epsilometer (E) test are recently introduced (27, 28). The MGIT system which is non-radiometric method, consists of an oxygen-quenched fluorescent indicator embedded in silicon at the bottom of a tube filled with Middlebrook 7H9 broth. Actively growing mycobacteria consume the oxygen dissolved in the medium, thereby releasing the indicator, whose fluorescence can be detected when the tube is viewed with a 365-nm UV light or in BACTEC 960 automate system. This system was proved to be reliable, rapid and easy to interpretation. The E test is a diffusion method with the ability to produce an MIC result. This method is much easier to perform, produces result quickly, reliable and less expensive.

The prevalence of MAC infection in Thailand is very high and resistance of MAC to clarithromycin will remain a therapeutic challenge in HIV infected patients (11, 17, 29). Ideally, treatment should be given on the basis of the *in vitro* determination of resistance. This requires a rapid and standardized method. The purpose of this study was to investigate the reliablility of the BACTEC MGIT 960 system and the E test for detection of clarithromycin resistance in MAC isolates in comparison with the NCCLS recommended method, broth microdilution. Information obtained could help in the selection of appropriate method for detection of clarithromycin resistance in MAC and thus provide information for management of MAC-infected patients.