CHAPTER VI

DISCUSSION

Mycobacterium avium complex (MAC) is an important opportunistic pathogen, particularly in patients infected with human immunodeficiency virus (HIV). The emergence of resistance during clarithromycin therapy and/or prophylaxis poses a therapeutic problem in human immunodeficiency syndrome (HIV) infected patients (17). Ideally, treatment should be given on the basis of the *in vitro* determination of resistance. This requires a rapid and standardized method. The NCCLS recommended methods for clarithromycin susceptibility testing of MAC are well standardized, but the radiometric method is relative expensive and requires radio-isotope use and the broth microdilution method is laborious and time-consuming to perform. In this study BACTEC MGIT 960 system (Becton Dickinson, U.S.A) and Epsilometer (E) test (E test; AB Biodisk, Solna, Sweden) were evaluated for their abilities to detect clarithromycin resistance for MAC isolates, and the results were compared with those of the broth microdilution method.

At present, there is no recommended interpretive breakpoint for antimicrobial susceptibility of MAC by the BACTEC MGIT 960. In this study, BACTEC MGIT 960-determined MICs was evaluated according to the broth microdilution interpretive breakpoint suggested by NCCLS. One hundred percent agreement between the BACTEC MGIT 960 and the broth microdilution was obtained. Evaluation of susceptibility testing performed with BACTEC MGIT 960 has not been published so far. However, Piersimoni et al. (28), who compared the MGIT system which is fully manual, with radiometric BACTEC 460TB method based on the interpretive breakpoint of BACTEC proposed by Heifets. They found that excellent agreement was demonstrated for all MAC isolates to clarithromycin and 100% of the result correlated within ± 2 $\log_2$ dilution step. They suggested that this system was reliable, rapid and easy to interpretation.

For the E test, previous study demonstrated an excellent correlation of the E test results with those obtained by agar dilution. Lebrun et al. (27), who compared the E test with the agar dilution method to assess the *in vitro* activites of clarithromycin against MAC, found that 70% and 100% of the results correlated within ±1 and ±2 $\log_2$ dilution step, respectively. No major errors resulting in misclassification in susceptibility or resistance categories were detected for the E-test MIC method. Thiermann et al. (23), who assessed the phenotypic reproducibility, found that E test 56/59 E test replicates were within ±1 $\log_2$ dilution step and 3/59 were ±2 $\log_2$ dilution step.

In the present study, 95% of the E-test-determined MICs were within ±1 $\log_2$ dilution step and 100% were within ±2 $\log_2$ dilution step of those determined by the broth microdilution method. When E-test-determined MIC was evaluated according to the broth microdilution interpretive breakpoint, excellent agreement was demonstrated for all MAC clinical isolates. Lebrun et al. (27) reported for the first time the E test for susceptibility testing of clarithromycin for MAC using the interpretive breakpoint for determining susceptibility at MIC of ≤8 μg/ml. They found that all susceptible MAC isolates had low-level E-test MICs of clarithromycin (ranged from 0.5 – 1.5 μg/ml) and no strain gave an intermediate MIC. In this study, when E-test determined MIC was evaluated according to the interpretive breakpoint used by Lebrun et al., 7 isolates (MIC 12-16 μg/ml) would be interpreted as intermediate to clarithromycin. However, MIC determined by broth microdilution method of these 7 isolates were of 8 μg/ml which were interpreted as susceptible (Tables 6 and 7). Moreover, sequencing analysis revealed the wild type genotype at A2058 and A2059 of these 7 isolates. Therefore, the E test MIC of ≤16 μg/ml was interpreted as susceptible in this study. In addition, all resistant MAC isolates had high-level MICs of >256 μg/ml. The isolate which had cut-off MIC of resistance was not found. It was therefore not possible to correlate the cut-off MIC for resistant determined by E test with that of broth microdilution method. However, in this study, E-test MIC for resistant was interpreted according to that of broth microdilution although 5% of MICs level determined by E test was two-fold higher than that determined by broth microdilution method (Table 8).

Most gram-positive and gram-negative bacteria contain multiple copies of the 23S rRNA gene in chromosome. Hence, a mutation involving only one of these genes would a rare cause of clarithromycin resistance among bacterial species. Previous studies in MAC have shown that they contain only a single chromosomal copy of the 23S rRNA gene (21, 101) and hence would be highly susceptible to mutation involving the gene.

Previous studies demonstrated that transition of adenine (A) to guanine (G) and transversion of adenine (A) to cytosine (C) or thymidine (T) point mutation at position 2058 or 2059 within domain V of the 23S rRNA gene are a cause of clarithromycin resistance in MAC (21-25). A2058 and A2059 are thought to be involved in macrolide binding for several reason. The *E.coli* ribosomal binding site for macrolides has been shown to involve the unpaired residues A2058 and A2059 of the 23S rRNA (103). Mutation in residue A2058 reduced macrolide binding affinity of the ribosomes, resulting in resistance bacterium (103, 121) to this drug. Although the reduced macrolide binding affinity of mutant ribosomes may be due to the actual base change within the binding site, there was evidence for a localized conformation change in the peptidyl transferase loop of the 23S rRNA. Nash and Inderlied (21), reported that in *M. avium*, ribosomal resistance to macrolides involves both a base mutation and conformational change in the ribosomal at the macrolide binding site.

In the present study, all clarithromycin-resistant strain had high level resistance to clarithromycin (MIC >256 μg/ml) as demonstrated by the three different methods. Sequencing analysis showed A2058G mutation in 2 isolates and A2058C mutation in 1 isolates. The result suggests the association of A to either C or G (A2058, -C or -G) mutation with high level resistance (MIC >256 μg/ml). This finding is consistent with the result of Jamal et al. (22), which demonstrated the change of A to either C or G at position 2058 or 2059 (A2058/2059, -C or -G) conferred high level resistance (MIC ≥100 μg/ml) (22). Thus our results support the fact that the A2058/2059 mutation in the 23S rRNA gene of MAC is linked to clarithromycin resistance.

Data from this study demonstrated that BACTEC MGIT 960 method and E test are reliable methods when compared with a broth microdilution. Thus additional consideration

such as resources in term of personel and facilities, as well as turnaround time and cost of a given assay will guide the choice of method for the detection of clarithromycin-resistant MAC. In our hands, the BACTEC MGIT 960 and E test were technically easy to perform, and the result were easier to interpret than broth microdilution method. The turnaround time for the unambiguous identification of resistance was 15, 12 to 14 (median, 13), and 13 days for the broth microdilution method, BACTEC MGIT 960 and E test, respectively. The price for each test was 520, 180 and 80 bahts for the BACTEC MGIT 960, E test and broth microdilution method, respectively.

BACTEC MGIT 960 show promise as a non radiometric method for detection of clarithromycin resistance in MAC. However this method is to expensive for determination MICs of each isolates. Therefore, only cut-off MIC of resistance (64 μg/ml) was determined to compensated high cost. This method will thus provide only qualitative result of resistance in state of MIC result. On the basis of these considerations the implementation of E test for determining susceptibility of MAC to clarithromycin may prove to be a good choice for many diagnostic mycobacteriology laboratories, because of the reliability, ease of use, speed and the price.