CHAPTER V

DISCUSSION

Three hundred and ninety-seven of HIV-infected patients were included for this study. The BACTEC MYCO/F Lytic blood culture bottle (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) was used to recover fungi, mycobacteria and bacteria. The sensitivity and specificity of AFB smear of blood culture, after the BACTEC system showed the signal of growth, were 87.4% and 99.3% respectively. The sensitivity of AFB smear was slightly low because of BACTEC system detected the rate of oxygen decrease as measured by increasing fluorescence determines the presumptive positivity of each sample (Esteban et al, 2000). In case of specificity, 2 of 302 cases were smear positive but culture negative. We suspected the patients infected with fastidious mycobacteria because these bacteria required a growth supplement; hematin or mycobactin (Dawson and Jennis 1980; Markesich *et al*, 1988). In this study, the supplements were not added to the solid media.

Ninety-five cases (23.9%) out of 397 patients were positive for mycobacteria by hemoculture. Thirty-three percent of these mycobacteria were *M. tuberculosis* complex and 38% were *M. avium*. The disseminated infection with *M. avium* is a frequency late complication of HIV infection. During the natural course of HIV-infection up to 15% of patients will suffer from this opportunistic infection (OI) within 12 months after their CD4 count has fallen below 50 cells/mm^3 (Nightingale *et al*, 1992). The recovery rate of mycobacteria from HIV-infected patients was the same rate as report of Di Lonardo *et al* (1995), 21.1% of 240 cases but not for *M. tuberculosis* and *M. avium* which were 92.9% and 5.8%, respectively. In Thailand, Chuchottaworn *et al* (1999) reported the mycobacterial hemocultures were positive for *M. avium* complex (MAC) in 58 patients (17.4%) and positive for *M. tuberculosis* in 34 patients (10.2%). The results of their study have proved that MAC infection, indeed, exists among Thai AIDS patients. So, the prevalence of MAC infection in Thailand was very high and comparable to that in the western countries. In Colombia, Muricia-Aranguen *et al*, (2001) reported that *M. tuberculosis* was identified in 4 cases (25%). *M. avium* was identified in 12 cases (75%).

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Sensitivity and specificity of AFB smear of sputum compared with culture were 43.9% and 97.8% respectively. Conde *et al* (1999) reported the sensitivity of AFB smear of sputum by using the Ziehl Neelsen technique after sedimentation at 3,000xg was only 31%. The sputum AFB sensitivity was lower than the ones reported in New Jersey, USA (Mathew *et al*, 2002) where sputum AFB smear had a sensitivity of 67.5% because the smears were screened by using auramine O stain followed by Kinyoun stain confirmation.

In this study, sputum from 171 (43.1%) of 397 patients were collected. Sixteen cases of mycobacteria were isolated from both blood and sputum as shown in Table 8. Same species of mycobacteria were isolated both blood and sputum from 12 patients; *M. tb* complex 3 cases, *M. avium* 6 cases, *M. intracellulare* 1 cases, and *M. scrofulaceum* 2 cases. Only 4 cases; *M. tb* complex and *M. avium* 1 case, *M. avium* and *M. tb* complex 1 case, *M. intracellulare* and *M. tb* complex 1 case, and *M. scrofulaceum* and *M. intracellulare* 1 case, mycobacteria isolated from blood and sputum were different because HIV-infected patient was low immunity. So, patients who have CD4 < 50 cells/mm^3 could be infect with mix organisms.

For the result of mycobacteria culture of environmental samples, *M. avium* was not detected. The reason may be the high rate of contamination in cultures and high number of rapid-growing mycobacteria. The contamination rate was 20.1% of the environment samples. Although, *M. avium* was not detected but other slow-growing mycobacteria including *M. intracellulare*, *M. scrofulaceum*, *M. kansasii*, unclassified MAC and *Mycobacterium* spp. were isolated. In a study examining the incidence of MAC organisms by von Reyn *et al* (1993), MAC organisms were isolated from 18 of 52 (35%) of the water samples in United States and Finland, whereas they were 4 of 39 (10%) samples in Zaire and Kenya. Yajko *et al* (1995) investigated samples which were collected from the home environment of 290 persons with HIV infection for mycobacteria. Although mycobacteria were recovered from numerous environmental samples, isolates reactive with MAC-specific probes were recovered from only 4 of 528 water samples and only 1 of 397 food samples. The species *M. avium* was recovered from one water sample (0.19%) and one food sample. In contrast, MAC organisms were recovered from 55% and *M. avium* from 27% of soil samples from the patients' houses. With used of serotype and multilocus enzyme electrophoresis analysis, some of soil isolates were found to be similar to isolates recovered from study patients. The results of their suggested that soil, rather than water, may be a significant

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reservoir of organisms causing MAC infection. Covert *et al* (1999) reported that MAC organisms were isolated from 6 (19%) of 32 NTM-positive samples collected from four water systems supplied by surface water, and *M. avium* was isolated from 1 (3%) of 32 NTM-positive samples from one of these systems. Overall, MAC organisms were found in 9% of drinking water supplies examined. The occurrence of MAC organisms reported in this study was lower than the occurrences found in other studies. The result may be attributed to the limited number of samples. Although, many studies reported that MAC recovered from environmental samples but the species *M. avium* was very few.

The result of identification of 27 slow-growing mycobacteria isolated by using PCR-restriction enzyme analysis method (Sansila *et al*, 1998) could not specifies species of the bacteria. Only one unspecified bacteria was isolated from patient's blood. The others were isolated from environmental samples. The isolate had about 4 amplified product sizes. (Hemoculture no. 1792) The products were about 320, 380, 420 and 550 base pairs. This isolate will be investigated further.

DNA fingerprints of *M. avium* from HIV-infected patients were analyzed. The number of IS*1245*-hybridization patterns varied from 0 to 31. Twenty isolates from 11 patients were not hybridized with IS*1245* probe. This indicated that the isolates did not have IS*1245* in their chromosome. The bacteria were confirmed as *M. avium* by PCR-restriction enzyme analysis method and dot blot hybridization method (Figure 19) developed by Sansila *et al* (1998). The result showed the isolates without IS*1245* belonged to *M. avium*. The isolates were also tested by the PCR amplification method developed in this study. The first primer set was, P1 and P2, specified to IS*1245* and the second primer set was, 16SC and 23SG, specified to 16S-23S spacer DNA. The second primer was used as self internal control. The results showed all of the isolates had only one amplified product size of about 380 base pairs. This, together with some studies reported that some isolates of *M. avium* did not contain IS*1245*. Bauer *et al* (1999) reported that 5 (2.8%) of 179 *M. avium* subsp. *avium* isolates carried on copies of IS1245 and were consequently not typeable by RFLP analysis. In addition, Panunto *et al* (2003) reported that 2 of 43 isolates of *M. avium* did not contain IS*1245*. Oliveira *et al* (2003) reported that 4 of 25 human isolates lacked IS*1245* reacted with AccuProbe reagent specific for *M. avium* and they were positive for amplification by PCR with IS*1245*- and IS*1311*-specific primers. However, the 4 strains were

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nevertheless repeatedly negative by IS*1245*-based RFLP analysis. The same membranes with all four isolates were positive when they were subsequently probed by RFLP analysis with a PCR-derived DT6-specific fragment.

As concluded in the previous studies (Picardeau *et al*, 1997; Ritacco *et al*, 1997), IS*1245* was found to be a useful tool for the differentiation of *M. avium* strains, and a considerable degree of polymorphism among was observed. Of the 76 *M. avium* isolates from 39 patients, 20 isolates from 11 patients (28.2%) carried no copies of IS1245 and were consequently not typeable by RFLP analysis. The remaining 56 isolates from 28 patients (71.8%) had of IS*1245*-hybridization bands. The *M. avium* isolates can be classified into 5 groups (I, II, III, IV and V). Group I had 2 to 4 bands. Group II had 17 to 31 bands. Group III had 13 to 14 bands. Group IV had 8 to 9 bands. Group V had no band. Group I was called "a low-copy-number group". This criterion was described previously by Bauer *et al* (1999). In this study, a less stringent cutoff of 80% was chosen to facilitate identification of clusters of isolates that differ a modest at fraction of the insertion sequence loci. Pestel-Caron and Arbiet (1998) observed the frequent transposition of IS*1245* in vivo among independent isolates collected at a single time, isolates collected from individual patients over time, and isolates collected from multiple patients. Bauer and Andersen (1999) confirmed the frequent observation of one- to two-band changes when they analyzed single colonies of *M. avium* from cultures. Thus, among isolates with ≥ 80% similarity, at least some of the variations in RFLP profiles could represent relatively recent events.

So, the pattern can be classified into 23 clusters (A to W). In each cluster, the hybridization pattern was identical except cluster K and V. In cluster K, four isolates were isolated from blood of a patient. Two isolates were isolated in the first time of admit. One of them had 26 bands and the other had 25 bands (Figure 16).

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Figure 16 Showed the duplicate of RFLP pattern (A and B) had different in number of band. Arrows indicate the position where changes occurred.

In cluster V, we also observed one- to two-band changes in the IS*1245*-based RFLP profiles among isolates which isolated from 2 patients (Figure 17). The patients were different in age and they were admitted in different hospital.

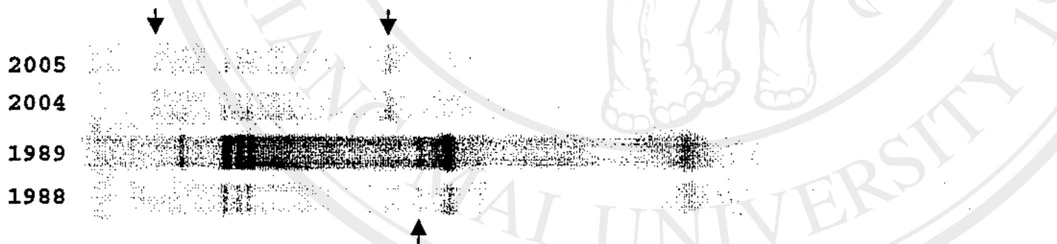

Figure 17 Showed the RFLP pattern (Cluster V) of *M. avium* isolates that isolated from blood culture of 2 patients. Arrows indicate the position where changes occurred.

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The largest cluster is cluster C that all isolates had 2 bands and had identical RFLP pattern. Eight isolates were collected from 4 patients and 2 different hospitals (Nakornping Hospital and Maharaj Nakorn Chiangmai Hospital). In general, the largest cluster of IS*1245*-RFLP pattern in other studies had more the number of bands. In Denmark, the largest cluster had 21 bands and all 15 MAC isolates were isolated from patients. Eight isolates were collected from HIV-infected patients and 7 isolates were collected from non-HIV-infected patients (Bauer *et al*, 1999). In Brazil, Oliveira *et al* (2003) reported only one isolate had 2 bands. An isolate was from

bone marrow of a HIV-infected patient, which exhibited a different PRA pattern of amplified product of *hsp*65 loci (*M. avium* PRA variant IV) and a non-clustered two-band IS*1245*-RFLP pattern.

In this study, a patient re-infected with *M. avium* was found. The patient was admitted at Maharaj Nakorn Chiangmai Hospital. The result of hemoculture was AFB positive and the bacteria were classified as *M. avium*. Also, *M. avium* was detected in sputum specimen. The IS*1245*-RFLP pattern of *M. avium* had 2 bands and classified as cluster C. Two months later, she was admitted again and the hemoculture result was still AFB positive but no growth of mycobacteria. One year later, *M. avium* was still detected from the blood. The IS*1245*-RFLP pattern of the bacteria had 9 bands and the pattern was classified as cluster R. The comparison of IS*1245*-RFLP pattern from the patient was shown in figure 18. Terriani *et al* (1996) and Oliveira *et al* (2000) found *M. avium* in bone marrow and other organs of abacteremic AIDS patients. *M. avium* may establish an infection in tissue which then gave rise to the release of small numbers from organisms into blood (Kemper *et al,* 1994). Intermittently, positive blood cultures may reflect relatively light burdens of *M. avium* in tissues, and the tissue reservoir may respond to treatment more slowly than blood.

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Figure 18 Showed the comparison of IS*1245*-RFLP pattern from the patient who was re-infected with *M. avium*. A was the IS*1245*-RFLP pattern of *M. avium* which detected at the first time. B was the pattern of *M. avium* which detected one year later.

Picardeau *et al* (1997) reported that the IS*1245*-RFLP patterns of *M. avium* recovered from 39 patients with relapse (Patients with a negative blood culture for 2 months and a subsequence positive culture was considered to have a relapse). The RFLP pattern showed identical patterns for strains isolated throughout bacteriological follow-up for 37 patients, indicating monoclonal infections. For 2 patients, RFLP patterns of strains isolated during follow-up were different from those of the initial isolates.

This study found that 23 IS*1245* patterns from 28 patients. So, the diversity of *M. avium* was 82 %.

%Diversity = (number of clusters/number of patients) x 100

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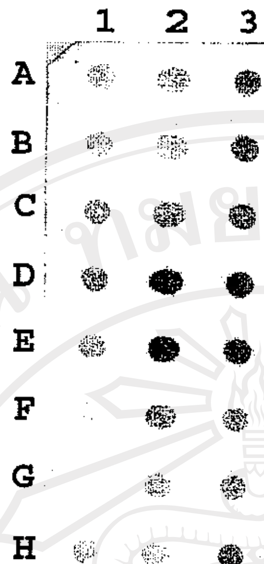

Figure 19 Dot Showed dot blot hybridization of *M. avium* which carried no copies of IS*1245* by MV222 probe

A1-F3 and G2, G3 = The 20 *M. avium* isolates which carried no copies of IS*1245*.

G1 = *M. intracellulare* ATCC 13950

H1 = *M. avium* ATCC 25291

H2 = M. *avium* no. 632

H3 = M. *avium* no. SST

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