CHAPTER IV

RESULTS

From August 2000 to June 2002, a total of 793 blood cultures and 296 sputa were collected from 397 HIV-infected patients. One hundred and forty-six patients (36.8%) were collected from Maharaj Nakorn Chiang Mai Hospital. One hundred and thirty patients (32.7%) were collected from Nakornping Hospital. One hundred patients (25.2%) were collected from Sanpatong Hospital. Twenty-one patients (5.3%) were collected from Sansai Hospital (Table 3).

Table 3 Number of patients from four hospitals

Hospital	**the number of patients (%)**
Maharaj Nakorn Chiang Mai	146 (36.8)
Nakornping	130 (32.7)
Sanpatong	100 (25.2)
Sansai	21 (5.3)
Total	397 (100)

1. CULTURE RESULTS

1.1 Blood culture results

Clinically significant microbial growths, including the growth of fungi, mycobacteria and bacteria, were detected in 169 patients (42.2%). According to the infection, the patients are classified as the following 2 groups, single organism infection and mixed organisms infection. The list and number of isolate of microorganisms are shown in Table 4.

1.1.1 Single organism infection

- Fungi 40 patients
- Mycobacteria 90 patients
- Bacteria 32 patients

1.1.2 Mixed organisms infection

- *Corynbacterium* spp. and *M. tuberculosis* complex 1 patient
- *Escherichia coli* and *M. avium* 1 patient
- *M. avium* and *Penicillium marneffei* 1 patient
- *M. scrofulaceum* and *Rhodococcus equi* 1 patient
- *M. avium* and *Salmonella enteritidis* (gr. B) 1 patient
- *Penicillium marneffei and Salmonella enteritidis* (gr. B) 1 patient
- *Enterobacter sakazakii, Escherichia coli* and *Klebsiella pneumoniae* 1 patient

Table 4 List of microorganisms detected from 169 patients

Organism	Number of isolates
Acinetobacter baumannii	1
Corynbacterium sp.	5
Cryptococcus neoformans	22
Enterobacter sakazakii	2
Enterococci (Beta type)	1
Escherichia coli	6
Klebsiella pneumoniae	1
Micrococcus luteus	1
Micrococcus sp.	2
Mycobacterium avium	63
Mycobacterium intracellulare	20
Mycobacterium scrofulaceum	20
Mycobacterium tuberculosis complex	52
Mycobacterium sp.	1
Unclassified *Mycobacterium avium* complex (MAC)	6
Penicillium marneffei	55
Proteus mirabilis	2
Rhodococcus equi	7
Salmonella enteritidis (gr. B)	24
Salmonella enteritidis (gr. D)	6
Staphylococcus aureus	4
Staphylococcus epidermidis	5
Staphylococcus sp.	1
Streptomyces sp.	2
Total	309

1.1.3 Comparison of AFB stain and culture results of blood culture

Ninety-five (23.9%) of the 397 patients were positive for *Mycobacterium* spp. isolated from blood samples. Hemoculture from 83 patients were positive with both Ziehl-Neelsen (ZN) staining and sub-culturing on LJ medium. Only 2 patients were positive for ZN staining but sub-culturing negative. Twelve cases were smear negative but sub-culturing positive. Three hundred cases were negative both smear and culture. Hence, sensitivity and specificity of ZN staining from positive signaling of BACTEC hemoculture were 87.4% and 99.3%, respectively when compared with the sub-culture results (Table 5). In addition, the mycobacteria were isolated from 162 (20.4%) of the 793 blood samples cultured.

Table 5 Comparison of AFB staining with sub-culture results

		SUBCULTURE (Number of patient)		
		POSITIVE	**NEGATIVE**	**TOTAL**
AFB Smear*	**POSITIVE**	**83**	**2**	**85**
	NEGATIVE	**12**	**300**	**312**
	TOTAL	**95**	**302**	**397**

*The AFB smears were done after the BACTEC system showed the signal of growth.

$$\text{Sensitivity} = \frac{\text{the both positive AFB and sub-culture} \times 100}{\text{the total positive sub-culture}}$$

$$= (83 \times 100)/95$$

$$= 87.4\%$$

$$\text{Specificity} = \frac{\text{the both negative AFB and sub-culture} \times 100}{\text{the total negative sub-culture}}$$

$$= (300 \times 100)/302$$

$$= 99.3\%$$

1.2 Sputum culture results

Two hundred and ninety-six sputum samples were collected from 171 patients. Thirty-four sputa collected from 26 patients were positive with ZN staining but 5 out of 34 were culture negative. While 66 sputa collected from 37 patients were positive with the culture, 37 out of 66 were smear negative. Two hundred and twenty-five sputa were both smear and culture negative. Only two of all samples (0.7%) were contaminated (Table 6 and 7). The sensitivity and specificity of ZN staining were 43.9% and 97.8%, respectively, when compared with culture results.

Table 6 The results of sputum culture

Organism	**Number of specimen**	**Number of patient**
Mycobacterium spp.	**66**	**37**
Contaminated	**2**	**1**
No growth	**228**	**133**
Total	**296**	**171**

Table 7 Comparison of AFB staining and culture results of sputum

		CULTURE (Number of specimen)		
		POSITIVE	**NEGATIVE**	**TOTAL**
AFB smear	**POSITIVE**	**29**	**5**	**34**
	NEGATIVE	**37**	**225**	**262**
	TOTAL	**66**	**230**	**296**

$$\text{Sensitivity} = \frac{\text{the both positive AFB and Culture x 100}}{\text{the total positive culture}}$$

$$= (29 \times 100)/66$$

$$= 43.9\%$$

$$\text{Specificity} = \frac{\text{the both negative AFB and culture x 100}}{\text{the total negative culture}}$$

$$= (225 \times 100)/230$$

$$= 97.8\%$$

Table 8 Mycobacteria isolated from blood and sputum in same patient

Blood	**Sputum**	**Number of case(s)**
M. tuberculosis complex	*M. tuberculosis* complex	3
M. tuberculosis complex	*M. avium*	1
M. avium	*M. avium*	6
M. avium	*M. tuberculosis* complex	1
M. intracellulare	*M. intracellulare*	1
M. intracellulare	*M. tuberculosis* complex	1
M. scrofulaceum	*M. scrofulaceum*	2
M. scrofulaceum	*M. intracellulare*	1

1.3 The culture results of water supply, soil and stool of livestock

All 492 samples were collected from five different types of sources; four hospitals, 2 jails, *M. avium*-infected patients' houses, river and communities. One hundred and thirty-three mycobacteria (27.0%) were isolated. Two hundred and sixty samples (52.8%) were negative for mycobacteria growth and 99 samples (20.1%) were contaminated.

1.3.1 Water sources

One hundred and ninety-seven water samples were collected from the sources as described above including public drinking water utilities, cisterns, bottled waters, drinking water treatment samples, ice samples from commercially available sources and Ping River. The mycobacteria were isolated 44 (22.3%) isolates from 197 samples (Table 9). One hundred and thirty-four (68.0%) samples were negative and 19 (9.6%) samples were contaminated.

Table 9 Numbers of detected mycobacteria in water samples

Water source (No. of samples)	No. (%) of positive samples for mycobacteria detection
Hospitals (70)	20 (28.6%)
Jail (12)	1 (8.3%)
Communities (51)	7 (13.7%)
Patients' houses (37)	12 (32.4%)
Ice (11)	3 (27.3%)
River (16)	1 (6.2%)
Total (197)	44 (22.3%)

1.3.2 Soil sources

The mycobacteria were isolated 84 (30.0%) isolates from 280 soil samples (Table 10). One hundred and twenty-three samples (43.9%) were no mycobacteria detection and 73 samples (26.1%) were contaminated.

Table 10 Numbers of detected mycobacteria in soil samples

Soil source (No. of samples)	No. (%) of positive samples for mycobacteria detection
Hospital (120)	44 (36.7%)
Jail (9)	3 (33.3%)
Communities (79)	20 (25.3%)
Patient's house (72)	17 (23.6%)
Total (280)	84 (30.0%)

1.3.3 Stool

Fifteen stool samples were collected from the livestock and pets from communities and patients' houses. Five out of 15 stool samples (33.3%) were positive for mycobacteria detection (Table 11). Three samples (20%) were negative for mycobacteria detection and 7 samples (46.7%) were contaminated.

Table 11 Numbers of detected mycobacteria in stool

Stool (No. of samples)	No. (%) of positive samples for mycobacteria detection
Swine (7)	5 (71.4%)
Bird (7)	0 (0%)
Chicken (1)	0 (0%)
Total (15)	5 (33.3%)

2. IDENTIFICATION OF MYCOBACTERIA

2.1 Growth rate

The study showed the growth of all *Mycobacterium* spp. strains from the patients. The growth took more than 7 days to appear on the media. So, all the bacteria are **slow-growing mycobacteria** (Grange JM, 1996.). In case of *Mycobacterium* spp. strains collected from their environment, both slow growing mycobacteria and **rapid-growing mycobacteria** were found. **Only the slow-growing mycobacteria were identified by PCR-REA in the experiment.** The results of this experiment are shown in Table 12.

Table 12 Numbers of slow-growing and rapid-growing mycobacteria identification from this study

Sample sources	Mycobacteria isolates	Slow-growing	Rapid-growing
Blood	162	162	0
Sputum	66	66	0
Environment*	128	57	71
Total	356	285	71

* Five samples were developed contamination.

2.2 Polymerase Chain Reaction – Restriction Enzyme Analysis (PCR-REA) by Sansila *et al.* (1998)

DNA from all 285 slow-growing mycobacteria strains could be amplified with primers 16SC and 23SG. The amplified products from the mycobacteria contained only single fragment about 380 base pairs (Figure 8A). The restriction endonuclease enzyme, *Hae*III, was used to digest the fragments. The digested products were run by electrophoresis and several patterns were shown. The patterns can be classified into 6 groups (Table 13). The first group, *Hae*III-digested products; 200, 120 and 55 base pairs were classified as *M. tuberculosis* complex 91 isolates. The second group, *Hae*III-digested products; 155, 115, 65 and 40 base pairs were classified as *M. avium* 72 isolates. The third group, *Hae*III-digested products; 180, 150 and 40 base pairs were classified as *M. intracellulare*, *M. scrofulaceum* and unclassified MAC. But after digested with *Msp*I, the products in the 3rd group exhibited 2 different patterns. Pattern I, *Msp*I-digested products; 220, 105 and 50 base pairs were classified as *M. intracellulare* and unclassified MAC 40 isolates. Pattern II, *Msp*I-digested products; 150, 105, 75 and 50 base pairs were classified as *M. scrofulaceum* 38 isolates. The fourth group, *Hae*III-digested products; 200 and 180 base pairs were classified as unclassified MAC 16 isolates. The fifth group, *Hae*III-digested products were no digested but *Bst*XI-digested products; 245 and 130 base pairs were classified as *M. kansasii* 1 isolate (Figure 8B). The last group, after digested the amplified products with *Hae*III, the patterns of digested products was not similar to the other group. They were classified as *Mycobacterium* sp. 27 isolates.

Table 13 Showed the PCR-REA identification

PCR product (bp)	*Hae*III-digested products (bp)	*Msp*I-digested products (bp)	Species	Number (isolates)
~380	200, 120, 55 **(Gr. 1)**	NA	*M. tuberculosis* complex	91
~380	155, 115, 65, 40 **(Gr. 2)**	NA	*M. avium*	72
~380	180, 155, 40 **(Gr. 3)**	220, 105, 50 **(Pattern I)**	*M. intracellulare* and Unclassified MAC	40
~380	170, 155, 40 **(Gr. 3)**	150, 105, 75, 50 **(Pattern II)**	*M. scrofulaceum*	38
~380	200, 180 **(Gr. 4)**	NA	Unclassified MAC	16
~380	No digestion* **(Gr. 5)**	NA	*M. kansasii*	1
~380	- **(Gr. 6)**	NA	*Mycobacterium* spp.**	27
				285

* The un-digested PCR product could be digested with *Bst*XI and the fragments were 245 and 130 base pairs.

** There were no the information for identification available.

NA = no analysis

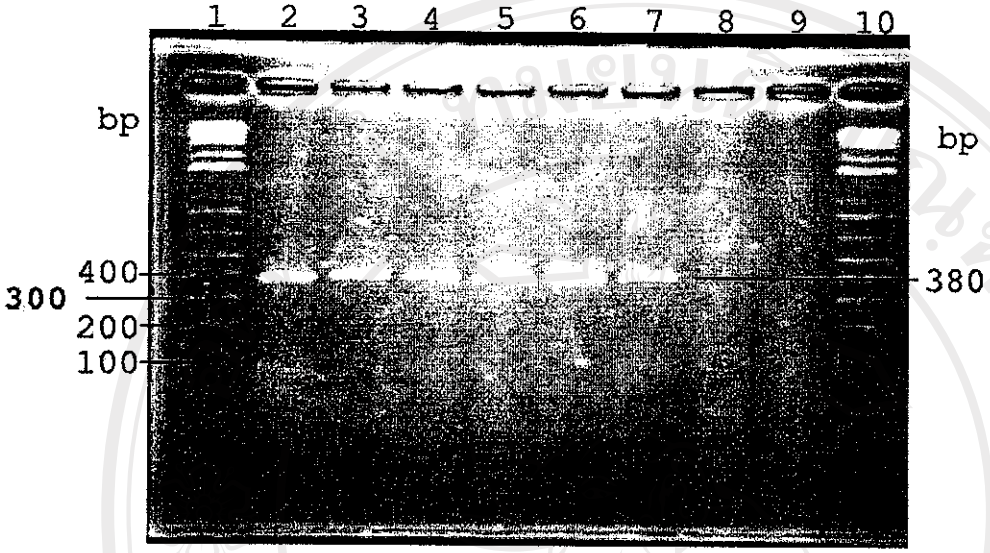

Figure 8A Showed the electrophoresis of about 380 base pairs PCR products of the slow-growing mycobacteria in 1.5% agarose gel

Lane 1, 10 1 Kb plus Marker

Lane 2 PCR product of *M. avium*

Lane 3 PCR product of *M. intracellulare*

Lane 4 PCR product of *M. kansasii*

Lane 5 PCR product of *M. scrofulaceum*

Lane 6 PCR product of *M. tuberculosis* complex

Lane 7 PCR product of unclassified MAC

Lane 8 Negative control in the system of master-mix preparation

Lane 9 Negative control in the step of DNA adding

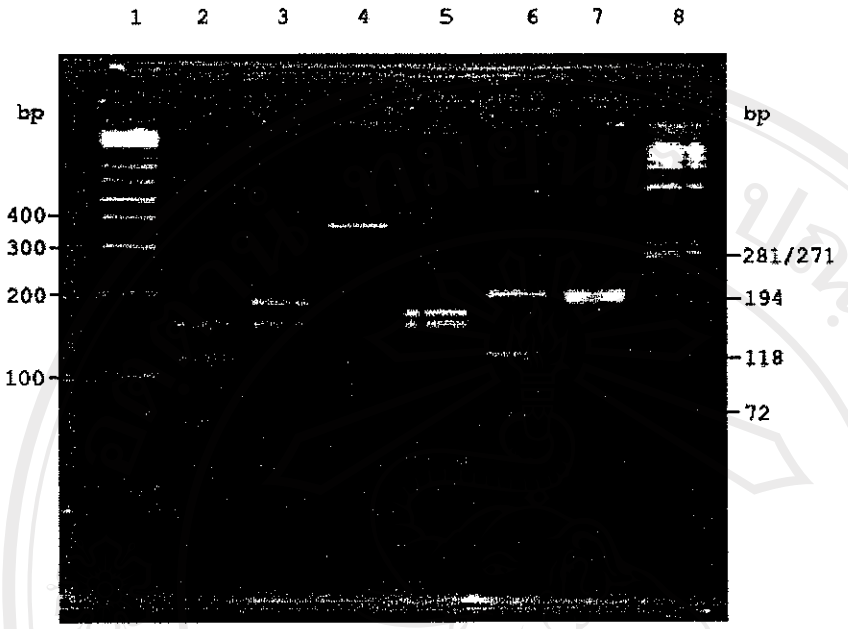

Figure 8B Showed the *Hae*III digested pattern of the slow-growing mycobacteria in 3% agarose 1000 gel

Lane 1 1 Kb plus Marker

Lane 2 PCR-REA products of *M. avium* (155, 115, 65 and 40 bp)

Lane 3 PCR-REA products of *M. intracellulare* (180, 155 and 40 bp)

Lane 4 PCR-REA products of *M. kansasii* (380 bp, non-digested)

Lane 5 PCR-REA products of *M. scrofulaceum* (170, 155 and 40 bp)

Lane 6 PCR-REA products of *M. tuberculosis* complex (200, 120 and 55 bp)

Lane 7 PCR-REA products of unclassified MAC (200 and 180 bp)

Lane 8 ϕX174 RF DNA/*Hae*III Marker

2.3 Dot blot hybridization

2.3.1 Amplified products of *Mycobacterium* spp. hybridization by MI231- and MV222-probe

In order to confirm the results of PCR-restriction enzyme analysis identification, the dot blot hybridization experiment was performed by using the probes, MI231 and MV222, which were specific fragments to *M. intracellulare* and *M. avium* respectively. The amplified products of the *Hae*III-digested patterns group 2, group 3 (pattern I) and group 4 were tested in this experiment.

2.3.1.1 Amplified products of Group 2

Seventy-two amplified products of these mycobacteria were dot blotted onto nylon membrane. Then, the membrane was hybridized by both probes. All of them can be hybridized by MV222-probe but can not be hybridized by MI231-probe.

2.3.3.2 Amplified products of Group 3, Pattern I

Forty amplified products of these mycobacteria were dot blotted onto nylon membrane and hybridized by MI231- and MV222-probes. All of them can not be hybridized by MV222-probe but all can be hybridized by MI231-probe.

2.3.3.3 Amplified products of Group 4

Sixteen amplified products of these mycobacteria were dot blotted onto nylon membrane. Then, the membrane was hybridized by both probes. All of them can not be hybridized by both probes.

From the results of PCR-restriction enzyme analysis and dot blot hybridization, the over all of slow-growing mycobacteria could be identified and were shown in Table 14. *M. tuberculosis* complex was 91 isolates. *M. avium* was 72 isolates. *M. intracellulare* was 40 isolates. *M. scrofulaceum* was 38 isolates. Unclassified MAC was 16 isolates. *M. kansasii* was 1 isolate. *Mycobacterium* spp. was 27 isolates (Figure 9).

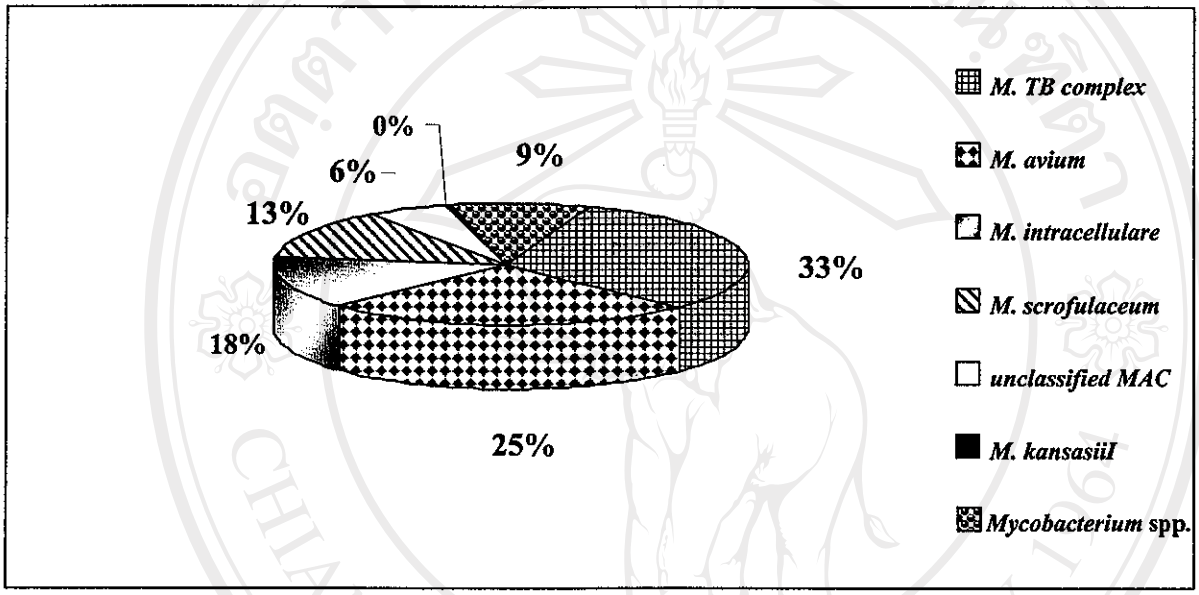

Figure 9 The circular graphic showed the percentage of each species of slow-growing mycobacteria detection

The *M. avium* were only isolated from 36 patients (9.1%) out of 397 patients but not found in the environment and were 31.6% of 228 isolates (Table14) of mycobacteria isolated from patients.

Table 14 Showed the species of slow-growing mycobacteria isolated in this study

Source	Blood	Sputum	environment	Total
***M. tuberculosis* complex**	52	39	0	**91**
M. avium	63	9	0	**72**
M. intracellulare	20	8	12	**40**
M. scrofulaceum	20	7	11	**38**
Unclassified MAC	6	3	7	**16**
M. kansasii	0	0	1	**1**
***Mycobacterium* spp.**	1	0	26	**27**
Total	**162**	**66**	**57**	**285**

3. RESTRICION FRAGMENT LENGTH POLYMORPHYSM (RFLP)

3.1 Hybridization of *M. avium* using IS*1245* as probe.

The *Pvu*II-digested chromosomal DNA of *M. avium* was hybridized with the IS*1245* probe. Fifty-six isolates could be hybridized with IS*1245* probe. Twenty isolates could not be hybridized with the probe indicating that these did not have IS*1245*. The others (56 isolates) had 2 to 31 IS*1245*-hybridized bands. A distribution of number of IS*1245*-hybridized bands was shown in Figure 10. The RFLP pattern could be classified into 5 groups. **The first group (I)** was from 17 isolates collected from 9 patients. There were 2 to 4 bands, 10, 5, and 2 isolates from 6, 2, and 1 patients showed 2, 3 and 4 bands respectively. **The second group (II)** was from 9 isolates collected from 4 patients. There were 8 to 9 bands, 5 and 4 isolates from 2 and 2 patients showed 8 and 9 bands respectively. **The third group (III)** was from 9 isolates collected from 5 patients. There were 13 to 14 bands, 4 and 5 isolates from 2 and 3 patients showed 13 and 14 bands respectively. **The fourth group (IV)** was from 21 isolates collected from 10 patients. There were 17 to 31 bands, 1, 2, 2, 4, 2, 3, 1, 2, 2, and 2 isolates from 1, 1, 1, 2, 1, 2, 1, 1, 1, and 1 patients showed 17, 18, 21, 23, 24, 25, 26, 28, 30, and 31 bands respectively. The others were from 20 isolates collected from 11 patients that did not hybridize with IS*1245* probe (Table 15).

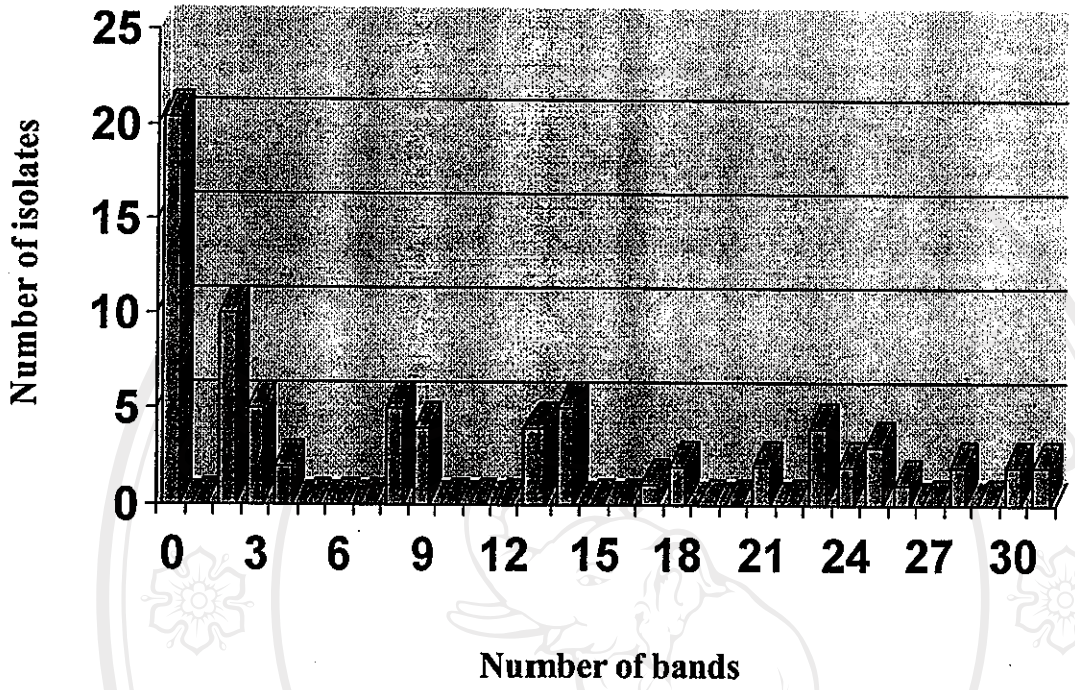

Figure 10 The distribution of isolates with different number of IS*1245*-hybrization bands

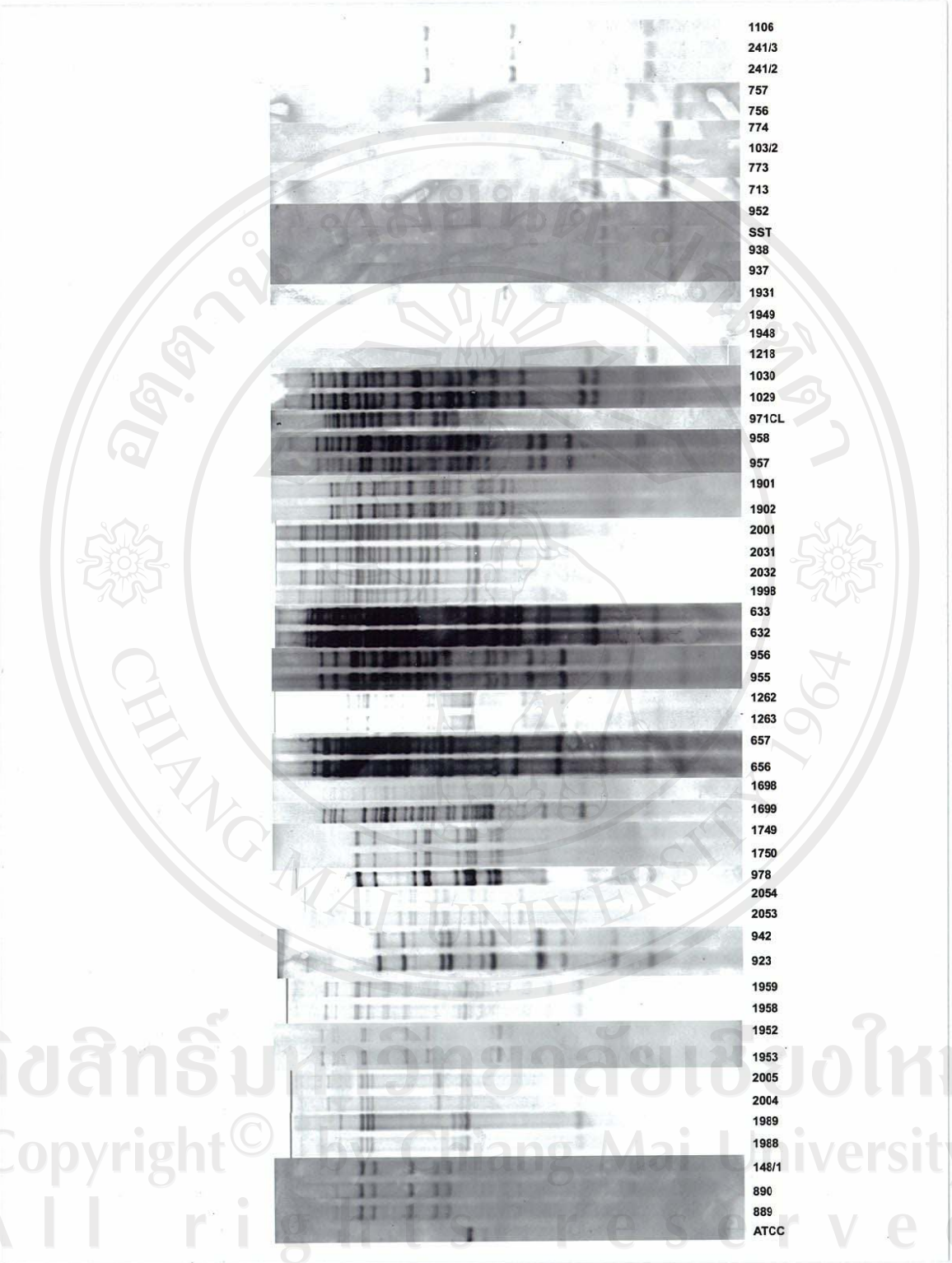

Figure 11 Showed the IS*1245* hybridization patterns of 56 *M. avium* isolates

Table 15 The numbers of bands, isolates and patients in each group

Group	Bands	Isolate (s)	Patient (s)
I	2	10	6
	3	5	2
	4	2	1
II	8	5	2
	9	4	2
III	13	4	2
	14	5	3
IV	17	1	1
	18	2	1
	21	2	1
	23	4	2
	24	2	1
	25	3	1
	26	1	
	28	2	1
	30	2	1
	31	2	1
V	0	20	11
Total		76	39

3.2 Analysis of RFLP pattern by GelCompar II software

The best images of hybridization pattern were selected and analyzed with the GelCompar II (version 2.0) program (Applied Maths, Kortrijk, Belgium). The result from the program also showed 4 groups (excluded the *M. avium* ATCC 25291 pattern) as manual grouping. There were 23 clusters (A to W), when more than 80% of similarity was grouped as cluster, of all 56 isolates *M. avium* in this study. The analyzed RFLP pattern was shown in Figure 12. The details of each group are shown in Table 16.

3.2.1 The first group (I)

The first group had 17 isolates from 9 patients. The patterns can be classified into 6 clusters (A to F). **Cluster A** was 3 isolates from a patient that had 3 bands. **Cluster B** was 2 isolates from a patient that had 4 bands. **Cluster C** was 8 isolates from 4 patients that had 2 bands. **Cluster D** was an isolate from a patient that had 2 bands. **Cluster E** was 2 isolates from a patient that had 3 bands. **Cluster F** was an isolate from a patient that had 2 bands.

3.2.2 The second group (II)

This group had 9 isolates from 4 patients. The patterns can be classified into 3 clusters (U to W). **Cluster U** was 2 isolates from a patient that had 9 bands. **Cluster V** was 4 isolates from 2 patients that had 8 and 9 bands. **Cluster W** was 3 isolates from a patient that had 8 bands.

3.2.3 The third group (III)

This group had 9 isolates from 5 patients. The pattern can be classified into 4 clusters (Q-T). **Cluster Q** was 3 isolates from 2 patients that had 14 bands. **Cluster R** was 2 isolates from a patient that had 13 bands. **Cluster S** was 2 isolates from a patient that had 13 bands. **Cluster T** was 2 isolates from a patient that had 14 bands.

3.2.4 The fourth group (IV)

The group had 21 isolates from 10 patients. The pattern can be classified into 10 clusters (G-P). **Cluster G** was 2 isolates from a patient that had 21 bands. **Cluster H** was 1 isolate from a patient that had 17 bands. **Cluster I** was 2 isolates from a patient had 23 bands.

Cluster J was 2 isolates from a patient that had 23 bands. **Cluster K** was 4 isolates from a patient that had 25 bands (3 isolates) and 26 bands (an isolate). **Cluster L** was 2 isolates from a patient that had 31 bands. **Cluster M** was 2 isolates from a patient that had 24 bands. **Cluster N** was 2 isolates from a patient that had 18 bands. **Cluster O** was 2 isolates from a patient that had 28 bands. **Cluster P** was 2 isolates from a patient that had 30 bands.

Table 16 The group, numbers of bands, isolates and patients in each cluster

Group	Cluster	Bands	Isolate (s)	Patient (s)
I	A	3	3	1
	B	4	2	1
	C	2	8	4
	D	2	1	1
	E	3	2	1
	F	2	1	1
II	U	9	2	1
	V	8,9	4 (2,2)	2
	W	8	3	1
III	Q	14	3	2
	R	13	2	1
	S	13	2	1
	T	14	2	1
IV	G	21	2	1
	H	17	1	1
	I	23	2	1
	J	23	2	1
	K	25,26	4(3,1)	1
	L	31	2	1
	M	24	2	1
	N	18	2	1
	O	28	2	1
	P	30	2	1

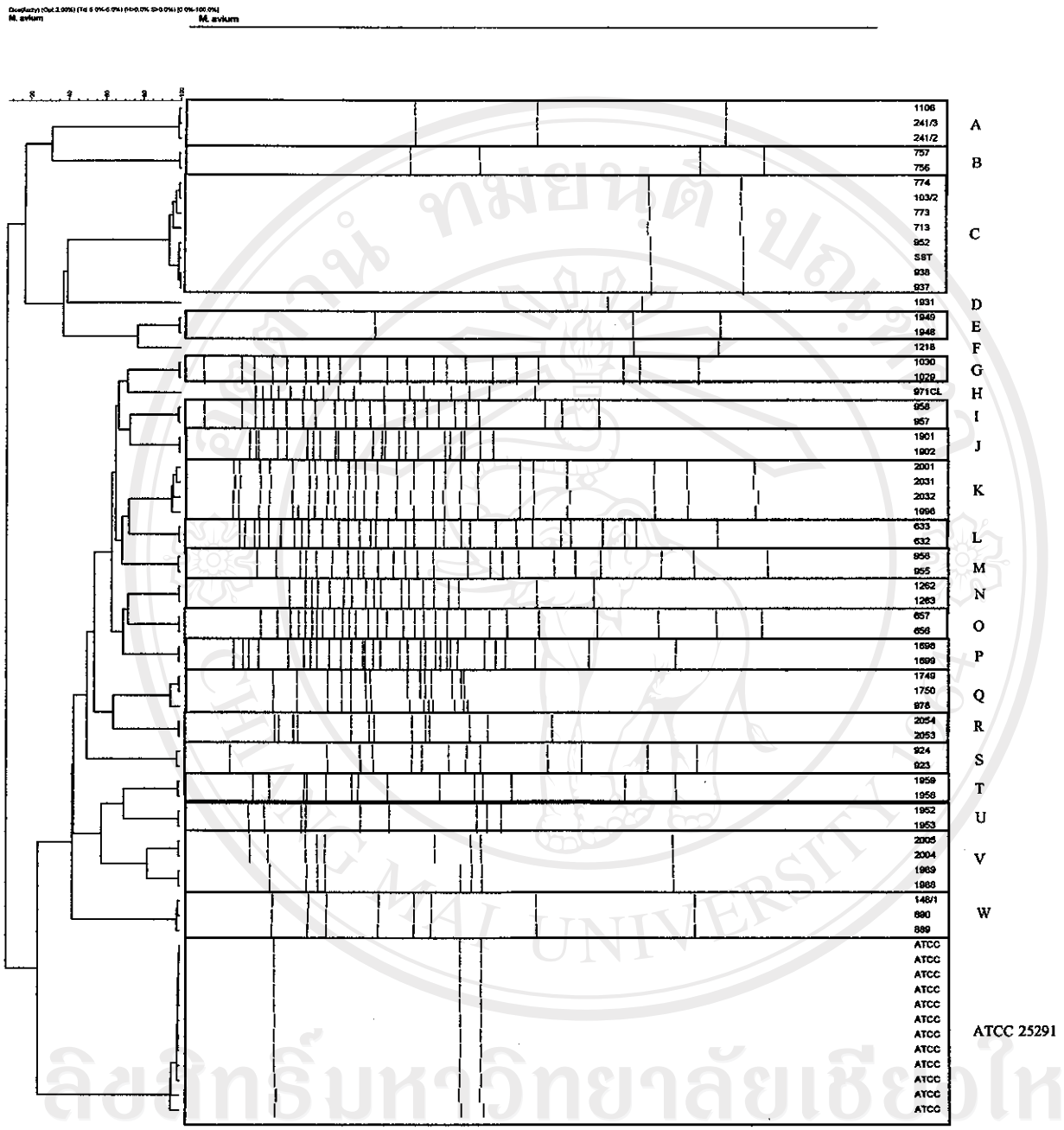

Figure 11 Dendrogram of the 68 IS*1245*-baseed RFLP profiles of *M. avium* isolates from blood, sputum and *M. avium* ATCC 25291. The numbers at the top represent percent relatedness. Isolates showing more than 80% similarity are boxed. Letters indicate the cluster to which the isolates belong. The column on the right present isolates names.

3.2.5 The fifth group (V)

The group had 20 isolates from 11 patients that could not hybridize with IS*1245*-probe. Although, the RFLP analysis was performed at least twice with each isolate. To confirm the results of RFLP analysis of these isolates, the new PCR amplification experiment was developed. The primers P1 and P2 that were specific to IS*1245* fragment 427 base pairs and primers 16SC and 23SG that were specific to 16S-23S rDNA spacer of 380 base pairs were used for the multiplex PCR. The specificity and sensitivity were tested.

3.2.5.1 Specificity

Five mycobacteria (*M. avium* ATCC 25291, *M. tuberculosis* ATCC 27294, *M. intracellulare* ATCC 13950, *M. scrofulaceum* ATCC 19981, and unclassified MAC) were used to test for the specificity. The results showed that only *M. avium* ATCC 25291 had 2 amplified products size of 380 base pairs and 427 base pairs (Figure 13). The others had only one product size (380 base pairs).

3.2.5.2 Sensitivity

The serial 10-fold dilutions of chromosomal DNA of *M. avium* ATCC 25291 were used to test sensitivity of the PCR method. The result showed that at the concentration of 100 pg of DNA, the 2 amplified products were stilled present (Figure 14).

3.2.5.3 Amplified results

The DNA of 20 isolates of *M. avium* that could not hybridize with IS*1245*-probe were tested with the multiplex. The results showed that all of them had 1 amplified-product size of 380 base pairs of 16S-23S rDNA spacer (Figure 15).

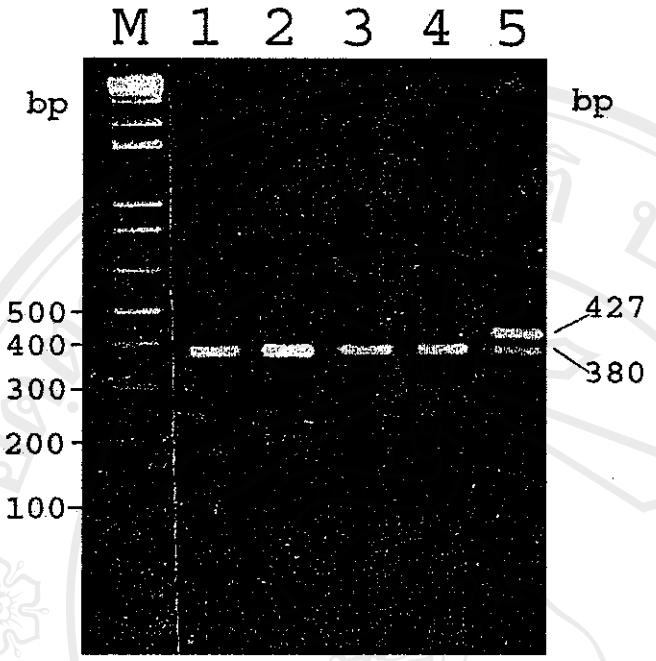

Figure 13 Showed the specificity of the multiplex PCR method

Lane M. 1 kb plus Marker

Lane 1. *M. tuberculosis* ATCC 27294

Lane 2. *M. intracellulare* ATCC 13950

Lane 3. *M. scrofulaceum* ATCC 19981

Lane 4. Unclassified MAC

Lane 5. *M. avium* ATCC 25291

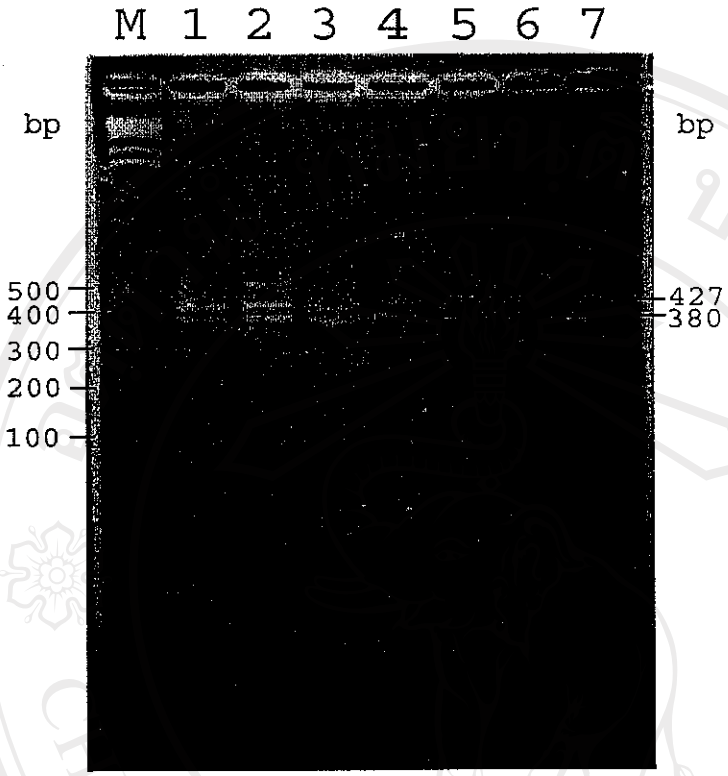

Figure 14 Showed the sensitivity of the PCR method

Lane M. 1 Kb plus Marker

Lane 1. DNA of *M. avium* ATCC 25291 = 1 µg

Lane 2. DNA of *M. avium* ATCC 25291 = 0.1 µg

Lane 3. DNA of *M. avium* ATCC 25291 = 0.01 µg

Lane 4. DNA of *M. avium* ATCC 25291 = 0.001 µg

Lane 5. DNA of *M. avium* ATCC 25291 = 0.0001 µg

Lane 6. DNA of *M. avium* ATCC 25291 = 0.00001 µg

Lane 7. DNA of *M. avium* ATCC 25291 = 0.000001 µg

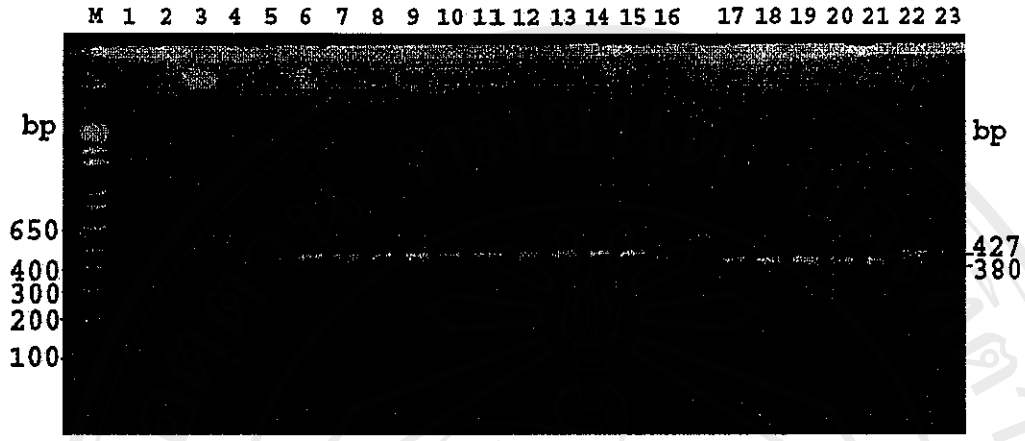

Figure 15 Showed the amplified products of 20 isolates of *M. avium* that could not hybridize with IS*1245*-probe

Lane M. 1 Kb plus Marker **Lane 1. 668**

Lane 2. 670 **Lane 3. 813**

Lane 4. 116/1 **Lane 5. 116/2**

Lane 6. 961 **Lane 7. 962**

Lane 8. 182/1 **Lane 9. 1061**

Lane 10. 1062 **Lane 11. 1286CL**

Lane 12. 1756 **Lane 13. 1757**

Lane 14. 1853 **Lane 15. 1854**

Lane 16. 1931 **Lane 17. 1932**

Lane 18. 1948 **Lane 19. 1984**

Lane 20. 1985 **Lane 21. *M. intracellulare* ATCC 13950**

Lane 22. *M. avium* ATCC 25291 **Lane 23. Negative control**

3.3 Clinical and demographic of all the isolates

The data of sex was analyzed. Nineteen patients were male (52.8%). Seventeen were female (47.2%). The distribution of patients by sex in each group of IS*1245* hybridization was shown in Table 17. The sex ratio of each group of the bacteria were not significantly difference (p=0.599, Chi-square test). All statistics in this study were analyzed under the STATA version 7 program.

Table 17 The distribution of the patients sex which were infected with *M. avium* from different IS*1245* hybridization patterns

Sex	Group I	Group II	Group III	Group IV	Group V	Total patients
Male	6 **(12)**	5 **(12)**	1 **(2)**	2 **(4)**	5 **(10)**	19 **(40)**
Female	3 **(5)**	4 **(8)**	4 **(7)**	2 **(5)**	4 **(8)**	17 **(33)**
Unknown	0	1 **(1)**	0	0	2 **(2)**	3 **(3)**
Total	9 **(17)**	10 **(21)**	5 **(9)**	4 **(9)**	11 **(20)**	39 **(76)**
Sex Ratio Male:Female	2:1	1:1	1:4	1:1	1:1	1:1

( ) is the number of isolates.