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# Distribution of *hsp65* PCR-Restriction Enzyme Analysis Patterns among *Mycobacterium avium* Complex Isolates in Thailand▽

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**A total of 227 clinical *Mycobacterium avium* complex isolates from Thailand were differentiated into species and types by using PCR-restriction enzyme analysis of *hsp65*. The distribution of types showed the predominance of *M. avium* I (77%) in blood specimens, whereas *M. intracellulare* I was more commonly found in pulmonary specimens (44.2%). In addition, infections with *M. avium* were more likely to be found in younger adults (20 to 39 years old), while infections with *M. intracellulare* were more likely to be found in older adults (≥60 years old). Our results provide the useful epidemiological information that some particular types have more invasive and virulent characters than others.**

The *Mycobacterium avium* complex (MAC) consists of two closely related species, *M. avium* and *M. intracellulare*, which are found widely in the environment, both in soil and water, and cause diseases in humans and animals (7). Both are capable of infecting diverse species, including birds, pigs, and humans, with consequences ranging from asymptomatic infection to clinically significant and even fatal disease. MAC is clinically important, as it is a frequent cause of disseminated disease and death in AIDS patients (5, 8). Clinically as well as genetically significant differences between *M. avium* and *M. intracellulare* have been shown. *Mycobacterium avium* is the most common MAC species isolated from AIDS patients and is also a pathogenic bacterium isolated from animals, whereas *M. intracellulare* is more frequently isolated from immunocompetent patients, especially from individuals with pulmonary illnesses (4, 9).

Based on the gene encoding the 65-kDa heat shock protein (*hsp65*), MAC can be identified and differentiated into species and types using PCR-restriction enzyme analysis (REA) (1, 2, 14). The present study demonstrates the usefulness of this method for rapidly differentiating MAC into *M. avium* types I, II, and III and *M. intracellulare* types I, II, III, and IV, providing useful epidemiological data.

Two mycobacterial reference strains, *M. avium* ATCC 25291 and *M. intracellulare* ATCC 13950, and 227 clinical isolates of *M. avium* complex bacteria from different patients, identified by the biochemical method, were submitted for molecular identification by using *hsp65* PCR-REA in the Molecular Mycology and Mycobacteriology Laboratory, Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, during 2003 and 2004. A total of 189 patients provided complete information on their sexes and ages; this group of patients consisted of 106 males and 83 females, with a mean age of 42.2 (range, 4 to 87) years. Clinical data about the human immunodeficiency virus (HIV) status of patients in this study were not completely available; only 67 (29.5%; 37 male and 30 female) of 227 patients confirmed that they were $HIV^+$ patients. Genomic DNA was extracted by a boiling technique and used as a template for the *hsp65* PCR-REA as previously described (1).

*Mycobacterium avium* ATCC 25291 and *M. intracellulare* ATCC 13950 were identified as *M. avium* I and *M. intracellulare* I, respectively. Of 227 MAC isolates, 146 isolates were identified as *M. avium* (64.3%), whereas 81 were *M. intracellulare* (35.7%). Interestingly, the 227 clinical isolates showed three distinct digestion patterns for *M. avium* and two for *M. intracellulare* (Fig. 1). According to PCR-REA patterns, 146 isolates of *M. avium* could be differentiated into type I (130 isolates [89%]), type II (15 isolates [10.3%]), and type III (1 isolate [0.7%]). Likewise, 81 isolates of *M. intracellulare* were differentiated into type I (62 isolates [76.5%]) and type IV (19 isolates [23.5%]).

When we considered the correlation of *M. avium* and *M. intracellulare* infections with sex and age (Table 1), it was noted that *M. avium* was found to infect mostly younger adults (age, 20 to 39 years; $P < 0.001$, $\chi^2$ test) and infected males more significantly than females ($P < 0.05$, $\chi^2$ test). These results may be due to the prevalence of HIV infection in younger males. In contrast, frequencies of infections with *M. intracellulare* were not different between males and females, but such infections were more likely to be found in older adults (age, ≥60 years; $P < 0.001$, $\chi^2$ test). Eight *M. intracellulare* isolates were obtained from pulmonary specimens from patients with pulmonary illnesses, five with chronic obstructive pulmonary diseases,

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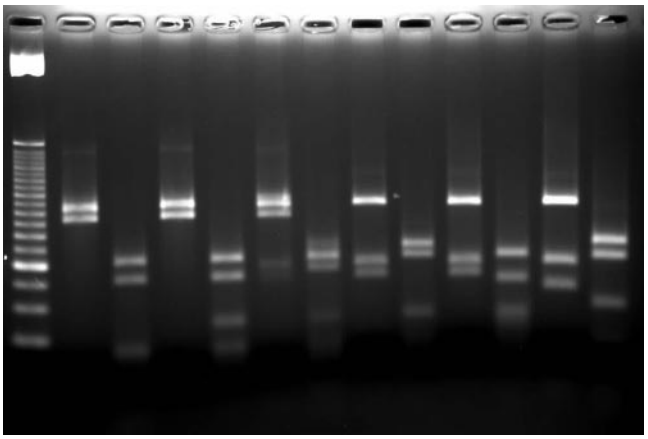

FIG. 1. Restriction patterns of *hsp65* amplicons after digestion with BstEII (lanes 1, 3, 5, 7, 9, and 11) and HaeIII (lanes 2, 4, 6, 8, 10, and 12). The digested bands of <60 bp were not used for interpretation. Lane M, 25-bp DNA ladder; lanes 1 and 2, *M. avium* I (BstEII 245/220 [values here and below are sizes of digested bands]; HaeIII 140/105); lanes 3 and 4, *M. avium* II (BstEII 245/220; HaeIII 140/105/60); lanes 5 and 6, *M. avium* III (BstEII 245/220; HaeIII 140/115); lanes 7 and 8, *M. intracellulare* I (BstEII 245/120/100; HaeIII 155/140/60); lanes 9 and 10, *M. intracellulare* IV (BstEII 245/120/100; HaeIII 140/90/60); lanes 11 and 12, an internal marker, *M. tuberculosis* (BstEII 245/120/80; HaeIII 160/140/70).

two with chronic bronchiectasis, and one with relapsed pulmonary tuberculosis. *M. intracellulare* I was isolated from all five patients (age, >60 years) with chronic obstructive pulmonary diseases and one (age, 21 years) with chronic bronchiectasis, whereas two *M. intracellulare* IV isolates were obtained from one patient (age, 39 years) with chronic bronchiectasis and one (age, 65 years) with relapsed pulmonary tuberculosis. These results were in agreement with those of a previous study showing that infections with *M. intracellulare* were more frequent among non-AIDS patients and more likely to be found in older patients (age, ≥50 years) (6).

The distributions of *M. avium* and *M. intracellulare* PCR-REA types varied among isolates from different sources (Table 2). Of the blood isolates, 77% were *M. avium* I, whereas 10.3, 9.2, and 3.5% were *M. avium* II, *M. intracellulare* IV, and *M. intracellulare* I, respectively. In contrast, *M. intracellulare* was the most common species, accounting for 51.9% of the isolates from the pulmonary specimens. The difference in the percentages of *M. avium* and *M. intracellulare* isolates from the pulmonary site was not statistically significant. Concerning the distribution of particular types, *M. avium* I and *M. intracellulare* I were found almost equally in 43.4 versus 44.2% of isolates, respectively. Furthermore, it was noted that *M. intracellulare* I was isolated from the pulmonary specimens (44.2%) much more often than from the blood specimens (3.6%) ($P < 0.01$, $\chi^2$ test). In contrast, *M. intracellulare* IV was isolated from blood significantly more often than was *M. intracellulare* I ($P < 0.01$, $\chi^2$ test). For other specimens, *M. avium* I was still the most common type (Table 2). Among 67 isolates from $HIV^+$ patients, 54 (80.6%) were identified as *M. avium* I, whereas the remaining isolates were *M. avium* II (11.9%) and *M. intracellulare* IV (7.5%). Most of the specimens (44 of 67, 65.7%) were blood isolates.

TABLE 1. Effect of age and sex on the distribution of MAC types

| Infection type | No. of patients by age (yr) and sex[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤19 | | | 20–39 | | | 40–59 | | | ≥60 | | |
| | M | F | Both | M | F | Both | M | F | Both | M | F | Both |
| *M. avium* I | 2 | 5 | 7 | 43 | 34 | 77 | 19 | 4 | 23 | 2 | 6 | 8 |
| *M. avium* II | 1 | 0 | 1 | 6 | 5 | 11 | 0 | 1 | 1 | 0 | 0 | 0 |
| *M. avium* III | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Total *M. avium* | 3 | 5 | 8 | 49 | 39 | 88 | 19 | 5 | 24 | 3 | 6 | 9 |
| *M. intracellulare* I | 1 | 0 | 1 | 4 | 1 | 5 | 3 | 5 | 8 | 18 | 14 | 32 |
| *M. intracellulare* IV | 0 | 0 | 0 | 3 | 4 | 7 | 2 | 2 | 4 | 1 | 2 | 3 |
| Total *M. intracellulare* | 1 | 0 | 1 | 7 | 5 | 12 | 5 | 7 | 12 | 19 | 16 | 35 |

[a] F, female; M, male.

In this study, MAC isolates were specifically selected from both pulmonary and disseminated infections. The PCR-REA could differentiate *M. avium* and *M. intracellulare* into three and two types, respectively. Expectedly, most clinical isolates of MAC bacteria from patients with disseminated infections were *M. avium* I ($P < 0.01$, $\chi^2$ test), as reported in previous studies (10, 13). In those studies, *M. avium* I was the predominant pathogen found in pigs and was the most common species isolated from human blood, whereas *M. avium* II was more frequently isolated from pulmonary specimens. Interestingly, *M. avium* II was shown to be the most predominant type among environmental isolates, and even *M. avium* III was more common than *M. avium* I (13). Our results were consistent with those studies in that *M. avium* I was the predominant variant among the blood isolates, but the majority of *M. avium* isolates from the pulmonary specimens were still *M. avium* I. These differences could result from the geographic variations between the isolates from the earlier study in the United States (13) and those from our study in Thailand. However, the distribution of MAC variants in our environment should be investigated in order to clarify the predominant variant in the environment.

*Mycobacterium intracellulare* was differentiated into two types, which have already been described in previous studies (3, 14); *M. intracellulare* I was a single type originally described by Telenti et al. (14), whereas *M. intracellulare* IV was an environmental isolate from India recently identified by Devallois et al. (3). *Mycobacterium intracellulare* II and III, the types

TABLE 2. Distribution of MAC isolates from different sources

| Source | No. of isolates by type[b] | | | | | Total |
|---|---|---|---|---|---|---|
| | Mav I | Mav II | Mav III | Min I | Min IV | |
| Blood | 67 | 9 | 0 | 3 | 8 | 87 |
| Pulmonary specimens[a] | 56 | 5 | 1 | 57 | 10 | 129 |
| Stool specimens | 4 | 0 | 0 | 0 | 0 | 4 |
| Tissue biopsy specimens | 1 | 1 | 0 | 0 | 1 | 3 |
| Cerebrospinal fluid | 2 | 0 | 0 | 0 | 0 | 2 |
| Joint fluid | 0 | 0 | 0 | 2 | 0 | 2 |
| Total | 130 | 15 | 1 | 62 | 19 | 227 |

[a] Pulmonary specimens include sputum, bronchial wash, and gastric wash specimens.
[b] Mav, *M. avium*; Min, *M. intracellulare*.

of clinical isolates described at PRASITE (http://app.chuv.ch/prasite/index.html), were not found in this study. Notably, among the *M. intracellulare* types, *M. intracellulare* IV was shown to be more invasive than *M. intracellulare* I, as 42.1% of the *M. intracellulare* IV isolates were blood isolates, relative to 4.8% of the *M. intracellulare* I isolates. The genetic difference between these two types would be valuable for further investigation. However, most infections caused by *M. intracellulare* were limited to the pulmonary area (51.9%) instead of invading the bloodstream (12.6%). In contrast, *M. avium* was the species predominantly isolated from the blood specimens (87.4%), whereas it was isolated less frequently from the pulmonary specimens (48.1%). This novel observation suggests that *M. avium* is likely more virulent than *M. intracellulare*, and among the *M. avium* isolates, *M. avium* I has a greater propensity for causing invasive, disseminated infections than other types do. These conclusions are in agreement with previous studies in which virulence-related determinants like the macrophage-induced gene (*mig*), the insertion sequence IS*1245*, and the hemolysin of MAC have been found mostly in *M. avium* (11, 12). However, the presence of those reported virulence determinants among the *M. avium* types should be further investigated, together with other virulence factors (currently undefined) that make some types of *M. avium* more invasive, more virulent, or longer survivors in the hosts. The characterization of the precise genetic changes that separate these variants will certainly provide the epidemiological connections and will define, if not all, some factors involved in virulent, pathogenic mechanisms and even routes of transmission.

Earlier studies demonstrated that, among AIDS patients, 98% of MAC infections caused by *M. avium* (5) and *M. intracellulare* were found in 13% of respiratory isolates but only 1.3% were found in blood isolates (15). Our results confirm those studies that showed that 92.5% of MAC infections among $HIV^+$ patients were due to *M. avium* (*M. avium* I, 80.6%, versus *M. avium* II, 11.9%), whereas 7.5% were due to *M. intracellulare*. It was noted that the *M. intracellulare* isolated from hemocultures of $HIV^+$ patients was *M. intracellulare* IV, emphasizing the particular genetic determinants responsible for an invasive characteristic of this type.

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