

The Studies of Rapid Identification of Non-tuberculous Mycobacterial Infections by PCR-RFLP

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ABSTRACT

Non-tuberculous mycobacteria (NTM) commonly exist in natural environment and can be easily found from water or soil. NTM, as opportunistic infectious bacteria, are able to induce various diseases, such as lung disease, skin or catheter-related infections, osteomyelitis, and even disseminated infections. This study aims to rapidly identify NTM infection via molecular diagnosis, and to study and compare the epidemic situations between NTM and *Mycobacterium tuberculosis* complexes (MTBC) in the Yunjianan area of Taiwan. Patients came from Chiayi Chang Gung Memorial Hospital in 2008. Various isolates from suspected patients of MTBC infections were cultured and examined by traditional mycobacterium culture and staining. Methods of molecular diagnosis including polymerase chain reaction coupled restriction fragment length polymorphism (PCR-RFLP) analyses were used for rapid identification of MTBC and/or NTM species. Positive clinical samples of bacterial cultures were acid-fast stained to confirm the existences of mycobacteria, and then subcultures were carried out. The growth rates and morphologies of bacteria were recorded. After subculture, a 123-bp MTBC-specific IS6110 DNA fragment was amplified by PCR to decipher the MTBC infection. If no 123-bp PCR product obtained, a 439-bp hsp65 DNA fragment was amplified via PCR to indicate the existence of NTM, and then PCR products were cut with BstEII and HaeIII, respectively. After gel electrophoresis, the results of RFLP were recorded and compared with database to identify the species of NTM.

Our results indicated that 10.7% of total isolates were positive for mycobacterium infections including 5.2% for MTBC and 5.5% for NTM infections. The samples with NTM infections were slightly higher than with MTBC infections in all suspected isolates. After PCR-RFLP analysis, our results indicated that the six most common species or types of infectious NTM are *M. intracellulare* (41.5%), *M. gordonae* (12.7%), *M. abscessus* (7.6%), Slow-growing NTM (7.3%), *M. avium* (6.6%), and *M. lentiflavum* (4.1%). All of them account for 79.8% of total NTM isolates. Furthermore, *M. intracellulare* extensively infected patients from all sources in this hospital. NTM infections account for the largest amount of isolates from patients with lung infections. The most common species of infectious NTM in the sputum samples are *M. intracellulare* (41.6%), *M. gordonae* (13.0%), and *M. abscessus* (7.5%). From these results, we conclude that *M. intracellulare* and *M. gordonae* are the most and second common species of infectious NTM in the Yunjianan area of Taiwan.

Recently, new species of NTM have been identified. We used the PCR-RFLP analyses that can identify more than 40 common species of NTM. In this thesis, our results identified some rare species of infected NTM that cannot be distinguished by PCR-RFLP analysis and two cases of MTBC and NTM mixed infections. Because the treated drugs between NTM and MTBC infections are different and various antibiotics are used for treatments of different NTM infections. Rapid identification of NTM infection and species or types of NTM, results of antibiotic sensitivity are the basis for medicine selection for NTM infection. Studies of NTM epidemiology and databases of drug sensitivities for various NTM species are two the major ways needed for more and more frequent NTM infections in the future.

Keywords : Bacterial infection、Mycobacterium、non-tuberculous mycobacteria、Molecular diagnosis、Polymerase Chain Reaction Coupled Restriction Fragment Length Polymorphism (PCR-RFLP)、Epidemiology、future、taiwan、after

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