IDENTIFICATION OF MYCOBACTERIA BY SEQUENCING OF *rpoB* GENE AND 16S rRNA

Yuko KAZUMI, Shinji MAEDA, and Isamu SUGAWARA

Abstract [Purpose] To classify a specific *Mycobacterium* among various mycobacteria utilizing sequencing of *rpoB* gene. To classify mycobacteria not identified by DNA-DNA hybridization (DDH) using sequencing of *rpoB* and 16S rRNA gene.

[Objects and methods] Classification of 106 Mycobacteria strains, one *Nocardia* strain, one *Rhodococcus* strain, four *Gordona* strains was made by using partial sequencing of *rpoB* and 16S rRNA (RIDOM). Thereafter, 38 mycobacteria clinical strains not identified by DDH were classified utilizing the DNA sequencing data.

[Results] Pairs of *M. kansasii* and *M. gastri*, *M. abscessus* and *M. chelonae*, *M. fortuitum* (ATCC49404) and *M. polcinum*, *M. peregrinum* and *M. septicum*, *M. farucinogense* and *M. senegalense* and *M. fortuitum* (ATCC49403), *Rhodococcus*, *Nocardia* and *Gordona* strains were classified using sequencing of *rpoB* gene. Even though sequencing of *rpoB* and 16S rRNA gene was utilized, it was impossible to classify *M. tuberculosis* complex, *M. avium* family, *M. marinum* and *M. ulcerans*, and *M. fortitum* subsp. *fortuitum* and *M. fortuitum* subsp. *acetamidolyticus*.

The 38 mycobacteria clinical strains not identified by DDH were successfully classified using sequencing of both *rpoB* and 16S rRNA. These sequencing analyses showed that *M. heckeshornense*, *M. branderi*, *M. intermedium*, *M. shimoidei*, *M. wolinskyi*, *M. malmoense* and *M. lentiflavum* could be identified. Thirty six clinical isolates (94.7%) and 32 clinical isolates (84.2%) were identified by *rpoB* sequencing and 16S rRNA sequencing (RIDOM), respectively.

[Conclusion] The classification ratio of mycobacteria including *Nocardia*, *Rhodococcus* and *Gordona* is 69.6% for sequencing of 16S rRNA and 89.3% for sequencing of *rpoB* gene. Sequencing of *rpoB* is useful for classification of mycobacteria due to its genetic diversity, but has some limitation in its application. In order to classify mycobacteria more accurately, it is important to combine sequencing of *rpoB* and 16S rRNA and biochemical/biological tests.

Key words: Identification of mycobacteria, 16S rRNA, Sequence, RIDOM, *rpoB* gene, Unidentified strain

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Original Article

USEFULNESS OF VARIABLE NUMBERS OF TANDEM REPEATS TYPING IN CLINICAL STRAINS OF *MYCOBACTERIUM AVIUM*

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Abstract [Objectives] We evaluated the usefulness of Variable Numbers of Tandem Repeats (VNTR) analysis, which was recently reported as a new typing method of *Mycobacterium avium* strains of animal origin, for strain differentiation of clinical isolates of *M. avium* in comparison with the standard IS*1245*-RFLP typing method. In addition, forty *M. avium* isolates recovered from sputum samples of same patient in different times were analyzed with VNTR typing method.

[Subjects and Methods] The subjects were twenty-four clinical isolates of *M. avium* stocked at Higashi Nagoya National Hospital and discriminatory power was evaluated with Hunter Gaston Discriminatory Index (HGDI). Furthermore, forty *M. avium* isolates recovered from sputum samples of one patient obtained at four different times were analyzed by using this VNTR typing method.

[Results] VNTR typing showed better discriminatory power for twenty-four clinical isolates than IS*1245*-RFLP method (HGDI : 0.975 vs 0.866). In the second study, polyclonal infection of four genotype strains with different allele profiles were detected. The ratio of mixture of the four different genotype strains varied during clinical course.

[Conclusion] We considered that VNTR typing method was very useful for discriminatory examination of *M. avium*.

Key words : VNTR typing, *Mycobacterium avium*, IS*1245*, RFLP typing, polyclonal infection

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INFLUENCE OF AGING ON TUBERCULOSIS INFECTION
— An Epidemiological Study of 1,141 Smear-positive TB Patients —

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Abstract [Objectives] To elucidate the influences of aging on the tuberculosis infection.

[Subjects and Methods] The subjects of this retrospective study were 1,141 smear positive pulmonary tuberculosis (TB) patients registered in Aichi prefecture between 1989 and 2003. All registration files were reviewed to identify epidemiological links of patients. When linked patients with an interval of the dates of registration of less than 10 years were found, the earliest case was considered as the source case, and the other patients were regarded as secondary cases.

An epidemic source rate (ESR) for a category of patients (e.g., age-group, etc.) was defined as following; ESR＝$NS/NA\times100$, where NA: Number of smear-positive pulmonary TB patients in a category A, and NS: Number of source cases in category A.

[Results] A total of 70 source cases were identifed and the ESR was 6.1%. The ESRs for different age-groups were; 14.3% for 10−19 years of age (NA＝14), 13.5% for 20−29 years (NA＝74), 14.6% for 30−39 years (NA＝48), 15.0% for 40−49 years (NA＝107), 6.9% for 50−59 years (NA＝145), 3.5% for 60−69 years (NA＝227), 3.8% for 70−79 years (NA＝293), 2.8% for 80−89 years (NA＝212), and 0% for 90−99 years (NA＝21). The ESR were significantly different between those aged 40 to 49 years and those aged 50 to 59 ($p<0.05$).

The ESR was significantly different between those aged 59 years and younger and those aged 60 years or older (11.6% vs 3.3%, $p<0.001$). The ESR was significantly different between those patients with cavitary lesion and those with non-cavitary lesion in the younger groups (14.3% vs 5.2%, $p<0.01$), as well as in the elder age-groups (4.8% vs 1.7%, $p<0.01$).

The rate in the younger groups was 6.3% for those with lower smear-positivity (Gaffky 1 to 4), compared with 15.3% for those with intermediate smear-positivitiy (Gaffky 5 to 8), and 32.4% for those with higher smear-positivity (Gaffky 9 and 10) (with $p<0.01$, $p<0.05$ respectively), while the rates were 3.1%, 3.9%, and 3.4%, respectively in the older groups.

[Conclusion] These findings suggest that the infectivity is significantly lower in older groups.

Key words: Smear-positive pulmonary tuberculosis, Cluster, Source cases, Epidemic source rate, Aging

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Short Report

COMPARISON BETWEEN DIRECT SMEAR BY ZIEHL-NEELSEN AND CONCENTRATED SMEAR BY FLUOROCHROME STAIN

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Abstract [Purpose] For the purpose of supporting more completely our assertion that two times concentrated sputum smear tests by fluorochrome stain are more sensitive than or at least equal to 3 times direct smear tests by Ziehl-Neelsen stain, we compare the sensitivity of concentrated smear by stain (conc-smear) and direct smear by Ziehl-Neelsen stain (di-smear).

[Object and Method] Retrospective study of sputum acid-fast smear tests in our hospital with tuberculosis ward from Jan. 1, 2003 to Sep. 30, 2005.

[Result] 170 of 899 sputums on which both conc-smear and di-smear were done, were smear positive by at least one of the two smear method. Of those 170, 167 (98.2%) were positive by conc-smear and 113 (66.5%) were positive by di-smear, and the difference was statistically significant ($p<0.001$). Of those 110 that were positive by both conc-smear and di-smear, in 65 (59.1%) smear grade by conc-smear were higher than that of di-smear, and in 3 (2.7%) smear grade by di-smear were higher than that of conc-smear. Smear grades in conc-smear were significantly higher than that of di-smear ($p<0.001$).

[Conclusion] In sensitivity and smear grades, conc-smear was superior to di-smear. Together with previous report, 2 times conc-smear tests are supposed to be superior to 3 times di-smear.

Key words: Pulmonary tuberculosis, Sputum, Ziehl-Neelsen stain, Fluorochrome stain, Direct smear, Concentrated smear

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Case Report

A CASE OF LUNG TUBERCULOSIS SHOWING NO CHEST RADIOGRAPH FINDINGS WITH RECURRENT HEMOPTYSIS

Satoko SEKINE, Takashi ISHIDA, Kengo OHSHIMA, Yoshinori OHTSUKA, and Mitsuru MUNAKATA

Abstract A 59-year-old male was referred to our hospital because of hemoptysis. A chest X-ray film and 7 mm-slice CT scan showed no abnormal finding. Bronchoscopy revealed hemorrhage in the right upper bronchus (B^1_a). Bronchial lavage of the lesion was performed, but *Mycobacterium tuberculosis* was not detected. Because of repeated hemoptysis, he was admitted to our hospital. Right bronchial artery angiograph showed vascular hyperplasia in the peripheral part of the upper lobal branch, and this lesion was suggested to be a bleeding point. There were no vascular malformations. Thin slice (0.5 mm-thick) CT scan showed mild infiltrative shadow in the right upper lobe. After admission, sputa smear for mycobacteria and PCR for *M. tuberculosis* became positive, and he was diagnosed as pulmonary tuberculosis. After starting antituberculous chemotherapy, hemoptysis disappeared, and sputa smear and culture for mycobacteria converted to negative. This case suggests that lung tuberculosis should be suspected in patients having hemoptysis, even though they had no chest X-ray film abnormality.

Key words: Hemoptysis, No chest radiograph findings, PCR for tuberculosis, Thin slice CT

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The 81st Annual Meeting Educational Seminar

CHLAMYDIA PNEUMONIAE INFECTIONS

Naoyuki MIYASHITA

Abstract *Chlamydia pneumoniae*, an obligate intracellular human pathogen, causes infections of the respiratory tract. It is a significant cause of both lower and upper acute respiratory illnesses, including pneumonia, bronchitis, pharyngitis and sinusitis. Most respiratory infections caused by *C. pneumoniae* are mild or asymptomatic. Some studies have suggested a possible association of *C. pneumoniae* infection and acute exacerbations of asthma and chronic obstructive pulmonary disease (COPD). Seroepidemiological studies showing antibody prevalence rates in a range of 50 to 70% suggest that *C. pneumoniae* is widely distributed and that nearly everybody is infected with the agent at some time.

C. pneumoniae can cause prolonged or chronic infections which may be due to persistence for months or years. These persistent infections have been implicated in the development of a number of chronic diseases including atherosclerosis, asthma and COPD. These persistent chlamydial infections can be established *in vitro* using several methods including cytokines, antibiotics and deprivation of certain nutrients. Despite differences in treatment, chlamydiae respond to form inclusions containing atypical reticulate bodies (RBs), which occasionally have been shown to be pleomorphic forms, termed aberrant form (AF). The AF is generally larger in diameter than typical RBs, and display a sparse densinometric appearance.

In general, it is likely that this aberrant developmental step leads to the persistence of viable but nonculturable chlamydiae within infected cells over long periods. Removal of several stress factors described above results in the condensation of nuclei, the appearance of late proteins, and the production of viable, infectious elementary bodies (EBs). Most of the major sequelae of chlamydial disease are thought to arise from either repeated or persistent chlamydial infection of an individual. The persistence would allow constant presentation to the individual immune response of these potentially deleterious immune targets. Since repeated infection can certainly be documented in many clinical settings, persistence is thought to also play a role.

Key words: Respiratory infection, Persistent infection, Asymptomatic infection, Type III secretion, Atherosclerosis

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