Clonal diversity of Mycobacterium tuberculosis in sputum specimen from individual patients of pulmonary tuberculosis
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Objectives: The isolation of Mycobacterium tuberculosis from sputum specimen is the principal tool to make the definite diagnosis on tuberculosis patients. During the isolation, sputum specimen treated with NALC-NaOH is usually inoculated and incubated into liquid media and onto egg-based media. In contrast to the ordinary bacteria, the isolate of M. tuberculosis will never be clonally separated on solid media, and is usually employed directly to phenotypic and genotypic characterization. In the present study, we determined clonal diversity of M. tuberculosis in sputum specimen taken from individual patients of pulmonary tuberculosis by using antimycobacterial susceptibility testing and IS 6110 restriction fragment length polymorphism (RFLP).

Methods: A total of nine patients were included in the study. Of nine patients, five were newly diagnosed as pulmonary tuberculosis, and the remaining four were during chemotherapy. The sputum specimen was treated with the standard NALC-NaOH, then directly plated on the Middlebrook 7H11 agar plates. After incubation, 10 to 15 well-separated colonies appeared on the agar were selected and subcultured. For individual colonies, antimycobacterial susceptibilities against eight agents were determined by broth microdilution test, BrothMIC MTB (Kyokuto Ltd, Japan) and DNA fingerprinting patterns were analysed using IS6110 RFLP as well as spoligotyping with low copy numbers.

Results: The results of antimycobacterial susceptibility tests indicated the significant differences among the individual colonies from a single specimen. Of nine sputa collected, three, including one newly diagnosed, were the mixtures of susceptible and resistant colonies against streptomycin, isoniazid and/or ethambutol. Whereas, DNA fingerprinting revealed that five sputa, including three newly diagnosed, contained heterogeneous cells on RFLP. However, there found no significant correlation between RFLP patterns and antimycobacterial susceptibilities.

Conclusions: The study indicates that, in some patients of pulmonary tuberculosis, the isolates of M. tuberculosis reveal clonal diversity in phenotypic and genotypic characterization. The diversity of the isolates from patients previously or presently treated with some antimycobacterial agents were likely, because antituberculosis chemotherapy always induce drug resistance through the accumulation of spontaneous mutations. However, it became apparent that polyclonal infection with M. tuberculosis in patients not previously treated is often the case. Possible infection with polyclonal strains of M. tuberculosis and heterogeneity in drug susceptibilities during the single episode of pulmonary tuberculosis is an important consideration from an epidemiological and therapeutic aspects.