

Original Article

MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS BY THE USE OF
IS6110 RESTRICTION FRAGMENT LENGTH POLYMORPHISM:
A STUDY FROM 2001 TO 2003

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Abstract [Objective] To analyze the situation of tuberculosis infection by DNA fingerprinting in the middle and eastern part of Osaka, Japan.

[Design] We performed IS6110 restriction fragment length polymorphism (RFLP) on 1200 isolates from tuberculosis patients who visited our hospital from January 2001 to December 2003. A cluster was defined as a series of isolates with more than 90% similarity by IS6110 RFLP and those with the same drug-susceptibility pattern. The isolates with fewer than six copies of IS6110 were considered to be clustered if the IS6110 RFLP patterns and the variable numbers of tandem repeats with 16 regions of ETR and MIRU "allele profile" were identical.

[Results] The number of samples in incremental study periods was 422 in 2001, 817 between 2001 and 2002 and 1200 between 2001 and 2003. The percentage of clustered cases was 27.8% in 2001, 19.1% in 2002 and 19.5% in 2003. The cumulative percentage of clustered cases was 27.8% in the first year, 29.7% over two years and 32.6% over three years. The percentage of clustered cases of isolates with a drug resistance was significantly lower (25.0%) than that of drug susceptible isolates (33.7%). Next, we investigated the clustered cases by gender and age. The percentage of clustered cases with isolates from young males and females (0–19 years

old) was 23.8%. In contrast, the percentage of clustered cases with isolates from 20–59 year-old females gradually decreased from 14.7% to 4.4%. Conversely, the percentage of clustered cases from young and middle aged male (20–59 years old) was higher (20.2%–32.4%) than that of females.

[Conclusion] The sharp increase in the cumulative cluster formation rate was curbed by the decline in the tuberculosis incidence rate in Osaka, Japan, after the first year of examination. We thought that this phenomenon suggests the success of the anti-tuberculosis measure in Japan.

Key words: *Mycobacterium tuberculosis*, Molecular epidemiology, DNA fingerprinting, IS6110 RFLP, 16 VNTR, Cluster formation rate

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Original Article

ANTIMYCOBACTERIAL SUSCEPTIBILITY AGAINST NONTUBERCULOUS MYCOBACTERIA USING BROTHMIC NTM

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Abstract [Purpose] Recently the incidence of pulmonary nontuberculous mycobacteria infection has increased among patients not only implicated with AIDS, but also without predisposing conditions. However, an effective antimicrobial therapy for the disease has not been established yet, because of the absence of highly active therapeutic drugs. We compared the *in vitro* antimicrobial activities of five antituberculous drugs, clarithromycin and fluoroquinolones against 92 clinical isolates belonging to three species of slowly growing nontuberculous mycobacteria.

[Material and Methods] *M. avium* (31 strains), *M. intracellulare* (44 strains), and *M. kansasii* (17 strains), all of which were isolated from sputum specimens of previously untreated patients with pulmonary nontuberculous mycobacteria infection, were used. The eight agents tested were streptomycin, ethambutol, kanamycin, isoniazid, rifampicin, clarithromycin, levofloxacin and gatifloxacin. The drug susceptibility of these strains in terms of MIC (minimum inhibitory concentration) was determined by BrothMIC NTM.

[Results] The MICs of rifampicin, clarithromycin, levofloxacin and gatifloxacin for all three species were low and gatifloxacin was more active than levofloxacin between two fluoroquinolones. Regarding clarithromycin, 100% of the strains were susceptible to 2 micrograms/ml or less and none of the strains were resistant on this level. In contrast, the MICs of ethambutol and isoniazid for *M. avium* and *M. intracellulare*

were high and less active *in vitro* than the other antimicrobial agents.

[Conclusion] These MIC studies suggest that rifampicin, clarithromycin, levofloxacin, and gatifloxacin have excellent *in vitro* antimicrobial activities against *M. avium*, *M. intracellulare* and *M. kansasii* and especially clarithromycin may be very useful as a drug therapy for previously untreated patients. In the treatment of pulmonary nontuberculous mycobacterium infection, further studies are needed to evaluate the clinical effects of these drugs and to observe the drug resistance, on the basis of the results of the drug susceptibility test by BrothMIC NTM.

Key words: BrothMIC NTM, Nontuberculous mycobacteria, Antimycobacterial susceptibility, Minimum inhibitory concentration (MIC)

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Original Article

IN VITRO ANTITUBERCULOUS ACTIVITY OF OFLOXACIN AND LEVOFLOXACIN
AGAINST MULTIDRUG-RESISTANT TUBERCULOSIS AND CLINICAL OUTCOMES

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Abstract [Objective] To investigate *in vitro* antituberculous activity of ofloxacin (OFLX) and levofloxacin (LVFX) against multidrug-resistant tuberculosis and to study the clinical outcomes.

[Subjects and Methods] *In vitro* antituberculous activity of OFLX and LVFX against multidrug-resistant strains of *Mycobacterium tuberculosis* isolated from 46 patients with pulmonary tuberculosis and a retrospective clinical analysis of 45 patients were investigated.

[Results] In susceptibility testing, resistance rates to OFLX or LVFX were higher in intractable cases (7/20: 35%) and in cases with prior chemotherapy using new quinolones (5/12: 42%). Sputum culture conversion was observed in 34 patients (76%), however 9 among them later reverted to positive culture. In a single variate proportional hazards model, risk factors related to poor outcomes (treatment failure or relapse) were resistance to OFLX or LVFX, advanced disease on chest radiograph, and the number of susceptible drugs four or less. In a multiple variate proportional hazards model, a risk factor

was resistance to OFLX or LVFX. Eighteen patients (40%) died, and among them, 10 died of tuberculosis. Survival time of treatment failure patients was significantly shorter than patients with sputum culture conversion.

[Conclusion] Resistance to OFLX or LVFX was considered to be a risk factor related to treatment failure and relapse in multidrug-resistant tuberculosis.

Key words : Multidrug-resistant tuberculosis, Ofloxacin, Levofloxacin, Antituberculous activity, Clinical outcome

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Case Report

A CASE OF SEVERE INTESTINAL TUBERCULOSIS, TREATED WITH CIPROFLOXACIN, KANAMYCIN AND PREDNISOLONE

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Abstract A 31-year-old man was admitted to our hospital because of frequent diarrhea. Colonoscopy showed ring ulcers on the rectum and ascending colon and chest X-ray showed abnormal shadows which were diagnosed as tuberculosis by sputum PCR. He started treatment with isoniazid (INH), rifampicin (RFP), pyrazinamide (PZA) and streptomycin (SM), however, eruption and ileus were seen. Then, he was retreated with ciprofloxacin (CPFX), kanamycin sulfate (KM) and prednisolone (PSL). Subsequently, we added RFP and further added calcium para-aminosalicylate (PAS). All these treatment was effective, and he was discharged from the hospital.

Key words: Ciprofloxacin, Kanamycin sulfate, Prednisolone, Intestinal tuberculosis, Eruption, Ileus

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A CASE OF PULMONARY TUBERCULOSIS COMPLICATED WITH AN ORTHOTOPIC LIVER TRANSPLANTATION

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Abstract The infectious disease is one of the most important complications related to the organ transplantation. Patients using immunosuppressive agents often present atypical tuberculosis and the treatment of such case is far more difficult in some cases due to the liver damage and/or the drug interaction. We report a case of pulmonary tuberculosis in a patient of 60-year-old man using tacrolimus after an orthotopic liver transplantation. He had liver transplanted orthotopically for the long-term history of chronic hepatitis B and subsequent liver failure on January 28, 2004. An abnormal shadow was first detected on his chest X-ray film on October, 2004. He was admitted to our hospital after the smear of the gastric juice showed some acid-fast bacilli and tubercle bacilli were confirmed by polymerase chain reaction (PCR). Tuberculin skin test was positive (erythema 10×10) and the computed tomography (CT) scan of his chest revealed a nodular opacity with some smaller nodules scattered around in the right upper lobe. We started four anti-tuberculous drugs other than pyra-

zinamide (PZA) and rifampicin (RFP), which included isoniazid (INH), ethambutol (EB), streptomycin (SM), levofloxacin (LVFX). The liver enzyme was transiently elevated (AST 123 IU/l, ALT 103 IU/l) but improved after desensitization against INH. The blood concentration of tacrolimus preserved between 5 and 7 ng/ml and there was no need to change the dosage.

Key words: Orthotopic liver transplantation, Pulmonary tuberculosis, Tacrolimus, Liver damage, Drug interaction, Desensitization therapy

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