

Original Article

ANTI-TUBERCULOSIS DRUG RESISTANCE SURVEY IN JAPAN, 2002 :
EXTERNAL QUALITY ASSESSMENT OF RESULTS

Tuberculosis Research Committee (RYOKEN)

Abstract [Objective] A nationwide anti-tuberculosis drug resistance survey for *Mycobacterium tuberculosis* was conducted by the Tuberculosis Research Committee (Ryoken) in Japan, 2002, to clarify the recent trend of drug resistant *M. tuberculosis*. The drug susceptibility testing (DST) results by participating laboratories were compared with the test results by the reference laboratory, in order to evaluate the quality of DST by the participating laboratories.

[Method] *Mycobacterium* strains were collected from patients who were admitted to the 99 participating hospitals between June and November in 2002. For each isolate, DST was performed at participating facilities and also at the reference laboratory (Research Institute of Tuberculosis: RIT) for four first-line anti-tuberculosis drugs, i.e., isoniazid (INH), rifampicin (RFP), streptomycin (SM) and ethambutol (EB). Each participating laboratory performed the DST with its routine method. The DST method for *M. tuberculosis* performed at the reference laboratory was the simplified proportion method on the standard 1% Ogawa medium as described in the national guidelines, and the results were regarded as the judicial ones. The DST results of each isolate from the participating laboratories were compared with the judicial results from the reference laboratory. The accuracy of DST done by the participating laboratories was evaluated in terms of the following indices; sensitivity for detecting drug resistant strains, the specificity for susceptible strain, the overall agreement, and kappa coefficient were calculated to evaluate the performance of local laboratories.

[Results] A total of 3,122 *M. tuberculosis* strains were obtained out of 4,134 mycobacterial strains collected from the participating facilities. Fifty, 23 and 17 local laboratories used Bitspectre-SR (Kyokuto pharmaceuticals), Welpack S (Nichibi) and Ogawa media for DST, respectively. MGIT (Becton Dickinson) and BrothMIC MTB-I (Kyokuto pharmaceuticals) were used in four laboratories each. The sensitivity,

specificity, efficiency and kappa coefficient for INH were 84.5%, 98.7%, 98.0%, and 0.798, respectively. Similarly for RFP, they were 90.3%, 99.7%, 99.5%, and 0.894, respectively. False susceptible results were frequently observed (2.1%) for SM compared with false resistance (0.5%), whereas the efficiency of SM was 97.4%. Similarly for EB, false resistances were frequently observed (2.6%) compared with false susceptibles (0.4%), whereas the efficiency of EB was 96.9%. The kappa coefficient for EB (0.470) was obviously lower than the others. The DST results with Ogawa method at local laboratories showed significantly lower sensitivity than those with Welpack S and MGIT.

[Discussion] The DST methods used at local laboratories were still mainly microtitre methods with egg-based solid media, but the number of laboratories using liquid DST methods increased in 2002 compared to 1997. The overall specificity and efficiency of DST for each anti-tuberculosis drug was over 95%, but the sensitivity was below 90.3%. Because of the frequency of drug resistance (up to 7.9% for SM in 2002), the efficiency and specificity may not be useful indicators for the quality assessment. The kappa coefficient for the agreement between local and reference laboratories' DST was clearly low in the case of EB, except for the laboratories using MGIT where kappa coefficient was higher than 0.8. The quality improvement of DST for EB could be achieved through the standardisation and automation.

Key words: Ryoken, Tuberculosis, Drug susceptibility testing, External quality assessment

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Original Article

EVALUATION OF ACCURACY OF CLINICAL DIAGNOSIS OF TB BY ANNUAL AUTOPSY REPORT

Hitoshi HOSHINO, Isamu SUGAWARA, Masako OHMORI, and Masako WADA

Abstract [Purpose] To investigate the accuracy of clinical diagnosis of TB in Japan in recent years and to compare them with previous studies.

[Method] Data (sex, age, clinical diagnosis, pathological diagnosis as cause of death) on deceased cases clinically or pathologically diagnosed ante mortem as having tuberculosis was collected from annual reports of the pathological autopsy cases in 1984, 1989, 1994, and 1999–2004. Information on TB death from population statistics in those 9 years also was collected and compared with data of autopsied cases.

[Result] Autopsy rate in these years was stably around 10%. Comparison of gender ratio and mean age between the two surveys showed similar numbers. During 1999–2004, 1725 death cases were diagnosed as TB clinically or pathologically. Number of pathologically proven pulmonary TB cases was 429 and that of miliary TB was 283. 55.7% of pulmonary tuberculosis and only 21.9% of miliary tuberculosis were correctly diagnosed before death. Out of 156 cases clinically diagnosed as non-TB diseases but proven as TB pathologically, 30.8% of clinical diagnosis was pneumonia and/or bronchitis, followed by diagnoses of interstitial pneumonia,

respiratory failure, pneumoconiosis and lung cancer. However, the main clinical diagnoses of 175 miss-diagnosed miliary TB cases were diseases other than pulmonary diseases such as renal failure, malignant diseases and sepsis.

[Conclusion] In order to reduce undiagnosed pulmonary TB cases and to prevent nosocomial TB infection, differential diagnosis among pneumonia and/or bronchitis cases should be done. In case of miliary TB, not only pneumonia but also diseases other than pulmonary diseases such as renal failure, malignant diseases and sepsis should be included in the list differential diagnosis.

Key words: Tuberculosis, Autopsy, Clinical diagnosis, Pathological diagnosis

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15 CASES OF PULMONARY *MYCOBACTERIUM SCROFULACEUM* INFECTION

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⁵Yasuko HARADA, and ²Yoshinari KITAHARA

Abstract [Objectives] We described clinical features of pulmonary *Mycobacterium scrofulaceum* disease.

[Materials and methods] We described 15 cases of pulmonary *Mycobacterium scrofulaceum* infection admitted to National Hospital Organization Omuta National Hospital from 1989 to 2003 and reviewed the clinical feature, the findings of chest radiograph, and clinical course.

[Results] Sex ratio was 8 male cases and 7 female cases, and the average age was 65.9 years old. Smoking history was found in 8 patients and occupational history of the dust inhalation was found in 7 patients with pulmonary *M. scrofulaceum* infection. There were 11 cases of tuberculosis-like form and 4 cases of nodular-bronchiectasis form according to the NTM Research society classification based on the findings of chest radiography. Improvement of the findings of chest radiography was seen in 4 patients by therapy, while no change or aggravation in 11 patients. Five patients died and among them, 3 died due to aggravation of pulmonary *M. scrofulaceum* infection.

[Discussion] Cases showing tuberculosis-like form were dominant, and most of them showed extensive lesions when they were diagnosed, and these facts were considered to be

major factors of difficulty in the treatment of this infection. The facts that 7 cases had occupational exposure to the dust, obstructive pulmonary disease in 3 cases, and 6 cases showed sputum culture positive for other nontuberculous mycobacteriosis, suggest that local resistance of lung might be attenuated, and this could be one of factors of onset and development of this infection. Only 4 cases showed improvement, while 5 cases died (primary disease death in 3 cases) and it was thought that the prognosis of the disease was in general poor.

Key words: *Mycobacterium scrofulaceum*, Pulmonary *M. scrofulaceum* disease, Nontuberculous mycobacteriosis

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Short Report

A SURVEY ON NOSOCOMIAL INFECTION OF TUBERCULOSIS
IN NURSING HOMES FOR ELDERLY IN OSAKA CITY¹Akira SHIMOUCI, ¹Shinichi KODA, and ²Hirotaoka OCHIAI

Abstract [Purpose] To ascertain tuberculosis (TB) infection control measures and incidence of TB in nursing homes for elder.

[Subjects and method] The questionnaire on TB infection control was distributed to all nursing homes in Osaka City in 2005.

[Results] The questionnaire was returned from 197 (90%) out of 219 facilities. In more than half of facilities, infection control committee was organized (57%) and automated ventilation system was installed (59%). In almost all facilities, residents had annual chest X-ray screening (94%). Respiratory symptoms were checked for residents and "day service" users in majority of facilities. 100% of employees had annual chest X-ray screening. However TB education session for employees was held annually in only 40% of facilities. Education materials on TB were distributed in 19%. Tuberculin skin test (TST) was conducted for new employees in 31%. TB patients were diagnosed in 22% of facilities in the past 3 years from 2002 to 2004. Incidence rate of TB is 75.2 per 100,000 for residents and 24.1 per 100,000 for employees. Analysis showed that TB incidence rate is higher in facilities with larger number of residents and in facilities where infection control committee

is organized, and facilities where TST is conducted for new employees.

[Discussion] In Osaka City, TB infection control was more often implemented in facilities where TB patient was diagnosed. When age structure is taken into consideration, TB incidence rate of employees or residents was lower than general population. Nosocomial TB infection does not seem to be occurring in nursing homes. However, as TB patients were diagnosed occasionally, TB infection control measures should be strengthened in Osaka City.

Key words: Nursing homes, Nosocomial tuberculosis infection control, Control measures, Survey by questionnaire, Osaka City

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————— Case Report —————

A CASE OF LIVER TUBERCULOSIS REQUIRING DIFFERENTIATION
WITH LIVER METASTASIS OF CANCER

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Hirokazu TOUGE, Shigenori ISHIKAWA, and Hiroyasu TAKEYAMA

Abstract A 79-year-old woman was admitted to our hospital because of general fatigue. Chest radiograph and computed tomograph showed bronchiectasis and centrilobular nodules in the right middle lobe and lingula, which had not changed from previous examination. Intrahepatic nodular lesions and swelling of the left cervical lymph nodes, supraclavicular lymph node, abdominal paraaortic lymph nodes and inguinal lymph nodes was observed. Biopsy specimen of the liver lesion demonstrated epithelioid cell granulomas. Biopsy of the right inguinal lymph node demonstrated epithelioid cell granulomas with caseous necrosis and culture of the specimen showed *Mycobacterium tuberculosis*. The patient was diagnosed as having liver tuberculosis and multiple tuberculous lymphadenitis. Antituberculous treatment with

isoniazid, rifampicin, ethambutol and pyrazinamide were started and continued for 6 months. All lesions improved after treatment. This was a rare case of liver tuberculosis that was difficult to distinguish from liver metastasis of cancer.

Key words: Liver tuberculosis, Tuberculous lymphadenitis

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————— Case Report —————

A CASE OF PULMONARY *MYCOBACTERIUM MAGERITENSE* INFECTION:
THE DIFFICULTY OF DIFFERENTIAL DIAGNOSIS OF
GRANULOMATOUS LUNG DISEASES¹Makoto MIKI, ¹Minoru SHIMIZUKAWA, ¹Hiroshi OKAYAMA, and ²Yuko KAZUMI

Abstract A 36 year-old female was pointed out of pulmonary abnormal shadows in the annual chest survey. Chest radiograph and computed tomography (CT) disclosed bilateral diffuse infiltrative shadows and tree-in-bud appearance in the right upper lung field and the left lingula. A sputum smear for acid-fast bacilli was negative. Histopathologically, the transbronchial lung biopsy specimen revealed non-caseous epithelioid granulomas with numerous giant cells. Acid-fast bacilli were cultured from her sputum, however, nontuberculous mycobacteria was not detected by DNA-DNA hybridization method. *Mycobacterium mageritense* was identified by 16S ribosomal RNA sequencing with 100% matching. The isolated colony of *M. mageritense* was resistant to nine anti-tuberculous drugs. Follow-up chest CT scan showed a gradual decrease of infiltrative shadows without therapy.

To the best of our knowledge, *M. mageritense* infections are rare, and this is the first case report of pulmonary infection in

the literature. We conclude that the pulmonary infection of *M. mageritense* is one of causes of granuloma formation, and in some case it is difficult to differentiate clinically from sarcoidosis.

Key words: *Mycobacterium mageritense*, Nontuberculous mycobacterial disease, granuloma, 16S ribosomal RNA sequencing, sarcoidosis

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The 81st Annual Meeting Educational Lecture

THE PERSPECTIVE OF PULMONARY MAC INFECTION TREATMENT

Atsuyuki KURASHIMA

Abstract Chemotherapy of pulmonary MAC (*Mycobacterium avium* complex) infection has been almost universally agreed with the multidrugs regimen that contains Clarithromycin (CAM), Rifampin (RFP), Ethambutol (EB), and aminoglycoside in case of advanced stage.

One of the reason for the multidrugs regimen which is similar to tuberculous chemotherapy is to inhibit the emersion of resistant MAC strain. The other reasons, enhancement of anti microbial activity and response to polyclonal infection are unique to the MAC chemotherapy. In the current MAC chemotherapy, both CAM and aminoglycoside are main axes because only they can suppress the growth of MAC alone respectively. Efficacy of CAM was revealed through the randomized controlled trials of disseminated MAC infection with HIV and that consequences applied to pulmonary MAC infection treatment. CAM is not effective unless exceed $2\ \mu\text{g/ml}$ blood concentration. RFP decreases CAM blood concentration remarkably, but the regimens contained RFP and CAM are superior clinically to the regimens without RFP. There seemed to be unknown pharmacological mechanisms with RFP. Although the advantage of aminoglycosides is easily achieved high blood concentration, if aminoglycoside dosage is exceed $15\ \text{mg/kg}$, the possibility of auditory disturbance increase.

About the duration of MAC chemotherapy, many guidelines recommended that one year continuation after the negative conversion of sputum culture. It is not the evidence but an expert opinion. We often experience recurrences several months later after the all drugs are ceased. The interval days to positive conversion of sputum culture from the day of

completion of chemotherapy are randomly distributed with weibull's equation. It suggests that exogenous re-infection may cause the recurrence of pulmonary MAC infection as pointed out by Wallace Jr.

Considering these issues, we have the conception of pulmonary MAC infection chemotherapy as follows.

1. full dose induction chemotherapy (two years)
2. maintenance chemotherapy (one year)
3. preventive chemotherapy (one year)

These conceptions have to be the problem validated.

However, these current chemotherapies are not effective adequately, we need the combination treatment with surgical resection when indicated as a localized focus for example. Generally chemotherapy could not cured the destructed bronchial lesion due to MAC infection as like as local bronchiectasis or cavities. Consequently, the chemotherapy just after the surgical resection of destructed focus is most appropriate period.

Key words: Non tuberculous mycobacteriosis, *Mycobacterium avium*, Relapse, Chemotherapy, Treatment period, Surgical resection

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PREVENTIVE MEASURES AGAINST TUBERCULOSIS IN WORKING FACILITIES AND COMPANIES

Chairpersons: ¹Kiminori SUZUKI and ²Ken SATOU

Abstract The health care program in working facilities and companies have played a significant part in prevention of tuberculosis. However, the ordinary national tuberculosis survey policy was abolished in April, 2005 and the tuberculosis survey for salary-earners is on the brink of drastic change. In this symposium the current status of the prevailing survey of tuberculosis in working facilities and companies was reviewed and the future direction of the tuberculosis survey in comparison to that in lung cancer survey was discussed.

1. Epidemiological trends of tuberculosis from the tuberculosis surveillance data: Masako OHMORI (Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association)

The estimated rate of tuberculosis case discovery by perio-

dical mass screening in the working facilities was 0.033% and it was higher than that in general adult population. The detection rate of tuberculosis in nurses who suffered from tuberculosis reached 40.4% by an aid of mass X-ray screening and 8.7% by contact tracing. The risk of onset of the disease was 4.3 times higher nurses than in general at the same years of age. The importance of infection control measures in the medical facilities was emphasized.

2. Current status and problems in tuberculosis control in a large-sized company: Yusuke NAKAOKA (Department of Occupational Health, Osaka Railway Hospital, West Japan Railway Company)

Some preventive modalities against TB such as periodical

medical check-up and awareness programs have been done for the purpose of prevention in our company. The prevalence of the disease has significantly reduced in number. The specific circumstances in large-sized company should be taken into consideration, and it is important for company workers and health professionals to recognize their roles in preventing the infectious disease.

3. Are there any differences between clinical cases and control people working for small-sized companies in the onset of tuberculosis? : Osamu NAKASHIMA, Kohei IMOTO (Taito Health Center, Tokyo) and Toru MORI (Research Institute of Tuberculosis, JATA).

We surveyed environmental conditions in working places and domestic conditions of employees who were working for small-sized companies located in Taito ward, based on written questionnaires. The companies were selected as those which had the patients of tuberculosis in the past one-year period, and the number of employees was less than ten. Compared with control people, TB patients had more frequent smoking habit ($p < 0.05$), and tended to have been less exposed to the sunshine at their residency and to have nutritionally poor meals and deficits of their meals. These results suggest that these factors alone or in combination may contribute to accelerated onset of tuberculosis.

4. Current status and problems in tuberculosis management among high prevalence population and in health check-up for personnel with unspecified and high occupational contact with tuberculosis patients: Hidetoshi IGARI (Division of Control and Treatment of Infectious Diseases, Chiba University Hospital), Kiminori SUZUKI (Chiba Foundation for Health Promotion and Disease Prevention)

Tuberculosis prevalence is as high as 500–1500 per 100,000 peoples among the homeless and construction workers living in "Hanba", a bunkhouse. We surveyed their medical conditions through periodical or extraperiodical health check-up. We retrospectively analyzed some medical factors contributing to successful treatment of the disease. Hospital admission and enhancement of counseling opportunities were two factors leading to the success of the treatment. The ambulance attendants have a significant possibility to contact patients

with TB and are high at risk of acquiring the infection. As there are often limited information on TB in patients in an emergency condition, it is difficult to protect themselves from its contagion properly. Periodical and extraperiodical health check-up is important for these personnel and application of QuantiFERON-TB 2nd generation to the personnel is new and useful for diagnosis of the latent tuberculosis infection.

5. A role of chest X-ray examination for lung cancer detection among company workers : Takeo TESHIMA (Koseikan Clinic, Miyagi Branch, Japan Anti-Tuberculosis Association)

Detection rates of lung cancer in company workers and in general population under the age of 60 years were compared. Chest X-ray survey was done using 10 cm × 10 cm indirect chest X-ray films of the chest. The detection rates of lung cancer in patients with definite or suspicious diagnosis in 265,620 company workers were 2.3 and 2.6 per 100,000, respectively. On the other hand the rates were 9.9 and 8.8 per 100,000 in a general population of 811,391. Twenty-four percent of patients with suspicious diagnosis were eventually made a definite diagnosis. The corrected detection rate reached to 21.0 for male patients and 8.3 for female patients and 12.1 totally. The detection rates of lung cancer in company workers and in general population under the age of 60 years exceeded the rate of pulmonary tuberculosis. Chest X-ray examination for the detection of lung cancer and pulmonary tuberculosis is still recommended under various working and social circumstances.

Key words: Working facilities and companies, Tuberculosis surveillance, Tuberculosis management, QuantiFERON, Lung cancer

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The 81th Annual Meeting Symposium

IMPROVEMENT OF ROUTINE WORKS AND QUALITY CONTROL
IN MYCOBACTERIAL LABORATORYChairpersons: ¹Katsuhiro SUZUKI and ²Takeshi HIGUCHI

Abstract Many new methods have been introduced into routine laboratory works in microbiology since 1990. Molecular biology, in particular, opened a new era and promoted a technician's skill much. PCR and hybridization technique have been ordinary one in many laboratories. Since old techniques such as smear and culture are still needed, amount of routine works is increasing gradually. Thus, improving efficiency and keeping quality of routine works are becoming more and more important issues. This symposium focused on such points, and four skilled technicians around Japan presented their own tips.

1. Coexistence of *M. tuberculosis* and *M. avium* complex (MAC) in the MGIT culture system: Yasushi WATANABE (Clinical Laboratory Division, NHO Nishi-Niigata Chuo National Hospital)

Sputum samples of some tuberculosis patients yielded only MAC in the MGIT culture system. Such co-infected cases presented problems to mislead proper treatment and infection control. The detection rate of MAC was significantly high, and the growth speed of MAC was significantly rapid in the MGIT culture system, compared to those of *M. tuberculosis*. Additionally, *M. tuberculosis* was not detected with even more quantity than MAC in the small amount of mixed samples. Higher sensitivity and growth speed of MAC are the important characteristics of the MGIT system.

2. Internal quality control with ordinary examination results: Akio AONO (Department of Clinical Examination, Double-Barred Cross Hospital, Japan Anti-Tuberculosis Association)

Our laboratory utilizes ordinary examination results as the internal quality control for specimen pretreatment, culture, and drug susceptibility testing. The contamination rate of MGIT culture system is useful for the evaluation of the decontamination process. It was 6.3% on average in our laboratory in 2005. The number of drug resistant strains is also useful to assess the performance of drug susceptibility testing. The incidence of each anti-tuberculosis drug resistance detected monthly in 2005 is up to 5 for isoniazid (INH), 4 for rifampicin (RFP), 7 for streptomycin (SM), 1 for ethambutol (EB), and 2 for pyrazinamide (PZA), respectively. If any serious deviation from the average number is observed, action for the investigation is taken. The analysis of the ordinary examination data is useful to implement a quality control efficiently, and to improve the total laboratory performance.

3. The advanced devices for solving problems of the smears and cultivation of Mycobacteria: Motohisa TOMITA (NHO Kinki-chuo Chest Medical Center)

Recently, the newly developed, standardized, commercially available kits including PCR and liquid media for confirmation and identification of mycobacteria are prevalent in Japan for the rapid diagnosis of *M. tuberculosis*. These tests are sensitive and accurate, but still expensive and technically demanding. The improvement of these methods, in particular, requires time-consuming process. We have optimized the culture technique, the identification method, and the drug-susceptibility testing for Mycobacteria in a time-saving manner. They should provide a basic grounding in the application of the techniques for anyone who is interested in these intriguing bacteria.

4. Ultimate quality control of specimens—teaching how to get a good sputum sample: Takeshi HIGUCHI (Kyoto University Hospital)

Modern techniques including molecular biology have been applied to routine laboratory works for rapid detection, identification, and drug susceptibility testing of mycobacteria. Even in using such techniques, however, poor quality specimens yield only poor results. To get a high quality specimen, particularly sputum samples, is very important. Therefore, laboratory technicians in our hospital have directly taught each patient how to expectorate good quality sputa since 2001. The teaching of patients has improved the rate of P1 samples from 21.5% to 36.6% by Miller and Jones visual score of sputum. The teaching has also improved the rate of smear positive P1 samples from 11.4% to 28.8%. To teach each patient how to get good sputa seems useful for keeping the laboratory quality high.

Key words: Mycobacterial laboratory, Quality control, Improvement, Teaching how to get good sputum

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