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ORIGINAL ARTICLE

Laboratory Investigation of a Nosocomial Transmission of Tuberculosis at a District General Hospital

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Background/Purpose: Nosocomial outbreak of tuberculosis (TB) is rarely documented and the transmission is usually difficult to confirm because of the long incubation period of the mycobacterial infection. In this report, we demonstrated the use of molecular genotyping methods together with contact tracing to identify the source case, the causative outbreak strain and transmission dynamics of *Mycobacterium tuberculosis*, and for the definite confirmation of a suspected outbreak.

Methods: *M. tuberculosis* strains were genotyped with IS*6110* restriction fragment length polymorphism, spacer oligonucleotide typing and minisatellite interspersed repetitive unit–variable number tandem repeat methods. Clinical data and contact tracing results were collected from medical records and the National TB Registry.

Results: In this episode, 66 health care workers (HCWs) were notified as TB cases. A total of 18 *M. tuberculosis* isolates from HCWs and patients were collected. IS*6110* RFLP results revealed that 9 out of 10 HCWs' and 7 out of 8 patients' isolates shared the same genotype. The causative isolate was identified as the Beijing genotype. The index case was a hospitalized respirator-dependent patient.

Conclusion: Thorough collection along with molecular diagnosis and genotyping of all *M. tuberculosis* isolates are recommended for the confirmation of any suspected nosocomial TB outbreak. [*J Formos Med Assoc* 2007;106(7):520–527]

Key Words: genotyping, nosocomial transmission, Taiwan, tuberculosis

Tuberculosis (TB) is a disease with a long incubation period; it can thus cause a nosocomial outbreak which is usually hard to confirm.[1] Nosocomial transmission of *Mycobacterium tuberculosis* has been previously reported to be associated with close contact with unrecognized and/or active TB patients,[2] autopsy,[3] surgery,[4] and medical procedures such as bronchoscopy,[5] endotracheal intubation, respiratory suctioning, aerosol or nebulized treatment and sputum induction.[6,7] Insufficient environmental control and inadequate administrative measures were also contributing reasons.[8–10] Since the early 1990s, nosocomial infection of TB among health care workers (HCWs) has drawn much more attention than before and is considered to be an important occupational hazard.[11]

During the peak severe acute respiratory syndrome (SARS) period (April to July, 2003) an outbreak of nosocomial pulmonary infection occurred in a district general hospital in Taipei. The suspected nosocomial outbreak of SARS alert led us to conduct a thorough contact investigation and it turned out to be a TB episode. A part of this episode has briefly been described previously.[12]

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The outbreak of SARS has further witnessed the importance of nosocomial infection control.[13] A combination of disease surveillance with epidemiologic and laboratory investigation has been successfully applied in several outbreak investigations of nosocomial TB infection.[11] TB remains the leading notifiable infectious disease in Taiwan.[14] The moderate incidence of TB will certainly increase the risk of potential transmission among HCWs in health care facilities. Even though some small clusters of sporadic TB cases have been recognized at schools, health care facilities and other institutions, no major nosocomial outbreak has ever been proven in Taiwan. In this report, we demonstrate the use of molecular genotyping methods together with contact tracing to identify the source case, the causative outbreak strain and transmission dynamics of *M. tuberculosis*, and for the definite confirmation of a suspected outbreak. It could serve as a standard protocol for any possible nosocomial TB outbreak investigation in the future.

Materials and Methods

Setting

The episode occurred in a private 700-bed district general hospital with approximately 20,000 annual inpatient admissions, in addition to providing medical services to approximately 2000 outpatients daily. Of the 700 beds, there are 18 beds on the 4th floor for chronic ventilator-dependent care and four negative-pressure isolation rooms on the 9th floor for highly contagious patients, including open TB cases. The main hospital building is structurally divided into two wings but without any physical partitions. The outbreak occurred primarily on the 4th floor of the main building (Figure 1). On the 4th floor of the east wing, there is a 48-bed ward for orthopedic, urology, and pediatric patients, in addition to an office for dieticians. On the same floor of the west wing is an 18-bed ward for mainly ventilator-dependent patients, an endoscopy suit, a pulmonary function test room, and neurologic and urodynamic study laboratories.

Study population

A total of 65 presumed and one confirmed HCW cases involved in this suspected outbreak were notified in 2003 and 2004, respectively. Initially, a female nurse (HCW 1) working on the 4th floor complaining of fever and vomiting was detected and identified by a routine health monitoring in April 2003. Another female nurse working in the same ward complained of fever, headache, and chest pain about 3 weeks thereafter. Both HCWs' chest X-rays (CXR) showed abnormality, and they were commenced on anti-TB treatment for TB. Subsequently, contact investigations, using CXR as a primary means to thoroughly screen for

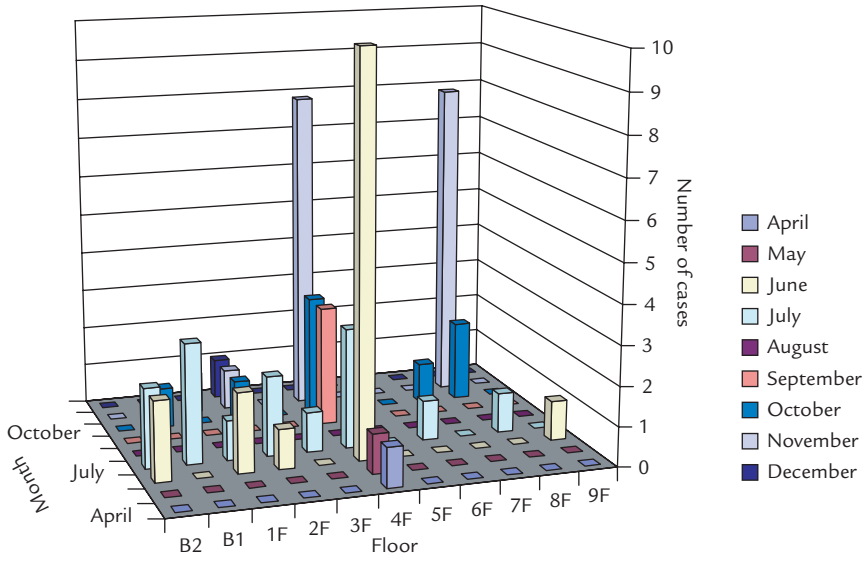

Figure 1. Trends over 62 notified tuberculosis cases on different floors of the main building and three cases from other buildings in the hospital complex from April to December 2003. One case was identified on the 8th floor of the main building in March 2004.

potential TB infectious cases, were conducted on all nurses, ancillary staff, medical technicians, and any other personnel who worked on the 4th floor. Tuberculin skin test was not performed because most of them had already received BCG vaccination. The algorithm of decision for the contact investigation was based on the "stone-in-pond" theory and a 3-month interval periodic CXR screening for 9 months.[15]

A female technician (HCW 2) working in the basement of the hospital was later confirmed to have TB by positive sputum culture for *M. tuberculosis*. An epidemiologic investigation afterwards revealed close contact (< 2 hours) between the technician and Patient Case 1 (P1) during a bone-scan process. Following this finding, almost the entire 1580-strong hospital staff including all full-time and part-time employees, volunteers and contracted personnel underwent CXR contact investigation for possible exposure. A retrospective review of the clinical data of all patients with past or recent primary TB history ever admitted to the 4th and other floors between November 2002 and December 2003 was conducted.

Mycobacterial strains

Clinical data and the results of contact tracing were collected from medical data, TB registries and patient interviews. Of 66 notified HCW cases, 61 (92.4%) submitted sputum and biopsy samples for bacteriologic and pathologic examinations. A total of 18 *M. tuberculosis* strains, 10 from HCWs and eight from patients, cultured in one of the clinical mycobacteriology laboratories of Taipei and Kaohsiung Veterans General Hospitals, Taipei Chronic Disease Hospital and a private Super Laboratory Co. were then submitted to the Reference Laboratory of Mycobacteriology of the Taiwan Centers for Disease Control, for confirmation and drug susceptibility testing by using standardized methods.[16]

Sequence analysis of rpoB and putative mut genes

The *rpoB* gene was amplified with primers *rpoB*-F (5′-TCGGCGAGCCCATCACGTCG-3′) and *rpoB*-R (5′-GCGTACACCGACAGCGAGCC-3′), which yielded a 541-bp fragment containing the hot spot region.[17] Primers for putative *mut* genes (*mut T*2, *mut T*4 and *ogt*) were designed according to Rad et al.[18] The amplification products were sequenced using an ABI 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Molecular genotyping

All isolates were assessed for epidemiologic dependence using IS*6110* restriction fragment length polymorphism (RFLP) profiling with modifications.[19] Spacer oligonucleotide typing (spoligotyping) was applied to identify and differentiate *M. tuberculosis* complex isolates in this study.[20] The characteristic Beijing genotype was defined as strains hybridized only to the last nine spacer oligonucleotides (spacers 35 to 43). Standard minisatellite interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) was performed as described previously with modification.[21] The RFLP and spoligotype patterns, and MIRU-VNTR profiles were scanned and analyzed using Bionumerics® software, version 4.0 (Applied Maths, Kortijk, Belgium).

Results

Ascertainment and characteristic of cases

According to The National Tuberculosis Register System, the hospital notified 86 new TB cases in 2001, 110 cases in 2002 and 181 cases in 2003, including outpatient and ward patients. Cases of pulmonary TB were defined as persons with or without symptoms having positive acid-fast bacilli smears, positive *M. tuberculosis* culture or biopsy demonstrating granulomatous changes. The trend of case notification is shown in Figure 1. In this episode, 66 HCW cases were confirmed with TB infections and about half (45.5%, 30/66) of cases were reported from the 4th floor of the main hospital building. The occupational distribution of notified cases were nurses (48.5%, 32/66), technicians (13.6%, 9/66), physicians (4.5%, 3/66), cleaners (4.5%, 3/66), nursing aides (4.5%, 3/66),

dieticians (3.0%, 2/66), and others (21.2%, 14/66). All 66 cases, previously with normal CXR, became abnormal in this episode. A total of 11 hospitalized and three outpatient clinic cases (P11, P13 and P14) were listed as presumed sources of infection (Table). The timeline of the suspected index patient cases is summarized in Figure 2.

Laboratory confirmation of an outbreak

There were 10 (15.1%, 10/66) HCW cases: seven staff members on the 4th floor, one nurse working on the 8th floor, another one stationed at the outpatient clinic on the 1st floor, and one nuclear medicine technician working in the basement, who had cultures positive for *M. tuberculosis* (from 8 sputa, 1 pleural effusion, and 1 excisional biopsy clinical samples). Besides, the other eight available *M. tuberculosis* isolates for genotyping were those from suspected source patient cases P2, P4, P11, P12, P13, P14, P7 (wife of P6) and P9 (son of P8).

RFLP, spoligotyping and MIRU-VNTR genotyping were performed on the 18 isolates obtained. Typing results revealed that 16 isolates had clustered RFLP patterns (9 from HCWs and 7 from patients) and the one from the nurse working on

Table. Characteristics of confirmed and suspected source cases of tuberculosis

Case	Age/Sex	Type of TB	Occupation	Job/Ward location	Culture date, result	RFLP bands, *n*	RFLP pattern	Cluster
HCW1	23/F	New; p	Nurse	4th F	8/18/03, TB	19	I	Y
HCW2	26/F	New; p	Technician	B2	5/14/03, TB	19	I	Y
HCW3	46/F	New; p	Janitor	4th F	7/21/03, TB	19	I	Y
HCW4	35/F	New; p	Nurse	1st F	6/30/03, TB	19	I	Y
HCW5	39/F	New; p	Technician	4th F	7/14/03, TB	19	I	Y
HCW6	50/F	New; p	Nursing aide	4th F	10/16/03, TB	19	I	Y
HCW7	25/F	New; p	Dietician	4th F	11/21/03, TB	19	I	Y
HCW8	25/F	New; p	Nurse	8th F	11/04/03, TB	18	II	N
HCW9	23/F	New; p	Nurse	4th F	11/17/03, TB	19	I	Y
HCW10	30/F	New; p	Dietician	4th F	3/03/04, TB	19	I	Y
P1	78/F	Old; relapsed; p		4th F	11/27/02, TB	NA	NA	NA
					1/11/03, TB	NA	NA	NA
					6/16/03, NTM	NA	NA	NA
P2	82/F	New; p		4th F	5/09/03, TB	19	I	Y
P3	75/F	New, p		4th F	4/24/03, C–	NA	NA	NA
P4	87/F	New; p		4th F	2/25/03, TB	21	III	N
P5	49/M	New; p		4th F	4/28/03, NTM	NA	NA	NA
P6	86/M	Old; p		4th F	repeated C–	NA	NA	NA
P7	77/F	New; p		4th F	7/12/03, TB	19	I	Y
P8	82/F	Old; relapsed; p		8th & 4th F	7/16/03, NTM	NA	NA	NA
P9	62/M	New; p		OPD	1/14/04, TB	19	I	Y
P10	81/M	Old; p		4th F	9/01/98, NTM	NA	NA	NA
					9/04/02, NTM	NA	NA	NA
P11	71/M	Old; relapsed; p & ep		OPD	11/25/03, TB	19	I	Y
P12	76/M	new; p		4th F	6/09/03, TB	19	I	Y
P13	76/M	new; p		OPD	6/20/03, TB	19	I	Y
P14	72/M	new; p		OPD	6/27/03, TB	19	I	Y

TB = tuberculosis; RFLP = restriction fragment length polymorphism; HCW = health care worker; P = patient; p = pulmonary; ep = extrapulmonary; OPD = outpatient department; C– = culture negative; NTM = nontuberculous mycobacteria; NA = not available.

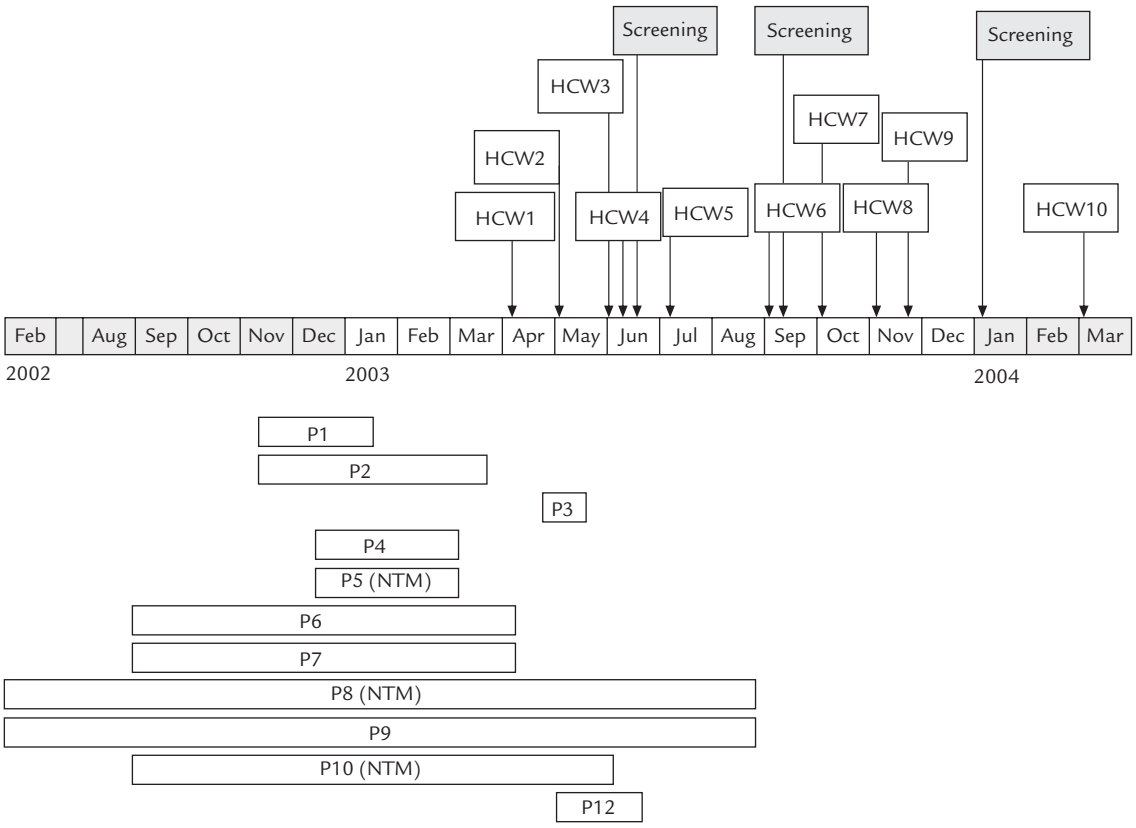

Figure 2. Timeline demonstrating the presumed chains of nosocomial transmission of tuberculosis. Patients 11, 13 and 14 are outpatient cases. HCW = health care worker; P = patient; NTM = nontuberculous mycobacteria.

the 8th floor and P4 each had the unique RFLP pattern (Figure 3). The clustered outbreak strain had a 19-band RFLP pattern, and the two exceptional strains had 18 and 21 bands, respectively. The putative mutator gene analysis indicated that the outbreak strain had missense mutations of *mut T*2 at codon 58 (GGA → CGA, Gly → Arg), *mut T*4 at codon 48 (CGG → GGG, Arg → Gly) and silence mutation at *ogt* at codon 12 (GGG → GGA, Gly → Gly), which matched the characteristics of the W-Beijing family. Those 16 clustered isolates showed typical Beijing genotype and identical MIRU-VNTR profile (2,2,3,3,2,5,1,7,3,5,3,3). Transmission was then suggested with matched DNA fingerprints. All 18 isolates investigated were susceptible to any first-line anti-TB drugs by the conventional agar proportion drug susceptibility testing method, and no resistance-related mutation was found by *rpoB* gene sequence analysis. Since the last positive culture of *M. tuberculosis* was collected in March 2004, no

Figure 3. IS*6110*-RFLP patterns of *Mycobacterium tuberculosis* strains: pattern I is the causative strain of outbreak cluster, pattern II is the strain isolated from health care worker on the 8th floor, pattern III is the strain isolated from patient 4, and pattern IV is the reference strain Mt14323.

TB case of HCW was notified afterwards, and the episode presumably came to an end.

Discussion

This study demonstrates the importance of strict hospital infectious control during SARS period, including close monitoring of the health condition of HCWs, could contain a nosocomial infection episode. Even though the long incubation period of TB hinders the exact transmission routes, applying molecular methods in the investigation of a nosocomial outbreak could prevail over the difficulty in confirming the transmission and the index case in an area with moderate prevalence of TB. We also observed that short contact with an active TB case during radiologic examination revealed high risk of infection. Furthermore, the identification of nontuberculous mycobacteria (NTM) in suspected cases might mislead the investigation and delay the responses to nosocomial infection control.

There are several limitations of this investigation. First, most of the HCW cases were in their early minimum TB stage with no apparent clinical symptoms and all induced sputum, pleural effusion and biopsy samples resulted in only 10 positive *M. tuberculosis* cultures for genotyping (Table). Second, the initial *M. tuberculosis* isolates of some suspected index patient cases were not available for genotyping. However, *M. tuberculosis* isolated from family members (P7 and P9) providing bedside care to two suspected index cases (P6 and P8) were later genotyped to be identical to the outbreak strain by RFLP. These results provided some indirect evidence for possible index cases of case P6 and P8 (Table). Therefore, a national archive of mycobacterial strains was recommended and it would become a sustainable resource in support of evidence-based retrospective investigation of temporal and spatial transmission. Third, the recent isolates of two highly suspected cases P1 and P10 turned out to be NTM rather than *M. tuberculosis.* They were residents on the 4th floor without isolation because they were considered cured old TB cases and had previous *M. tuberculosis* isolated. The complication of NTM might have caused missed or delay in diagnosis of an active TB case and unintentionally result in a nosocomial outbreak.

In this investigation, 16 isolates with identical RFLP pattern clearly indicates a nosocomial infection. Case P1 was considered the primary index case of this outbreak. Of the 10 HCWs, nine cases were clustered. Eight of them worked on the 4th floor, and a technician who worked in the basement had casual contact with P1, who was hospitalized in the respiratory care ward (RCW) on the 4th floor. Among those seven patients (P2, P7, P9, P11, P12, P13, P14) infected with the same strain, two (P7 and P9) were family members of previously hospitalized RCW patients on the 4th floor (P6 and P8). In addition, P2 was recognized after being transferred to another Taipei municipal hospital and notified as a TB case later. The P2 case, with no TB history and a normal CXR at the time of admission, was hospitalized in the RCW on the 4th floor in December 2002 and might have been exposed to ward mate P1, whose CXR showed reactivation of pulmonary TB and was admitted since November 2002. Being reported by another hospital in Southern Taiwan, P11 was an outpatient case who visited the clinic on the 1st floor during the episode. Cultures from his sputum and surgical wound showed positive *M. tuberculosis* that matched the genotype of the outbreak strain. Since one of the HCWs (HCW 4) working at the outpatient clinic was also infected with the outbreak strain, there might be a second infection source. P12 stayed on the 4th floor during the outbreak. Two outpatient cases (P13 and P14) infected with the clustered outbreak strain, and with no prior hospitalization and obvious contact history, might have been infected in the community.

Overall, both the RCW on the 4th floor and outpatient clinic on the 1st floor were recognized as locations with the highest risk for possible *M. tuberculosis* transmission. Most of the patients in the RCW were ventilator-dependent, thus generating highly concentrated infectious nuclei; while in the outpatient clinic, there might be unrecognized

open TB cases in the waiting area without proper isolation. It has been found that aerosol droplets of *M. tuberculosis* may remain viable for days in the environment.

The possibility of laboratory cross-contamination was carefully evaluated according to the culture date, origin and procedures. All *M. tuberculosis* isolates received were subcultured and reconfirmed before further genotyping investigation. Several nosocomial investigations revealed that the frequently observed causative strain was W-Beijing genotype, including the outbreak strain identified in this episode.[22] According to the results of surveillance conducted in 2003, the overall prevalence rate of Beijing family genotypes was 44.4% and 51.6% in Taiwan and Northern Taiwan, respectively.[23] Several studies indicated the unique features of the Beijing strain, including its early but ephemeral immune response, more severe pneumonia, more deaths, poorer BCG vaccination protection in animal models, and frequent association with multidrug resistant TB outbreaks in institutions.[24] Based on the mutation analysis results of genes involved in the repair of DNA (*mut* genes), the outbreak Beijing strain identified in this episode presumably had increasing adaptation and persistence ability to hosts as suggested.[18] Identical MIRU-VNTR pattern was found in *M. tuberculosis* strains isolated from Malaysia and China.[25] It is not surprising that Taiwan is geographically and economically closely related to Mainland China, which has a high prevalence rate of the Beijing genotype of *M. tuberculosis*.[26] Close surveillance of this prevalent strain is necessary to prevent any possible outbreaks in future. Nosocomial transmission of TB has been frequently reported, especially after the observed resurgence of the disease and the increased incidence of multidrug-resistant (MDR) TB in the 1990s.[27] The identified outbreak strain has a RFLP pattern different from the MDR W-Beijing strain reported (Figure 3). For many cases notified in such a short period of time, we could have been facing a highly virulent and contagious Beijing strain of *M. tuberculosis*. Fortunately, the causative strain in this outbreak was susceptible to all first-line anti-TB antibiotics.

In conclusion, missed and delayed diagnosis and treatment of TB among hospitalized patients on the 4th floor were the main causes of this outbreak. With the help of molecular genotyping methods, epidemiologic links between the patients and HCWs have been confirmed. Thorough collection along with the molecular diagnosis and genotyping of all *M. tuberculosis* isolates are recommended for the confirmation of any suspected nosocomial TB outbreak.

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