Journal of Hospital Infection 102 (2019) 116–119

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Short report

Nosocomial *Mycobacterium tuberculosis* transmission by brief casual contact identified using comparative genomics

J. Seto [a,*], Y. Otani [b], T. Wada [c], Y. Suzuki [a], T. Ikeda [a], K. Araki [d], K. Mizuta [a], T. Ahiko [a,d]

[a] *Yamagata Prefectural Institute of Public Health, Yamagata, Japan*
[b] *Shonai Public Health Centre, Yamagata, Japan*
[c] *Nagasaki University, Nagasaki, Japan*
[d] *Murayama Public Health Centre, Yamagata, Japan*

ARTICLE INFO

Article history:
Received 18 October 2018
Accepted 2 January 2019
Available online 7 January 2019

Keywords:
Casual contact
Comparative genomics
Mycobacterium tuberculosis
Recent tuberculosis transmission
Variable number tandem repeat typing

SUMMARY

This paper reports a case of nosocomial transmission of *Mycobacterium tuberculosis* by brief casual contact. Routine variable number tandem repeat typing in Yamagata Prefecture, Japan found that *M. tuberculosis* clinical isolates from two patients showed indistinguishable genotypes. The patients had an epidemiological relationship of sharing a waiting room in a hospital on the same day. As comparative genomics detected only two single nucleotide variants between the isolates, it was concluded that recent tuberculosis transmission occurred in the waiting room. These results indicate that the physical separation of infectious tuberculosis patients is an essential control measure for preventing unpredictable nosocomial transmission by casual contact.

Introduction

The nosocomial transmission of *Mycobacterium tuberculosis* is a threat for healthcare facilities worldwide [1]. Therefore, several recommendations have been developed for preventing nosocomial tuberculosis (TB) transmission. In particular, the triage and physical separation of infectious TB patients is strongly recommended for breaking the chain of transmission [1,2]. However, it is difficult to confirm the effectiveness of those control measures [1]. One reason for this is that nosocomial TB spread by casual contact is difficult to identify due to the long incubation period [3].

Comparative genomics can adequately distinguish genetic differences among *M. tuberculosis* isolates [4,5]. Other genotyping methods that analyse parts of the *M. tuberculosis*

* Corresponding author. Address: Yamagata Prefectural Institute of Public Health, 1-6-6 Toka-machi, Yamagata-shi, Yamagata 990-0031, Japan. Tel.: +81 23 627 1373; fax: +81 23 641 7486.
E-mail address: setoj@pref.yamagata.jp (J. Seto).

https://doi.org/10.1016/j.jhin.2019.01.002

genome, such as variable number tandem repeat (VNTR) typing, are often used. However, genotypes that appear to be indistinguishable with VNTR may differ in their whole-genome sequences, suggesting the mixing of *M. tuberculosis* isolates not due to recent TB transmission [4]. Comparative genomics targeting the *M. tuberculosis* whole genome (i.e. approximately 4.4 million base pairs) can strongly support the possibility of recent TB transmission by counting the number of single nucleotide variants (SNVs) among different *M. tuberculosis* genomes. Walker *et al.* showed that recently transmitted *M. tuberculosis* isolates have five or fewer SNVs [4].

This study demonstrated the effectiveness of applying comparative genomics for the analysis of *M. tuberculosis* genomes to identify nosocomial TB transmission by brief casual contact. The results also indicate the need to use comparative genomics to progress public health actions.

Methods

Study setting

Yamagata Prefecture, located in the northern part of Japan's main island of Honshu, is subdivided into four topographically separated areas, and has an area of 9323 km^2. In 2017, Yamagata Prefecture had 7.4 TB cases/100,000 population among 1.1 million inhabitants. Since 2009, public health officials in Yamagata have conducted TB control using VNTR typing of *M. tuberculosis* clinical isolates to discover recent TB transmission events [6].

VNTR typing

This study used 24-locus VNTR typing ($24_{Beijing}$), optimized for the *M. tuberculosis* Beijing lineage that is predominant in Japan [7]. Polymerase chain reaction (PCR) was performed using a hot-start Ex-taq DNA polymerase (Takara Bio Inc., Shiga, Japan) or the SapphireAmp Fast Master Mix (Takara Bio Inc.) or both. These two PCR protocols have been described previously [8]. The sizes of the amplified PCR fragments were confirmed via agarose gel electrophoresis and with a microchip electrophoresis system (MCE-202; Shimadzu Corp., Kyoto, Japan). The number of repeats for each locus was determined based on the sizes of PCR products in published allelic tables [9].

Comparative genomics

The comparative genomics analysis of *M. tuberculosis* clinical isolates in this study was performed using platforms manufactured by Illumina (San Diego, CA, USA), as described previously [10].

Ethical approval

This study was approved by the Ethics Committee of Yamagata Prefectural Institute of Public Health (Approval No. YPIPHEC 18-06).

Results

In October 2014, through comprehensive VNTR typing, *M. tuberculosis* clinical isolates from two unrelated TB patients in Yamagata Prefecture, Japan were found to have indistinguishable profiles [6]. Patient 1, an elderly woman with low social activity, was diagnosed with pulmonary TB in June 2012 (Table I). Two years later, Patient 2, a middle-aged woman working at a company, was diagnosed with pulmonary TB. On performing contact investigations with patients using the interferon-γ release assay and chest radiographic analysis, a family member of Patient 1 and a family member and a colleague of Patient 2 received preventive therapy for latent TB infection. However, the epidemiological relationship of the two patients had not been recognized prior to the VNTR typing because they inhabited different administrative districts. The distance between their residences was approximately 73 km.

Subsequently, retrospective investigations of hospital records by two public health centres demonstrated that the two patients attended a hospital in the morning of the same day in June 2012. Specifically, on the day when Patient 1 with diagnosed TB was hospitalized, Patient 2 visited a department unrelated to respiratory medicine in the same hospital. Furthermore, Patient 1 had stayed in the waiting room near the main entrance to fulfil hospitalization procedures, and Patient 2 had also stayed in the same waiting room for approximately 10 min to pay the consulting fee. Accordingly, the public health centres considered that Patient 1 transmitted TB to Patient 2 during their brief casual contact in the waiting room. However, the hospital did not accept this scenario due to the insufficient discriminative power of VNTR typing [4,5], and the fact that nosocomial TB transmission by brief casual contact had not been reported previously.

Three years later, the opportunity arose to analyse the genome sequences of the two *M. tuberculosis* isolates; namely, Fmu18 isolated from Patient 1 and Hsho18 isolated from Patient 2 after a further 26 months. Comparison of their genome sequences (GenBank accession nos. SAMD00136662 and SAMD00136663) revealed that the Hsho18 isolate had two SNVs compared with the Fmu18 isolate. One SNV was in a gene of uncertain function (Rv1947), and the other SNV was a point

Table I
Characteristics of two tuberculosis (TB) patients involved in nosocomial transmission by brief casual contact, Yamagata Prefecture, Japan

Patient characteristic	Patient no.	
	Patient 1	Patient 2
Age	80s	50s
Sex	Female	Female
Notification date	June 2012	August 2014
Residential area	Murayama region	Shonai region
Distance from residence to hospital[a]	11 km	84 km
Site of disease	Pulmonary	Pulmonary
AFB sputum smear test	Positive	Positive
Treatment history	Initial	Initial
Birthplace	Japan	Japan
Specimen collection date	June 2012	August 2014
Mtb isolate no.	Fmu18	Hsho18

AFB, acid-fast bacillus; Mtb, *Mycobacterium tuberculosis*.
[a] The hospital in which Patient 1 was hospitalized and TB transmission occurred.

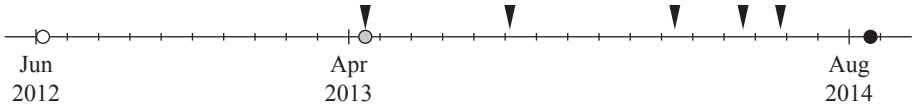

Figure 1. Schematic representation of the time course of Patients 1 and 2. Patient 1 was diagnosed with tuberculosis in June 2012 (white circle), and shared a waiting room in a hospital with Patient 2. Patient 2 was diagnosed with atypical pneumonia in April 2013 (grey circle), and was subsequently diagnosed with tuberculosis in August 2014 (black circle). Arrowheads indicate the ongoing prescriptions of levofloxacin.

mutation in *gyrA* related to fluoroquinolone resistance (Asp94Gly). Concordantly, drug susceptibility testing of the Hsho18 isolate showed that it was resistant to levofloxacin.

To resolve the reason for the acquisition of levofloxacin resistance in the Hsho18 isolate, the hospital records of Patient 2 were investigated. This revealed that Patient 2 presented with a fever, cough and leg pain, and was diagnosed with atypical pneumonia 10 months after contact with Patient 1. Levofloxacin was prescribed for more than one year (Figure 1). Therefore, it was concluded that the selective pressure of this inappropriate monotherapy regime resulted in levofloxacin resistance of Hsho18.

All the results of epidemiological investigations based on the genomic comparison data were reported to the hospital. The hospital accepted the possibility of nosocomial TB transmission by brief casual contact. Thereafter, the infection control committee of the hospital improved the flow of transferred TB patients. Following this improvement, TB patients are transported directly to a medical ward with ventilation systems without taking them through the waiting room, which many unspecified persons utilize.

Incidentally, as of December 2018, no other clinical isolates of *M. tuberculosis* showing the same VNTR profile as the two isolates characterized in this study had been detected in Yamagata Prefecture, Japan. Therefore, further transmission of these two *M. tuberculosis* isolates is not likely to have occurred.

Discussion

This paper reported a case of nosocomial transmission of *M. tuberculosis* by brief casual contact. Given that one SNV between the two isolates (except for the iatrogenic drug-resistant mutation) fulfills the criteria of recent TB transmission, that is, five or fewer SNVs between *M. tuberculosis* genomes [4], the inference that Patient 1 transmitted TB to Patient 2 in the waiting room of the hospital would be reasonable.

Results indicate that the genotyping of *M. tuberculosis* can contribute to the identification of recent TB transmission events. In this study, comprehensive VNTR typing triggered additional epidemiological investigations, and a further comparative genomics analysis supported the hypothesis of recent TB transmission as was proposed by the public health centres. Similar approaches have been used in previous studies [10,11]. Since the technology of genome analysis has the limitations of high cost and the requirement for specific expertise in addition to low versatility [5], VNTR-typing-based comparative genomics may remain an effective framework not only for public health actions in national or subnational settings, but also for TB infection control in healthcare facilities.

This study demonstrated a case of nosocomial TB transmission by brief casual contact identified by a combination of epidemiological investigations and a comparative genomics analysis of *M. tuberculosis* clinical isolates. The established evidence led to the development of a consensus with hospital staff and the strengthening of infection control procedures for TB in the hospital where transmission occurred. The results indicate that healthcare facilities should physically separate patients with infectious TB from other people [1], even in common use spaces, to prevent unpredictable nosocomial transmissions by casual contact.

Conflict of interest statement
None declared.

Funding source
This research was supported by AMED under Grant Numbers JP17fk0108114 and JP18fk0108063.

References

[1] World Health Organization. WHO policy on TB infection control in health-care facilities, congregate settings and households. Geneva: WHO; 2009. Available at: http://www.who.int/tb/publications/2009/infection_control/en/ [last accessed September 2018].
[2] Jensen PA, Lambert LA, Iademarco MF, Ridzon R. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care settings, 2005. MMWR Recomm Rep 2005;54:1—141.
[3] Pan SC, Chen CC, Chiang YT, Chang HY, Fang CT, Lin HH. Health care visits as a risk factor for tuberculosis in Taiwan: a population-based case—control study. Am J Public Health 2016;106:1323—8.
[4] Walker TM, Ip CL, Harrell RH, Evans JT, Kapatai G, Dedicoat MJ, et al. Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study. Lancet Infect Dis 2013;13:137—46.
[5] Takiff HE, Feo O. Clinical value of whole-genome sequencing of *Mycobacterium tuberculosis*. Lancet Infect Dis 2015;15:1077—90.
[6] Seto J, Wada T, Suzuki Y, Ikeda T, Mizuta K, Yamamoto T, et al. *Mycobacterium tuberculosis* transmission among elderly persons, Yamagata Prefecture, Japan, 2009—2015. Emerg Infect Dis 2017;23:448—55.
[7] Seto J, Wada T, Iwamoto T, Tamaru A, Maeda S, Yamamoto K, et al. Phylogenetic assignment of *Mycobacterium tuberculosis* Beijing clinical isolates in Japan by maximum *a posteriori* estimation. Infect Genet Evol 2015;35:82—8.
[8] Seto J, Wada T, Suzuki Y, Ikeda T, Mizuta K, Mitarai S, et al. Convenient PCR method for variable-number tandem-repeat typing of *Mycobacterium tuberculosis* clinical isolates. J Microbiol Methods 2017;139:12—4.
[9] Iwamoto T, Yoshida S, Suzuki K, Tomita M, Fujiyama R, Tanaka N, et al. Hypervariable loci that enhance the discriminatory ability

of newly proposed 15-loci and 24-loci variable-number tandem repeat typing method on *Mycobacterium tuberculosis* strains predominated by the Beijing family. FEMS Microbiol Lett 2007;270:67—74.

[10] Seto J, Wada T, Suzuki Y, Ikeda T, Araki K, Umetsu Y, et al. A case of laboratory cross-contamination of *Mycobacterium tuberculosis* identified using comparative genomics. Int J Tuberc Lung Dis 2018;22:1239—42.

[11] Walker TM, Crook DW, Peto TE, Conlon CP. Whole-genome sequencing identifies nosocomial transmission of extra-pulmonary *M. tuberculosis*. QJM 2016;109:819—20.