explain about PHC-based DOTS and address any concerns they may have about their treatment. After discharge, the patients are then asked to visit the PHC every day to continue their treatment. Patients not only receive drug treatment at the PHC, they are also offered food and drink on each visit, as well as the opportunity to talk about other problems, such as health concerns and social relationship issues. Around 10 patients per day visit the PHC on average, and three to four nurses are present to receive them. The nurses deal with the problems themselves or consult social welfare offices and other relevant organisations, such as non-government organisations working with homeless people, when deemed necessary. At the end of each successfully completed treatment course, a small ceremony is organised by the nurses to congratulate the patient.

METHOD

This qualitative study was conducted via in-depth interviews with ex-homeless TB patients using a guideline. Seventy homeless patients received PHC-based DOTS during the study period between September 2007 and October 2010. Those who met the following criteria were approached: 1) they had completed treatment at Shinjuku PHC, 2) they were willing and available to participate, and 3) they had no serious mental health problems. Eighteen people gave written informed consent. Data collection was discontinued after 18 interviews, when theoretical saturation was achieved.[11]

All interviews were conducted in Japanese, by the same researcher (LK), in a room without the presence of a nurse. None of the participants received financial incentives to participate in the study, although refreshments were served during the interviews. Each interview lasted for approximately 60 min, and participants were asked to talk freely about their experience of and their life after DOTS. All interviews were recorded using an integrated circuit recorder and were transcribed verbatim.

The participants were also asked to complete a simple self-administered questionnaire to collect basic demographic information. The nurses were consulted to triangulate the data regarding changes among the participants before and after DOTS. The study was approved by the ethics committee of the Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association (reference number: 21-5).

Data analysis

The interview data were analysed by performing interpretive content analysis, which is one of the most extensively used analytical tools in qualitative studies in various fields, including public health.[12] The method was chosen because it was deemed most appropriate in understanding the social reality, i.e., the experience, of the participants. Analysis first involved familiarisation with the text through review of all the transcripts. A thematic framework was then designed using an iterative process, and segments describing any changes that participants had experienced in the context of DOTS were retained for further analysis. These segments were then organised by means of open coding and abstraction, and a model of empowerment was developed.

RESULTS

Table 1 shows the participants' demographic information; Table 2 summarises the various changes experienced by the participants. Patient empowerment was defined in terms of these various changes, which were divided into five sub-categories: improvement in mental health, health behaviour, living environment, interpersonal relationships, and attitude towards and relationship with society.

To explore empowerment in the context of PHC-based DOTS, we then developed a model in which we argue that several of the changes were directly attributable to the participants' treatment under PHC-based DOTS, which addressed some of their most fundamental emotional needs (Figure). From the analysis of the interviews, three kinds of emotional need were identified: the need to feel cared for as a person, the need to have efforts recognised and the need to feel (re)attached to society; each of these is discussed below.

Need to feel cared for as a person

The need to feel cared for as a person was identified by many participants. For example, improved self-worth was experienced by almost all participants,

Table 1 Demographic background of the participants

Characteristic	*n*
Age, years	
26–76 (median = 58.8)	
Sex	
Male	18
Female	0
Co-morbidities	
Alcohol dependence	3
Hypertension	2
Diabetes	4
Others	1
None	8
Sleeping place at the time TB was diagnosed	
Street, parks	3
Shelter and other ad hoc housing	9
Other public or private place not designed for use as regular sleeping accommodation	4
Government subsidised apartment	3
Employment status at the time TB was diagnosed	
Full-time	4
Part-time/day labourer	5
Unemployed	9

TB = tuberculosis.

Table 2 Five sub-categories of patient empowerment and corresponding codes

Patient empowerment sub-category	Code	Sample quotes
Mental health	Feel happy Feel hopeful Feel confident Improved self-esteem	'I overcame TB, so now I think I can overcome other difficulties. And also . . . perhaps even try for new things.' (Y09) 'Sure . . . I feel I have a stronger will. Like, I won't be beaten so easily now.' (S03) 'Oh before . . . I thought if I die, I just die. But now, I don't want to die like this. I want to live for a long time . . . calmly, and peacefully.' (T13)
Health behaviour	Eat well/healthier Drink/smoke less Seek medical help Other personal hygiene behaviours	'I try to eat healthier . . . like more vegetables. I also try to eat at regular times.' (Y09) 'I try to reduce salty food.' (N01) 'I drink much less now . . . I mean, the most important thing is to stay healthy.' (O05)
Living environment	Acquire certificate of residence Move to a better place Buy furniture/electrical appliances	'I'll apply for a certificate of residence . . . then I can start looking for jobs, like everyone else.' (K02) 'I bought several kitchen appliances . . . so that I can cook for myself. That way, I can lead a much more decent life.' (M18)
Interpersonal relationships	Better able to express themselves Better able to communicate politely Able to build relationship of trust Better able to understand other people's feelings	'I've learnt to bow my head. And no, I don't hesitate to do that. I feel grateful to other people . . . and I should show that I appreciate them.' (S11) 'Before . . . I didn't care. I just said what I wanted to say, but that attitude wasn't right. I now try to understand people's feelings . . .' (T07) 'I now try to think what words mean before I speak . . . what do people feel if I say this or that? Because I don't want to hurt people by my words. Before? I was like, who cares!' (S14)
Attitudes towards society	Accept social rules and regulations Feel indebted Want to give something back	'Oh, I feel very indebted now . . . I mean, not just to the doctors and nurses, but to society in general. My treatment was paid for by the country's money . . . and so I feel I should give something back.' (T06) 'Sure I feel bad . . . my treatment paid for by society's money and now receiving money from society at my age . . . when I'm still supposed to be working. It's a bad thing. When my health fully recovers, I want to start working and return this money.' (O08)

TB = tuberculosis.

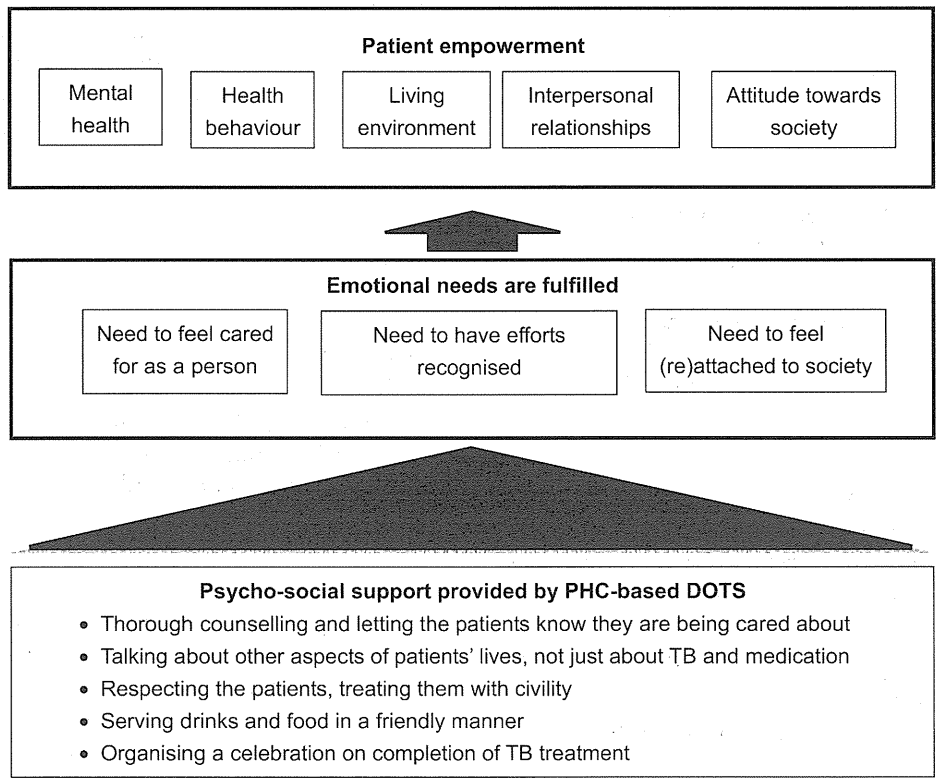

Figure Model of empowerment through PHC-based DOTS. PHC = Public Health Centre; TB = tuberculosis.

and many attributed this change to feeling that the nurses genuinely cared for and took interest in them as individuals, and not just as any other homeless person with TB. Participant T07, who assessed himself as reckless and cared little about himself or others before developing TB, describes how his experience of being cared for as a person by the nurses changed the way he valued himself:

> LK: 'So how do you feel now?'
>
> T07: 'Well . . . it's like I'm a completely different person. I'm happy, and I really like the way I am now . . . I used to live a pretty self-destructive life, you know. Didn't care . . . but now I feel I should take a better care of myself . . . I mean, I've never really had people worry about me, so . . . oh, at first . . . I really wondered . . . why on earth they (nurses) cared for me so much? And why are they so polite, and so kind? I didn't understand . . . (but that experience) changed me, like you know, I shouldn't continue like this. I should take care of myself more . . . for example, I'm more careful about what I eat . . . like more vegetables. I also smoke less . . . and trying to cut down on alcohol as well!'

For some, improved self-worth also had a positive effect on interpersonal relationships. Participant N01, who, at the time of the interview, was undergoing alcohol treatment, explains how the way he was treated at the PHC not only changed him but also affected his relationships with others:

> 'Coming here was such a relief . . . I could let it all out, and they (the nurses) would listen. It was so good. They would never look down on me, but were always so kind, so polite. Coming here really gave me strength to continue the treatment. You know, many people just give up (the treatment). But they made me feel I could do it . . . but not just that . . . I also feel much gentler towards other people . . . I can talk kindly, politely . . . not get angry so quickly, like I used to.' (N01)

Several others also commented that talking to the nurses, and feeling that they were being cared for, helped them become milder, more forgiving and less assertive in their attitude towards other people.

Need to have efforts recognised

The need to have efforts recognised was identified from conversations with several participants who said that they had become more confident. Participant T07 speaks once again about how the way the nurses recognised his efforts improved his self-confidence:

> LK: 'You said that you have become more confident . . . is that because you overcame TB?'
>
> T07: 'Overcoming TB was one . . . but I'm not sure if I'd have finished the treatment by myself. I am really grateful for the nurses . . . they would never use degrading words . . . never demoralise us. You know, sometimes I wanted to give up, and actually once I did try to run away! But they soon found me [laughs]. The nurse got really angry and scolded me, but you know, I didn't feel bad because of the way she got angry . . . I understood that she was worried. And I kind of felt . . . that she knew I was doing my best. That in turn gave me strength, like I have to live up to her expectations . . . she was not always saying *'ganbatte, ganbatte'* ('Try harder! Try harder!'). Because I'm already trying hard! I think she (the nurse) got really angry (when I tried to quit treatment) because she knew that I was trying.'

Having treatment completion recognised and praised also seems to have had a similar effect. At the completion ceremony, many participants felt proud and confident, as indicated below:

> 'At the end of the long treatment . . . and when I attended the ceremony, I feel, oh I did it! I was able to finish it! I didn't give up and that really gave me a lot of confidence. I feel I can now try other things . . .' (N01)
>
> '. . . and all the nurses came out and praised me (at the ceremony) . . . sure I felt good. Like a job well done. If I could overcome this (TB), I can overcome other difficulties.' (O05)

Need to feel (re)attached to society

Lastly, the need to feel (re)attached to society was identified from conversations with almost all the participants, who said that their attitude towards and relationship with society had improved. For example, some participants commented that before becoming sick they had felt they could do without being involved with society. After completing DOTS, however, their thinking had changed, and they expressed their gratitude not only to the individual nurses but also to society in general.

> 'I thought . . . I could do without society but I guess I was wrong. I'm really glad that I overcame TB and I'm really thankful to society for that . . . I realise that I'm part of all this (society) after all.' (S16)
>
> 'It was like DOTS linked me to the society again . . . which I thought I'd left years ago. But I didn't . . . and I am glad that I didn't. Because I'm so glad that I'm alive today, and I admit that is because I was saved by society.' (N01)

A sense of social (re-)connectedness and gratitude made many participants feel that they wanted to give something back to society. Some participants wanted to become re-employed, not only to support themselves but also so that they could give something back to society. Others expressed their wish to do voluntary work, including TB-related activities.

For others, reconnecting with society through PHC-based DOTS pushed them to try to get back, or

closer, to the life they used to live before becoming homeless. This led some to try to manage everyday expenses and save money so that they could buy basic furniture and cooking appliances, and others to try to get jobs so that they could move into subsidised accommodation. Participant S16, who worked in the construction industry before becoming homeless, said that everything had become meaningless when he lost his job. However, he believes that his experience of TB treatment with PHC-based DOTS gave him a second chance:

> S16: 'I really think . . . DOTS gave me a second chance . . . To live my life again . . . properly.'
>
> LK: 'Is that why you continue with your part-time work (cleaning), which at first you said you didn't really . . . take it well?'
>
> S16: 'Haha . . . yes, I mean, at first, I thought 'me? cleaning?' . . . but then I realised, look, this could be the last chance to come off the street, and so I took it. And once I started working, I realised that this life is so much better than sleeping rough and just waiting for days to pass, not caring about anything. So I will continue this job until my body no longer moves . . . also, now that I have what I can call a home, I want to make it as comfortable as I can. I've already bought a second-hand sofa, you know. If I continue like this, then I can also start buying things to decorate the room.'

DISCUSSION

To our knowledge, this is the first study in a high-income country to focus on the positive impact of DOTS on homeless patients beyond their treatment outcome.

The concept of empowerment is certainly not new in TB control; however, it has usually been conceptualised as a means to achieve treatment adherence, and not necessarily as an 'effect' of DOTS. Likewise, many interventions have been conducted to that end, for example by providing culturally sensitive information,[13,14] motivating the patient to take an active part in his or her treatment,[15] and organising TB clubs.[16,17] On the other hand, studies that have focused on the wellbeing of TB patients have tended to use 'empowerment' in a utilitarian sense, in that they have evaluated the post-treatment life of the patients in terms of whether or not former patients have become involved in TB control activities.[18–20]

Our study has given empowerment a broader meaning, and explored the possible contribution of PHC-based DOTS in effecting such empowerment. In doing so, we are certainly not ignoring the possible effects of illness in and of itself. Many studies in the field of sociology and psychology have examined the association between patient empowerment and experience of illness.[21–25] Several of our participants also commented that the very experience of overcoming TB made them more confident and made them aspire to a better future.

In discussing the role of PHC-based DOTS, we have also taken into consideration the effect of socio-economic support. Studies on TB control, particularly among the homeless, in other industrialised countries, including the United States, the United Kingdom and Canada, have consistently emphasised the importance of social protection interventions in DOTS.[26–28] In Shinjuku City, too, PHC-based DOTS was implemented with the recognition that the provision not only of medication but also of accommodation was essential to prevent default, and such support in turn may have exerted a positive influence on the participants.

Our study results have nevertheless highlighted another critical aspect of DOTS in empowering the patients, i.e., the psycho-emotional support that served to meet the various emotional needs of the participants, as discussed in the results. By exploring the socio-economic context of homelessness among our participants, we seek to explain how meeting the three particular needs encouraged the participants to achieve the various changes.

According to the report on the state of homeless people in Tokyo, as of 2007, the average age was 59 years, with 47% aged over 60.[29] Setting aside various personal reasons, it has been recognised that one of the principal macro socio-economic forces behind people in their fifties and sixties becoming homeless was the shrinking labour demand in the Japanese construction industry in the mid-1990s, which once acted as the last safety net against homelessness.[30,31] Many had thus once worked and lived ordinary lives. The report indicates that before becoming homeless, 70% of these people had lived in stable accommodation and respectively 43% and 29% had worked full-time. Furthermore, 70% continued to earn some income by working after they became homeless, and 83% wished to get off the street by finding full-time or part-time employment again. These characteristics could be applied equally to our participants: they had an average age of 59 years, and many had worked in the construction industry at some point in their lives. All of them expressed a wish to either find full-time employment or receive welfare but still work part-time to support themselves. Unlike the new, younger generations of homeless people in Japan, whose origin and sense of values are quite different from those of older generations,[32] many had once had a place in society, with a clear sense of identity and belonging—these had simply been temporarily lost in the process of becoming homeless. We thus argue that by addressing the need to feel cared for as a person, the need to have their efforts recognised and the need to feel re-attached to society, PHC-based DOTS has helped participants to regain their sense of identity

and belonging, which has in turn encouraged them to try to return to the life they used to have.

Do our findings contradict the large body of literature that criticises DOTS for its disempowering effects? We do not believe so. When we examine past studies from similar resource-rich countries that have questioned the value of DOTS as opposed to self-supervised treatment, the core of their argument seems to be that DOTS is ineffective because it is implemented in an authoritarian manner.[33,34] The problem thus lies in how DOTS is implemented, and not in the actual nature of the treatment. For example, a study that evaluated the relative effectiveness of DOTS by reviewing past practices concluded that the treatment completion rate was likely to exceed 90% when DOTS was delivered as a patient-centred approach, with various enablers.[35] Several studies have also shown the importance of health care worker-patient relationships in delivering effective DOTS, especially among the socio-economically vulnerable.[36–38] A report on the experience of controlling TB among the poor in New Jersey and New York stresses the back-to-basics human touch as a crucial factor in their success, with health care workers delivering not only anti-tuberculosis medications but also a sense of care and empathy.[36] 'Authoritativeness' is therefore not necessarily the inherent nature of DOTS. Rather, as Zachariah et al. argue, TB services 'are not just about the science of treatment, they are also about something much more fundamental: dignity, social fairness, social injustice and a willingness to serve'.[39]

With regard to the generalisability of our study, we by no means wish to claim that the experience of Shinjuku's PHC-based DOTS can simply be reproduced in other settings. Compared to many medium- and high-burden countries, Japan is rich in financial and human resources. Furthermore, the TB situation among the homeless in Japan is quite different, even when compared to similarly industrialised countries such as the United States or the United Kingdom, in that it is not necessarily compounded by such issues as the human immunodeficiency virus, drug use and illiteracy. We nonetheless believe that the theoretical implication of our study can still be useful, and that addressing the emotional needs of the patients is just as important as meeting their socio-economic needs in delivering patient-centred DOTS. Certain types of enablers may only be feasible in high-income countries. However, it does not cost much to make a patient feel welcome, worthy and cared for as a person.

The more specific emotional needs of the patients are likely to be culturally constructed. Health care providers should therefore be trained to strengthen not only their technical skills but also their interpersonal skills, so that they become more sensitive to the various emotional needs of the patients, and respond appropriately. Our study has demonstrated that when DOTS is implemented in such a way, not only does it become an effective means of controlling TB, it is also an effective tool of empowerment and, in the case in Shinjuku, it provided a springboard for homeless patients to rethink and reconstruct their lives.

Finally, although the aim of this study was to explore the empowerment of those patients who completed DOTS, a small note could be added about those who were lost to follow-up. During the study period, the default rate was 8.6%. Of the six patients who defaulted, three returned and eventually completed their treatment. The reasons for the remaining three patients failing to complete their treatment were not necessarily related to alcohol or any other apparent risk factors, according to the nurses. The patients simply 'disappeared', and the nurses said that they had not picked up any warning signs before their disappearance. The question of how to reach those patients who have no apparent problems but who still disappear and fail to complete treatment is an issue to be considered in future studies. On the other hand, of the 70 patients who had received DOTS during the study period, 17 had alcohol or other mental health problems, and yet 13 of these completed their treatment. Although limited, such data suggest that PHC-based DOTS could have a similar empowering effect even for those patients with underlying conditions, precisely because, under PHC-based DOTS, their specific socio-economic and emotional needs would be identified and addressed.

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RÉSUMÉ

CONTEXTE : Depuis 2000, le centre de santé publique (PHC) de Shinjuku, une ville comportant une des plus importantes populations sans abri à Tokyo, Japon, a mis en œuvre une stratégie DOTS basé sur le PHC pour les patients tuberculeux (TB) sans domicile avec un succès épidémiologique important. Des évidences anecdotiques indiquent par ailleurs que les patients sans domicile qui étaient sous DOTS ont ressenti différentes modifications positives. Toutefois, de telles expériences n'avaient pas encore été analysées de manière systématique.

OBJECTIF : Explorer les modifications ressenties par les patients TB sans domicile et discuter du rôle possible du DOTS basé sur le PHC dans ces expériences.

SCHÉMA : Une recherche qualitative au travers d'interviews approfondies avec 18 anciens patients sans abri qui ont achevé le DOTS au PHC de Shinjuku-city. Les données ont été analysées par la méthode d'analyse interprétative du contenu.

RÉSULTATS : Diverses modifications ressenties par les participants ont été classées en cinq sous-catégories de responsabilisation, notamment l'amélioration de la santé mentale et des relations interpersonnelles. Certaines de ces modifications ont été attribuables aux participants sous DOTS basé sur le PHC ; ce dernier, en veillant à répondre à leurs divers besoins émotionnels, a contribué à stimuler la responsabilisation du patient. En se basant sur notre observation, on a élaboré un modèle de responsabilisation via un DOTS basé sur le PHC.

CONCLUSION : Le DOTS basé sur le PHC n'a pas seulement réussi à traiter la TB avec succès mais a responsabilisé les patients sans domicile en répondant à leurs besoins émotionnels. Dans ce processus, le savoir-faire humain des infirmières a joué un rôle critique.

RESUMEN

MARCO DE REFERENCIA: Desde el año 2000, el centro de salud pública (PHC) de Shinjuku, una ciudad que cuenta con una de las poblaciones más grandes de personas sin domicilio en Tokio, ha aplicado la estrategia de DOTS dirigida a estas personas sin domicilio con diagnóstico de tuberculosis (TB) y ha logrado resultados epidemiológicos muy satisfactorios. Algunos hechos observados indican que estos pacientes que recibieron el DOTS alcanzaron además otros cambios positivos. Sin embargo, esta experiencia no se ha analizado aun de manera sistemática.

OBJETIVO: Explorar los cambios experimentados por los pacientes sin domicilio fijo que recibieron el tratamiento contra la TB y analizar el posible papel de DOTS de base hospitalaria en la modificación de su experiencia.

MÉTODOS: Se llevó a cabo una investigación cualitativa mediante entrevistas detalladas a 18 ancianos pacientes sin domicilio fijo, que completaron el protocolo DOTS en el PHC de la ciudad Shinjuku. Los datos se analizaron por el método de análisis de contenido interpretativo.

RESULTADOS: Los diversos cambios operados en los participantes se categorizaron en cinco subcategorías de empoderamiento, entre ellas una mejor salud mental y mejores relaciones interpersonales. Algunas de estas modificaciones se atribuyeron al seguimiento del DOTS al PHC, el cual respondió a las diversas necesidades emocionales y favoreció el empoderamiento de los pacientes. Con base en estos resultados se construyó un modelo de empoderamiento incorporado al DOTS de base PHC.

CONCLUSIÓN: La estrategia DOTS con sede PHC fue muy eficaz, no solo en el tratamiento de la TB, sino también en el empoderamiento de los pacientes sin domicilio fijo, al atender sus necesidades emocionales. En este proceso cumplieron una función primordial las competencias humanas del personal de enfermería.

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DIAGNOSTICS

Sub-speciation of *Mycobacterium tuberculosis* complex from tuberculosis patients in Japan

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SUMMARY

Mycobacterium tuberculosis is the major causative agent of tuberculosis in humans. It is well known that *Mycobacterium bovis* and other species in the *M. tuberculosis* complex (MTC) can cause respiratory diseases as zoonosis. We analyzed the MTC isolates collected from tuberculosis patients from Japan in 2002 using a multiplex PCR system that detected *cfp32*, RD9 and RD12. A total of 970 MTC isolates that were representative of the tuberculosis cases throughout Japan, were examined using this method. As a result, 966 (99.6%) *M. tuberculosis*, two *Mycobacterium africanum* and two *Mycobacterium canettii* were identified using a multiplex PCR system, while no *M. bovis* was detected. Two isolates that lacked RD9 were initially considered to be *M. canettii*, but further analysis of the *hsp65* sequence revealed them to be *M. tuberculosis*. Also two *M. africanum* were identified as *M. tuberculosis* using the −215 *narG* nucleotide polymorphism. Though PCR-linked methods have been used for a rapid differentiation of MTC and NTM, from our cases we suggest careful interpretation of RD based identification.

1. Introduction

Tuberculosis (TB) is a disease caused by the *Mycobacterium tuberculosis* complex (MTC), which includes *M. tuberculosis* sensu stricto, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium caprae*, *Mycobacterium canettii*, *Mycobacterium microti*, and *Mycobacterium pinnipedii*, as of 2012. *M. bovis* is a common zoonotic pathogen that can infect a wide range of hosts. *M. bovis* primarily infects cattle, but can be transmitted between humans, especially among HIV-infected patients.

On the other hand, Non-tuberculous mycobacteria (NTM) also known as mycobacteria other than tuberculosis (MOTT) are acid fast bacilli that do not belong to *M. tuberculosis* complex or *Mycobacterium leprae*. These mycobacteria are environmental organisms and are found in natural bodies of waters, biofilms, soil, water damaged walls, etc. NTM may also be found in drinking water supplies.

Recently it has been estimated that approximately 5–10% of the global TB burden may be due to *M. bovis* [1]. There are several reports regarding the infections caused by MTC other than *M. tuberculosis* sensu stricto, but only limited data is available on the total incidence or prevalence of mycobacterial disease caused by specific MTC members [2,3]. Japan is still a medium prevalence country for TB, with an incidence of 18.2/100,000 in 2010 [4]. Bacteriologically-identified TB cases in Japan are diagnosed based on the detection of MTC, in general, using commercial identification kit like Capilia TB (TOUNS, Japan). Although all mycobacterial isolates from patients in Japan are submitted for species identification, very few isolates will be subjected to further analysis to identify the MTC species. It is possible to differentiate MTC members by conventional laboratory culture and chemical testing methods; however, the procedures are time-consuming and not practical in clinical laboratories. The lack of surveillance for the specific

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causative agents of TB is mainly due to the absence of simple tests in diagnostic laboratories. Differentiation of MTC members is important for the accurate diagnosis of mycobacterial disease, public health surveillance and appropriate case management. Recently, polymerase chain reaction (PCR) based methods have been developed to differentiate TB from bacille Calmette-Guérin (BCG) disease in HIV-infected infants, where accurate diagnosis has implications for both clinical management and public health policy [5]. In this study, we utilized a simple multiplex-PCR diagnostic method to differentiate MTC members known to cause disease in humans. The method amplified one conserved MTC locus [6] (*cfp32*), and two chromosomal region-of-difference (RD) loci [7] (RD9, RD12). When necessary, additional loci [6] (MiD-3, RD4, RD7, *hsp65*, −215 *narG*) were characterized to allow rapid and conclusive differentiation of the relevant MTC species. Detailed information about the pathogenic MTC in Japan will provide a clear perspective for the management of patients diagnosed with TB and zoonotic situations.

2. Methods

2.1. Clinical isolates of the M. tuberculosis complex

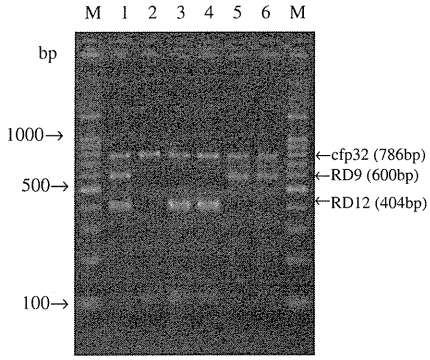
A total of 970 MTC isolates were randomly but systematically selected from the collection of the Ryoken anti-tuberculosis drug resistance survey in 2002 [8]. The Tuberculosis Research Committee (Ryoken) is a nationwide coalition of TB hospitals in Japan. The Committee has been conducting nationwide drug resistance surveys approximately every 5 years since 1957 with the participation of almost all the TB hospitals across the country and the surveys have resulted in high reliability in our country. In the survey in 2002, the MTC isolates numbered 3122 and a total of 99 major TB hospitals all over Japan participated and the entire country was divided into six geographical regions. All patients who had chemotherapy for culture-confirmed TB in the participating hospitals were eligible for the study and corresponded to approximately 10% of all MTC isolates in that year. Also in the drug resistance survey, children were indeed included and the patients consisted of 2211 males and 911 females, with a mean age of 61.1 ± 18.5 years (range 0–100) and 61.0 ± 22.4 years (0–100), respectively. In that survey a total of 2011 MTC isolates were recovered by using BACTEC MGIT 960 (Nippon Becton Dickinson, Japan) or BacT/ALERT 3D (Sysmex-Biomerieux, Tokyo, Japan), and the others were isolated using solid culture media. MTC isolates were identified by the lateral flow immunoprecipitation method (Capilia TB: TOUNS, Numazu, Japan), DNA–DNA hybridization method (DDH mycobacteria: Kyokuto Pharmaceuticals, Tokyo, Japan), and/or direct 16S rRNA sequencing [9]. The obtained 16S rRNA sequence data was subjected to Ribosomal Differentiation of Microorganisms (RIDOM) on the web site (http://www.ridom-rdna.de) for similarity searches. In our study a total of 970 MTC isolates were analyzed and the number of isolates was 1/3 of the drug resistance survey performed in 2002. This may have resulted in a bias in the selection of number, age and hospitals. However, including all the major TB institutions of the country, the adjustment for the regions made no substantial change in the estimates of the prevalence figures, which suggests that the results obtained in our study accurately represent the actual prevalence of MTC isolates differentiation in Japan. Also in age, in Japan in 2002, among children aged 0–4 years childhood tuberculosis notification rates was 1.4/100,000, aged 5–9 years was 0.6/100,000, aged 10–14 years was 0.7, on the other hand among total the rate was 25.8/100,000 [4]. These data indicates that Japan is a low-burden country in younger children tuberculosis, and then we can recognize that isolate selection in our study lesser influenced the our observed results in a younger age and our study result would not contradict the fact that no *M. bovis* was isolated in our study (Pages 5, lines 149–155). We couldn't analyze too much more isolates and if we had analyzed much more isolates, the result might have been slightly different from our study. But there have never been larger reports about MTC isolates differentiation in our country. After all at least our study result actually indicated the differentiation of 970 MTC isolates in 2002 in Japan and in this point we think that our study is of value to report the proper situation of MTC isolation in Japan.

2.2. Species identification of M. tuberculosis complex

DNA specimens were prepared using the ISOPLANT extraction kit (Nippon Gene, Japan). A multiplex PCR was performed to detect *cfp32*, RD9 and RD12, using a Type-it Microsatellite PCR Kit (QIAGEN, Japan) [6,10–13]. The expected sizes of the PCR products generated using each primer pair were 786, 600, and 404 bp, respectively. DNA templates from *M. tuberculosis* (H37Rv, ATCC 27294) were used as positive controls. The amplified products were analyzed on 2% agarose gels (Figure 1) and automated gel electrophoresis (QIAxcel system, QIAGEN, Japan). When MTC species other than *M. tuberculosis* sensu stricto were identified, further analyses were performed detecting RD4, RD7 and MiD3 [7,14–16]. The expected RD loci for each MTC species are summarized in Table 1.

2.3. Analysis of hsp65 for unidentified species

Any isolates showing discrepant or abnormal RD identification patterns that did not correspond to the typical morphotype were further analyzed by sequencing *hsp65* [17]. If the nucleotide at the position corresponding to 631 of the homologous *hsp65* of *M. tuberculosis* H37Rv was cytosine, the isolate was considered to be *M. tuberculosis* sensu stricto.



1. *M. tuberculosis* H37Rv ATCC 27294
2. *M. bovis* ATCC 19210
3. *M. africanum* ATCC 25420
4. *M. microti* ATCC 19422
5. strain A (this study)

Figure 1. Electrophoretic separation of multiplex PCR products.

Table 1
Summary of MTC PCR typing panel.

	16S rRNA	Rv0577 (*cfp32*)	IS*1561*′ (MiD3)	Rv1510 (RD4)	Rv1970 (RD7)	Rv2073c (RD9)	Rv3120 (RD12)
M. tuberculosis	+	+	+	+	+	+	+
M. bovis	+	+	+	−	−	−	−
M. africanum subtype I	+	+	+	+	−	−	+
M. africanum subtype II	+	+	+	+	+	−	+
M. caprae	+	+	+	+	−	−	−
M. canettii	+	+	+	+	+	+	−
M. microti	+	+	−	+	−	−	+

2.4. −215 narG polymorphisms for two M. africanum specimens identified using multiplex-PCR

Two isolates showing *M. africanum* pattern using multiplex-PCR were further analyzed by sequencing −215 *narG* polymorphisms [18]. The primers used for the amplification of a 276-bp fragment were as follows: −215 *narG* F; 5′-AGA TGT CCA CCG TCG CTG TTA G-3′ and −215 *narG* R; 5′-CGC ACT CGC TGG ACG TTA C-3′. Then the amplified product was sequenced. At nucleotide −215 prior to the start codon of *narG*, the thymidine residue is specific for *M. tuberculosis*, whereas *M. africanum*, *M. bovis*, *M. bovis BCG*, *M. microti* carry a cytosine residue at this position.

2.5. Ethical considerations

Additional ethics committee approvals were not required for this type of laboratory-based study. The original study (Tuberculosis Research Committee (RYOKEN), 2007) followed national guidelines for epidemiological research. In that study, the clinical information was taken from each patient directly after obtaining informed consent.

3. Results

A total of 970 MTC isolates (89 from the Hokkaido-Tohoku area, 292 from the Kanto area, 121 from the Chubu-Hokuriku area, 404 from the Kinki area, and 64 from the Shikoku-Kyusyu area) were randomly selected as representative of TB prevalence in each region. Of 970 MTC, 966 (99.6%) isolates yielded all three expected PCR products, *cfp32*, RD9 and RD12, and as such were considered as *M. tuberculosis* sensu stricto. No RD pattern corresponding to *M. bovis* was identified in this population. Two isolates (0.2%), which were *cfp32*- and RD9-positive but RD12-negative, were categorized as *M. canettii* (Figure 1). However, these isolates showed stable rough colony surface morphology on solid media. Therefore, *hsp65* was sequenced and the strains were found to have the genotype of *M. tuberculosis* sensu stricto (Table 2). Consequently, the isolates were identified as *M. tuberculosis*. The other two isolates were *cfp32*- and RD12-positive, but RD9-negative. Both isolates were MiD3- and RD4 positive. However, one isolate was RD7-positive but the other was negative. Thus, the RD7-positive isolate (0.1%) was considered to be *M. africanum* subtype II, and the other (0.1%) was subtype I (Table 1). These two *M. africanum* strains were isolated from patients of Japanese nationality and sputum samples. The isolate identified as *M. africanum* subtype I was from the Shikoku-Kyusyu area (southern island of Japan). The other isolate identified as *M. africanum* subtype II was from the Kanto area where Tokyo is located. Interestingly, no previous cases were reported of *M. africanum* identified specimens infected with human in Japan. To confirm the result of multiplex-PCR identifying two *M. africanum* specimens, further we analyzed −215 *narG* polymorphism by sequencing. Then the single nucleotide polymorphisms (SNPs) specific to *M. tuberculosis* at the −215 *narG* position were detected and the two specimens were presumably considered as *M. tuberculosis*. In conclusion, all 970 MTC isolates were confirmed as *M. tuberculosis* sensu stricto.

Table 2
Differential distribution of known single-nucleotide changes among MTC species.

MTC species	Partial *hsp65* nucleotide sequence
M. tuberculosis H37Rv	ACCCTGCTCAAGGG**C**GCCAAGGAGGTCGAGACCAAGGAGC
M. canettii	ACCCTGCTCAAGGG**T**GCCAAGGAGGTCGAGACCAAGGAGC
Strain A (this study)	ACCCTGCTCAAGGG**C**GCCAAGGAGGTCGAGACCAAGGAGC
Strain B (this study)	ACCCTGCTCAAGGG**C**GCCAAGGAGGTCGAGACCAAGGAGC

The marked base (in bold font) in each sequence shows the single nucleotide polymorphism in *hsp65* of *M. canettii* at position 235 (C-to-T transition) in the 441-bp PCR product. This nucleotide corresponds to position 631 of the homologous *hsp65* of *M. tuberculosis* H37Rv. The nucleotide sequences of strain A and B were the same as that of H37Rv.

4. Discussion

In 2011, 8.7 million people fell ill with tuberculosis, including 1.1 million cases among people with HIV. TB is second only to HIV/AIDS as the biggest killer worldwide due to a single infectious agent. In 2011, an estimated 1.4 million people died from TB, including 430,000 among people who were HIV-positive [19]. In that situation, significant advances have been made for these years in the diagnosis of tuberculosis using novel nucleic acid molecular targets, but the differentiation of members of the MTC to the species level is not routinely performed. This is the first nationwide survey of MTC in Japan. In the present study, though the results of multiple-PCR detected two *M. africanum* strains and two *M. canettii* strains at first, all of the isolates from active TB patients in Japan turned out to be *M. tuberculosis* sensu stricto using sequences, finally. Despite the expectation prior to this study, no *M. bovis* was isolated. *M. bovis* is a zoonotic agent and is generally a foodborne infection. Zoonoses have been recognized for many centuries, and over 200 have been described. They are caused by all types of pathogenic agents, including bacteria, parasites, fungi, and viruses. Especially tuberculosis is important, because mycobacteriosis, including tuberculosis, is common among non-human primates and ruminants. *M. bovis* and *M. bovis* BCG are intrinsically resistant to PZA and drug-resistant strains of *M. tuberculosis* due to varying natural resistance are increasing. Differentiation within the MTBC is useful for epidemiological purposes, especially with respect to understanding transmission dynamics of zoonoses. Reports from European and American countries where dairy farming is common show a number of *M. bovis* infections in humans [3]. There are many dairy farms in Japan, but people commonly boil or pasteurized milk for drinking. Additionally, there was no tradition of drinking milk in Japan until about 120 years ago. This could be one reason why almost no *M. bovis* infections have been reported to date. Furthermore, only four cows were reported to be infected with *M. bovis* in Japan from 2006 to 2010. These data also support the relevance of this study (National Institute of Animal Health, 2012: [Animal

Infectious Disease Database. http://kdh.dc.affrc.go.jp/kdh/find.php]). The incidence of bovine TB is increasing in cattle herds of developed countries that have a wildlife reservoir of *M. bovis*, such as the UK, New Zealand and the USA. The increase in the incidence of bovine TB is thought to be due, at least in part, to a wildlife reservoir of *M. bovis* [20]. In developed countries it has been suggested that the burden of bovine TB in humans ranges from 0.5 to 7.2% of TB cases, while in developing countries, where very few little data is available, this figure is as high as 15% [21,22]. Monitoring bovine TB in humans is valuable for guiding public health policy and is important for the study of zoonotic TB epidemiology. Our literature searches only identified one vaccination-independent *M. bovis* infection causing pulmonary tuberculosis in Japan [23], although several cases of *M. bovis* BCG infection have been reported in Japan as a consequence of BCG vaccination [24–26]. Disseminated BCG infection after vaccination most commonly occurs in the setting of human immunodeficiency virus infection or other causes of immunodeficiency [27]. BCG is the most widely used vaccine, an attenuated derivative of a virulent strain of *M. bovis* and protects a wide range of mammals against experimental infection with TB [28]. Information on diversity of BCG policies between countries and across time may be helpful for better interpretation of TB diagnostics [29]. In our research, two isolates were suspected to be *M. canettii*, but the colony morphology was different from the type strain; further analysis revealed these strains to be *M. tuberculosis*. These two isolates of *M. tuberculosis* lacked RD12, which demonstrates the limitation of the current multiplex PCR for identification of strains with large sequence polymorphisms. Further analyses will be required to clarify the proportion of unexpected exceptions with this type of identification system. Based on our literature searches, there have been no reports of the isolation of *M. africanum* in Japan and this study indicated that there was virtually no case of *M. africanum* in Japan. *M. africanum* is endemic to West Africa [30–32] where it causes up to half of all cases of sputum smear-positive pulmonary tuberculosis (PTB) [32]. Infection with *M. africanum* responds to regular TB treatment in most cases. Previously, biochemical methods distinguished between West African *M. africanum* and East African *M. africanum*. Phylogenetic trees now group the former East African *M. africanum* with *M. tuberculosis* sensu stricto and distinguish two variants of the West African *M. africanum*: *M. africanum* type 2, which lacks regions of difference (RD) 7 through 10, and type 1, which lacks only RD9 compared with *M. tuberculosis* sensu stricto [33]. *M. africanum* disease is rare in industrialized countries and is commonly diagnosed in immigrants from Africa. Cases have been described in countries outside Africa, such as the United States, Brazil, China, Australia, Turkey, Denmark, Italy, Germany, the United Kingdom, France, Portugal and Spain [34–40], and also in animals in Norway, Croatia and Bangladesh [41]. To identify species within the *M. tuberculosis* complex (MTC), several biochemical assays have been used. However subjective results and intraspecies variations are confounding. Recent comparative genomic analyses have provided valuable information on the Region-of-difference (RD) in the chromosome of MTC to indicate that specific identification of MTC can be achieved by the detection of these regions. In our study, firstly genetic regions (*cfp32*, RD9, RD12, RD4, RD7, MiD3) were chosen as targets for a species distinguishable multiplex PCR and then four samples were further examined by the sequence analyses of *hsp65* and −215 *narG* according to previous publications. These approach that is, multiplex PCR and sequence analyses have often been used in other labs in order to clarify the discrepant and abnormal RD identification or to confirm the sequences [42,43]. As the RD differentiation has been developed recently, also there have been some reports concerning that sequence variations at primer binding sites may often occur [44]. In our study, there was a possibility that mutations in the priming sites led to false negative detection of RD which related to the identification of *M. africanum* and *M. canettii*. It is possible that false negative or false positive results of RD region exist also in other cases. Really PCR-based methods targeting RDs can be easily performed in local clinical laboratories with low expense and have come into wider use but we should always keep in mind that there was a possibility that mutations in the priming sites led to false negative or false positive results of RD region. We think that is not always the limitation of our approach but we need to reconsider more about the misinterpretation cases. DNA sequencing of all culture isolates in a clinical laboratory is not practical, but our cases suggest that we sometimes need to care the interpretation of the PCR based RD assay. Zoonotic TB remains a serious threat to human health in developing countries where its prevalence is accurately unknown, and even in the developed countries there is uncertain *M. bovis* infection. Although PCR-based methods have been developed, there is no still practical commercial differentiation methods of the MTC. The ability to differentiate between members of the MTC could potentially allow for the monitoring of zoonotic exposure leading to human TB infection and recognizing accurate epidemiological information for the clinician, guiding contact tracing and source case identification. In our 970 MTC isolates differentiating study, no *M. bovis* was detected. When we think about the background of consumption of dairy products or breeding animals in Japan, we can agree with our study result. In other words, at least *M. bovis* infection to human in our country is next to zero. It is important we know that *M. bovis* and *M. bovis* BCG are intrinsically resistant to PZA and paying attention to the choice of chemotherapy regimen, but considering TB infection as zoonosis, it would be more important to recognize the meaning of differentiating MTC from the perspective of the epidemiological situation. Therefore we think that our study is very valuable in that our isolates are from wider area of Japan and this is the first report referring to many isolates (970 MTC) differentiation.

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Ethical approval: Additional ethics committee approvals were not required for this type of laboratory-based study. Clinical information about each patient was recorded directly from the patient after obtaining informed consent in the Ryoken survey. The survey followed national guidelines for epidemiological research. The study protocol, including ethical issues, was approved by the Ryoken General Assembly.

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特集 1

予防指針に基づく対策の進展―先駆的な取り組み

接触者健診の手引き(改訂第5版)の主な変更点

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本手引きの今回の改訂（第５版）は，平成23年５月に改正された「結核に関する特定感染症予防指針（厚生労働省告示）」の中に，接触者健診に当たっては「インターフェロンγ遊離試験（IGRA）及び分子疫学的調査手法を積極的に活用することが重要である」と明記されたこと，およびIGRAの検査方法が増えたことなどを踏まえて修正を加えたものである。改訂作業は，平成25年度厚生労働省新型インフルエンザ等新興・再興感染症研究事業「地域における効果的な結核対策の強化に関する研究（研究代表者：石川信克）」の分担研究の一環として実施した。主な変更点は以下のとおりである。

１．IGRAの適用について

手引き「第４版」の公表時点では，第３世代のQFT検査（クォンティフェロン® TBゴールド：QFT-3G。以下，QFT）が国内で利用できる唯一のIGRAであった。その後，QFTとは測定原理の異なる手法として「Tスポット®.TB」（以下，T-SPOT）が平成24年11月に健康保険適用となり，接触者健診においてもすでにQFT-3GまたはT-SPOTのいずれかの手法で実施されていることを踏まえて手引きの内容を修正した。

（１）QFTとT-SPOTの比較

QFTとT-SPOTの性能比較については，わが国の接触者健診適用例での比較検討に関する知見がまだ少ない。特にT-SPOTについては接触者健診に適用されてからの期間が比較的短いため，検査後の追跡調査（例：陰性者からの発病率の分析など）を含めた評価は今後の課題である。現時点では，潜在性結核感染症（LTBI）のスクリーニングを目的とした接触者健診における両者の検査性能はほぼ同等と考えられるので，各地域の検査体制（地方衛生研究所で実施，民間検査機関に委託など），経費負担，および利便性などを考慮して各保健所等がいずれかを選択して実施するよう記述した。なお，免疫抑制状態にある者に適用し

た場合などを含めた詳しい性能比較については，本手引きでの説明を省略し，日本結核病学会予防委員会作成の指針（IGRA使用指針）[注]などを参照していただくこととした。

注：現在作成中であり，本稿作成時には公表されなかったが，日本結核病学会のホームページ内の委員会報告に掲載される予定である。

（2）IGRAの的中度と有病率

スクリーニングの検査性能に関する代表的な評価指標は，「感度」と「特異度」であるが，実際の健診においては，「陽性的中度」と「陰性的中度」も問題となる。IGRAを例にすると，陽性的中度は「IGRA陽性と判定された人のうち，実際に結核感染のあった人の割合」であり，陰性的中度は「IGRA陰性と判定された人のうち，実際に結核未感染である人の割合」と定義される。この二つの的中度には，感度・特異度のほか，検査対象となった集団内における対象疾病の有病率（IGRAの場合は，対象集団人口に占める結核感染者の割合）が大きな影響を及ぼす。第５版では，有病率（結核感染者の割合）の異なる集団に対してIGRA（感度90％，特異度99％と想定）を適用した場合の的中度の計算結果（表）を例示して解説を加えた。例えば「結核感染者の割合が１％」の集団にIGRAを適用した場合，陽性的中度は47.6％にとどまり，「偽の陽性」（結核未感染なのに，IGRA陽性）が多くなる。一方，「結核感染者の割合が50％」の集団にIGRAを適用した場合は，陰性的中度が90.8％であり，「偽の陰性（いわゆる見落とし）」が約１割を占めることになる。このため，結核感染者の割合が高い接触者集団に IGRAを適用した

表　有病率（結核感染者の割合）の異なる集団に対してIGRAを適用した場合の「的中度」

（※IGRAの感度= 90%，特異度= 99% とみなして計算）

①有病率= １％の場合

		感染「あり」	感染「なし」	計
検査結果	(+)	9	**10**	19
	(−)	1	980	981
計		10	990	1,000

陽性的中度=９/19=47.6%（偽陽性率=52.4%）
陰性的中度=980/981=99.9%

②有病率= 50%の場合

		感染「あり」	感染「なし」	計
検査結果	(+)	450	5	455
	(−)	**50**	495	545
計		500	500	1,000

陽性的中度=450/455=98.9%
陰性的中度=495/545=90.8%（偽陰性率=9.2%）

場合は，IGRA陰性者の中に真の結核感染者が含まれていることを念頭に置いた事後管理（例：健診を終了せず，胸部X線検査による経過観察を半年間隔で少なくとも2年間は確実に行うなど）が必要であること解説した。

（3）乳幼児に対するIGRAの適用拡大

今回の改訂にあたっては，乳幼児へのIGRAの適用拡大が大きな議論となった。第4版までは，乳幼児に対する第2世代のQFT-2Gとツベルクリン反応検査（ツ反）の性能比較の成績などを根拠に，乳幼児の結核感染診断法としてはツ反を優先していた。しかし，

①QFT-3GはQFT-2Gと比べて感度が高くT-SPOTと同等であること[1)]

②小児の活動性結核患者（LTBIではなく，結核発病者）に対するQFT-3Gの感度は，成人結核患者を対象とした場合と同等であるという知見が得られたこと[1)]

③健診対象がBCG既接種の乳幼児の場合，IGRAよりもツ反を優先するための科学的根拠が乏しいこと

などを理由に第5版では，乳幼児であってもIGRAを接触者健診の基本項目と位置づけて実施することとした。ただし，乳幼児の活動性結核（発病後）に対するIGRAの感度をそのまま乳幼児のLTBI(発病前)にも適用できるかは不明である。小児の結核感染診断におけるIGRAの有用性を検討したsystematic reviewにおいても，IGRAは5歳未満の「未発病感染例」を正確に検出できない可能性があることを指摘している[2)]。このため，乳幼児のLTBIに対するIGRAの感度不足の可能性を考慮して，IGRA単独ではなく，ツ反の併用が望ましい。たとえば，BCG既接種の乳幼児の健診においてIGRA陰性であっても，ツ反が「強陽性」の場合は「感染あり」とみなすなどの対応が考えられる。

検査の手順として，先にIGRAを実施し（その結果が陰性の場合に）引き続きツ反を実施するという方法では，結果として少なくとも3回の受診を必要とすることから，できるだけIGRAとツ反を同時に実施することが望ましい。これは健診方法の大きな変更であり，かつ，IGRAのための乳幼児の採血は困難を伴う場合があることから，健診を実施する施設の状況，および事例のBCG接種歴や感染リスクなどに応じて，従来どおり，ツ反を優先することも選択の一つとした。特にBCG未接種児の場合は，ツ反発赤径10mm以上を「陽性」とする判定基準を適用できるので，ツ反を優先する意義がある。

ただし，ツ反を優先する場合であっても，乳幼児の活動性結核の見落としを防ぐために，患者との接触歴等から感染リスクが高いと推定される乳幼児には，IGRAの併用を推奨することとした。

（4）高感染率集団でのIGRA再検査

結核感染が明らかな者でも，感染初期にはIGRAおよびツ反で陽性反応を検出できない。QFTを用いたこれまでの研究によれば，感染を受けてからIGRA陽転までの期間は通常2～3カ月と推定されている[3)4)]。しかし，結核感染率が極めて高かった集団感染事例においてQFTによる追跡検査を

長期間実施した研究によれば[4)5)]，感染曝露から２カ月後の陽性確認が最も多いものの，３～６カ月の間に陽転化したと考えられる者も少なくないことが報告されている。そこで，結核患者との最終接触から２カ月後の健診で実施したIGRAの陽性率が非常に高かった場合など，結核感染率が極めて高いと推定される集団に対しては，IGRA再検査を最終接触の６カ月後にも実施するよう推奨することとした。

２．「感染性期間」の始期の推定方法について

接触者健診の企画にあたっては，結核患者の感染性期間（患者の接触者に結核を感染させる可能性のある期間）の推定が重要である。米国CDCのガイドラインでは[6)]，基本的に結核診断日の「３カ月前」からを感染性期間とすることが勧められている。しかし，わが国では，感染症法に基づき「結核にかかっていると疑うに足りる正当な理由のある者」に対して，知事等が接触者健診を勧告する（従わなければ強制措置が可能）という人権制限的な制度であること，および感染・発病リスクの高い集団を優先して段階的に（同心円方式により）接触者健診を進める場合の最初の優先集団（第一同心円）を念頭に置いた場合は，症状出現時点や感染性結核を疑う画像所見の出現時期などを感染性期間の始期と考えて健診を企画することを基本としていた。わが国では，米国と比べて胸部X線検査の機会（定期健診，他疾患で入院時の検査など）が多いので，「一律３カ月前」ではなく，基本は「症状出現時期」とし，比較できる胸部Ｘ線所見があれば，それを参考にして判断する（事例によっては，逆に３カ月よりも大幅に長期間となる場合もあり）という考え方であった。

しかし，「診断時に喀痰塗抹陽性（３＋）であっても，咳の出現は１カ月前からで，健診歴がなく参考にできる過去の画像所見もない」といった事例が少なくないことや，症状出現時期の聞き取りが（特に高齢者では）十分にできない事例も多いことから，感染性期間の始期の推定方法を一部修正することとした。具体的には，喀痰塗抹陽性（または胸部Ｘ線検査で空洞あり）の患者については，過去のＸ線所見や菌検査所見等を遡って分析することにより感染性期間の始期の推定が可能である場合を除いて，基本的に「結核診断日の３カ月前，又は初診時の胸部Ｘ線検査ですでに空洞所見を認めた例では初診日の３カ月前」を始期とする方法を提案した。また，患者登録直後の（第一同心円の）接触者健診により新たな結核患者（発病者）が発見された場合は，感染から発病までの期間（集団感染事例の観察では，感染源患者の症状出現から７～８カ月後の発病例が最も多い）[7)]も考慮して，感染性期間の始期を遡及することを追加記載した。

３．結核菌分子疫学調査の推進について

わが国は今，結核の中蔓延国から低蔓延

国への移行期にある。低蔓延下では，新登録結核患者の感染源や感染経路を通常の実地疫学調査（接触者調査等）のみでは追えなくなるケースが多くなると推定される。そこで，低蔓延下での対策を視野に入れて，結核菌分子疫学調査と実地疫学調査を組み合わせた手法の有用性を解説するとともに，社会ネットワーク分析（social network analysis：SNA）の活用などについて，以下のような内容を追加記載した。

低蔓延下での対策としては，地域における菌陽性結核患者の菌株を網羅的に収集して分子疫学調査（例：VNTR法）を実施し，経年的にデータを蓄積することにより，同一菌株に感染した患者の発生状況や，これまで未知であった感染経路などが明らかになる。特に結核罹患率の低い地域では，網羅的な結核菌分子疫学調査と保健所による実地疫学調査を組み合わせることにより，①実地疫学調査で浮かび上がった患者間の関連性に科学的裏付けを付与できるほか，②実地疫学調査だけでは探知の困難な未知の感染伝播を発見できること，③新たな感染リスク集団の探知に役立つこと，④既感染発病（内因性再燃）が多いとされる高齢者でも最近の感染（再感染を含む）による発病が少なくないことが示唆されたこと，および⑤結核集団感染事例の追跡に寄与すること，などの多くの有用性が示されている[8]。

都道府県等の広い地域を範囲として結核患者から検出された結核菌について網羅的な分子疫学調査を実施すると，遺伝子タイピングの一致する菌株群（クラスタ）が数多く発見される。しかし，同一クラスタ内の患者間の関連性（接触歴や同一施設の利用歴など）が分子疫学調査の結果判明前の段階で把握されている例は，家族内感染などを除くと，一部に限られるのが現状である。その理由の一つとして，従来の実地疫学調査では，患者の症状出現後の接触者の把握に重点が置かれ，患者の発病前の社会的活動状況や生活歴等の調査が不十分であったことがあげられる。

そこで最近は，SNAの活用が注目されている。結核の疫学調査におけるSNAは，個々の患者について結核診断前の社会的活動状況（特に屋内で時間を過ごすことが多かった場所や活動状況など）についてもアンケートや面接などで詳細に調査し，相互の関連性が不明だった患者間の共通性を見出す（散在する点と点を線で結ぶ）技法といってもよい。SNAは通常の接触者健診では認識できなかった結核の感染経路の把握に役立つ手法であり，低蔓延下において注意すべき潜在的な感染経路（感染リスクの高い場所や行動，集団など）を探知することにより，LTBI治療の恩恵を受ける多くの接触者（従来の方法では健診対象となっていなかった接触者）の把握に寄与するといわれている[9]。わが国の結核患者向けの詳細な調査票の提案や調査データの電子化，および分析方法の標準化など，SNAを進めるにあたっての課題は多いが，分子疫学調査とSNAを組み合わせた手法は結核低蔓延下の対策として有望であり，今後の国内での試行を含めた実践研究が必要である。

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結核低蔓延地域における網羅的な結核菌反復配列多型（VNTR）分析の有用性

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要旨：〔目的〕国内の結核低蔓延地域において結核菌VNTR分析を広範に実施し，低蔓延下の結核対策における同分析の有用性を明らかにする。〔方法〕2009～2011年に山形県内で新規登録された菌陽性肺結核患者266人中184人（69.2%）から分離された結核菌の24領域VNTR分析を実施した。VNTRパターンが23領域以上一致した菌株を同一クラスタと定義し，各クラスタの由来患者間の関連性の有無を実地疫学調査結果から検証した。〔結果〕184株中49株（26.6%）が17クラスタを形成した。関連性がある事例として，6クラスタ内に院内感染3事例，家族内感染3事例，施設内感染1事例を見出した。このうち，院内感染，施設内感染各1事例ではVNTR分析により未知の伝播が明らかにされた。最大クラスタ（12株）のVNTRパターンは，2007年発端の集団感染事例の初発患者パターンと一致した。〔考察〕結核低蔓延地域では，広く収集した患者由来菌株のVNTR分析と実地疫学調査の結果を組み合わせることで，未知の感染伝播の発見，新たな感染リスク集団の探知，および集団感染の追跡に役立つなど，VNTR分析の高い有用性が確認された。

キーワーズ：結核低蔓延，結核菌，VNTR分析，分子疫学，実地疫学

はじめに

わが国の2011年の結核新登録患者数は22,681人，罹患率（人口10万対）は17.7であり，国際的には結核中蔓延国に位置付けられている。今後，早期に低蔓延国（罹患率10未満）の仲間入りを果たし，その先の制圧（elimination）を目指すにあたっては，結核患者一人ひとりの感染源・感染経路の特定が重要となる。これを支援する方法として結核菌の遺伝子タイピングを用いた分子疫学調査が必須とされている[1]。遺伝子タイピングのうち，現在国内において主流となりつつある反復配列多型（variable number of tandem repeat, VNTR）分析は，地方衛生研究所を中心として実施体制の整備が進んでいる。しかし，VNTR分析を保健所の実地疫学調査と組み合わせて活用した報告はまだ少ない[2][3]。これまでは主に結核集団感染事例における活用報告であり，都道府県レベルの地域全体の結核患者から分離された結核菌株を広く収集し，そのVNTR分析結果と結核患者の実地疫学調査情報を組み合わせてVNTR分析の有用性を検証した報告はない。

既に結核低蔓延に近い状況にある山形県（2011年罹患率11.3）では，結核菌VNTR分析を，感染症の予防及び感染症の患者に対する医療に関する法律第15条に基づく積極的疫学調査の基本項目と位置付けた。これにより2009年からは，原則として菌陽性結核患者全例の結核菌株について保健所が医療機関あてに譲渡を依頼し，譲渡を受けた菌株について山形県衛生研究所がVNTR分析を実施する体制となった。そこで本研究では，山形県全域の患者由来菌株を広く収集してVNTR分析を実施し，VNTR分析の結果と実地疫学調査情報を組み合わせた検討を行うことにより，低蔓延地域の結核対策におけるVNTR分析の有用性を明らかにすることを目的とした。

対象と方法

〔対象〕

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2009～2011年（3年間）の山形県における新登録菌陽性肺結核患者のうち，保健所が医療機関に依頼して患者由来菌株の譲渡を受けた全例を対象としてVNTR分析を実施した。また，対象患者については必要に応じてVNTR分析結果と保健所による実地疫学調査の結果を組み合わせて感染源・感染経路の分析を行った。なお，これらの調査・分析は法律に基づく行政権限として県の保健所および衛生研究所が実施したものであり倫理上の問題はないと判断されたが，研究目的の分析を含むため，山形県衛生研究所倫理審査委員会の承認を得て実施した。

〔VNTR分析の方法〕

結核菌1白金耳を300 μlの蒸留水に懸濁し，100℃10分間加熱処理した結核菌粗抽出液を鋳型とした。PCR法によるVNTR領域の増幅は前田らの方法[4]に準拠した。得られたPCR産物は0.5×TBE緩衝液を用いた2% Nuesieve 3:1 Agarose（Lonza社）でアガロース電気泳動を行い，コピー数を算定した。遺伝子増幅産物の鎖長が長くコピー数の判定が困難なPCR産物については，マイクロチップ電気泳動（*i*-チップ/コスモアイ，日立化成工業株式会社）により鎖長を算出することでコピー数の判定を行った。

VNTR分析領域の設定は，識別能を高めるため，および多発性大規模感染株（putative expanding cluster types, pECTs）[5]として提唱されている結核菌株の24領域VNTRパターン（9種類）との比較を可能とするために，国内の標準法として提唱されているJATA（12）[4][6]12領域に，JATA（15）3領域[7]（QUB-11a, ETR A, QUB-18），超可変3領域[8]（QUB-3232, v3820, v4120），および国際比較6領域[9]（MIRU04, MIRU16, MIRU40, ETR C, Mtub30, Mtub39）を加えた計24領域とした。

〔VNTR分析によるクラスタ形成と関連性評価〕

VNTR分析で同一パターンを示す菌株群（クラスタ）の定義を，「24領域のVNTRパターンのうち23領域以上が一致した菌株」とした。本研究では，結核菌の偶発的な遺伝子変異を考慮して，VNTRパターンが1領域異なる場合も同一クラスタと判定した。クラスタを形成した菌株の由来患者について，保健所による実地疫学調査結果と組み合わせて関連性を分析した。また，pECTs-VNTRパターン9種類のいずれかと24領域のVNTRパターンが完全一致した菌株を認めた場合にも，当該患者の実地疫学調査結果を参照し，感染経路などの分析を試みた。

各クラスタ内の患者間の疫学的関連性の定義は，保健所の実地疫学調査結果との組み合わせにより明らかな関連性（明らかな接触歴など）が判明した事例を「関連あり（Related）」とした。これには，VNTR分析を実施する前の実地疫学調査で既に患者間の関連性が示唆されていた事例のほか，VNTR分析によるクラスタ形成を根拠に保健所が追加の実地疫学調査を実施して関連性が判明した事例も含めた。また，クラスタ内の患者間の明らかな接触歴は確認できないものの実地疫学調査で関連する要素（同じ時期に同じ施設の利用歴ありなど）を見出した事例を「関連の可能性あり（Probably related）」，関連が全く不明の事例を「関連なし（Unknown）」とした。

結　果

2009～2011年（3年間）の山形県内の新登録菌陽性肺結核患者数は266人であった。このうち，医療機関から患者由来菌株の譲渡を受けてVNTR分析が可能であった

Table 1 Parameters of 184 pulmonary tuberculosis patients identified in Yamagata Prefecture, Japan between 2009 and 2011

Parameters	Area inhabited				Total
	a n (%)	b n (%)	c n (%)	d n (%)	
Total	103	6	28	47	184
Sex					
Male	58 (56.3)	4 (66.7)	19 (67.9)	26 (55.3)	107 (58.2)
Female	45 (43.7)	2 (33.3)	9 (32.1)	21 (44.7)	77 (41.8)
Age group (years)					
≦29	7 (6.8)	0	0	2 (4.3)	9 (4.9)
30–39	9 (8.7)	1 (16.7)	0	5 (10.6)	15 (8.2)
40–49	7 (6.8)	0	1 (3.6)	0	8 (4.3)
50–59	4 (3.9)	0	1 (3.6)	0	5 (2.7)
60–69	4 (3.9)	1 (16.7)	1 (3.6)	3 (6.4)	9 (4.9)
70–79	17 (16.5)	1 (16.7)	6 (21.4)	8 (17.0)	32 (17.4)
80–89	44 (42.7)	3 (50.0)	14 (50.0)	26 (55.3)	87 (47.3)
≧90	11 (10.7)	0	5 (17.9)	3 (6.4)	19 (10.3)
Medical history					
Initial	100 (97.1)	6 (100.0)	23 (82.1)	44 (93.6)	173 (94.0)
Retreatment	3 (2.9)	0	5 (17.9)	3 (6.4)	11 (6.0)