Table 1 Basic characteristics and TB-related findings of the study participants

		Homeless persons (n = 263)	Caregivers (n = 173)
Basic characteristics			
Sex, female	n (%)	2 (0.8)	94 (54.3)
Age, years	mean median (range)	58.0 59 (30-74)	48.2 48 (30-71)
30-49	n (%)	40 (15.2)	97 (56.1)
50-59	n (%)	108 (41.1)	52 (30.1)
60-74	n (%)	115 (43.7)	24 (13.9)
Current smoker	n (%)	189 (73.0)	63 (37.1)
Current drinker	n (%)	134 (51.9)	54 (32.0)
Length of time spent living and/or working in the Airin district, years	mean median (range)	13.5 10 (0.1-58)	10.3 5 (0.1-64)
<5 years	n (%)	63 (25.9)	73 (43.2)
5-10 years	n (%)	46 (18.9)	33 (19.5)
≥10 years	n (%)	134 (55.1)	63 (37.3)
TB-related findings			
Past history of TB	n (%)	33 (12.6)	6 (3.5)
Past exposure to TB patients	n (%)	50 (19.8)	32 (18.7)
Cough and/or sputum	n (%)	64 (24.6)	6 (3.5)
General fatigue	n (%)	51 (19.6)	9 (5.2)
Elevated body temperature	n (%)	9 (3.5)	1 (0.6)
Abnormal chest X-ray finding	n (%)	68 (25.9)	7 (4.1)
Active TB case	n (%)	4 (1.5)	0 (0.0)
Positive QFT result	n (%)	133 (50.6)	42 (24.3)
Indeterminate QFT result	n (%)	4 (1.5)	1 (0.6)

Abbreviations: TB, tuberculosis; QFT, QuantiFERON-TB Gold In-Tube.

The number of missing values was 20 for length of time spent in the Airin district, 10 for past exposure to TB patients, 4 for smoking, 5 for drinking and 3 for cough and/or sputum, general fatigue and elevated body temperature among homeless persons, and 4 for length of time spent in the Airin district, 2 for past exposure to TB patients, 3 for smoking and 4 for drinking among caregivers.

None of the participants had a history of extra-pulmonary TB or used immunosuppressive agents.

The trends for active TB prevalence among homeless people varied considerably by location. In New York City, the prevalence had a substantial decline, that is, 1,502 per 100,000 in 1992 to 171 per 100,000 in 2004 [23]. In the Airin district, where approximately 80% of residents were estimated to be homeless people, the prevalence declined substantially from 1400 per 100,000 in 2000 to 680 in 2005, and declined further to a small extent to 653 per 100,000 in 2007 [22].

The substantial decline in the active TB prevalence in New York City and the moderate decline in the Airin district among homeless people were attributable

Table 2 Characteristics of active TB cases

	Case 1	Case 2	Case 3	Case 4	Total*
Homeless or caregivers	Homeless	Homeless	Homeless	Homeless	4
Sex	Male	Male	Male	Male	4
Age	62	63	66	66	Mean 64.3
Current smoker	-	+	+	-	2
Current drinker	-	+	-	+	2
Length of time spent living and/or working in the Airin district, years	10	9	20	13	Mean 13.0
Past history of TB	-	+	-	-	1
Past exposure to TB patients	-	-	-	-	0
Cough and/or sputum	+	+	+	+	4
General fatigue	+	-	+	+	3
Elevated body temperature	-	-	-	-	0
Abnormal chest X-ray finding	+	+	+	+	4
QFT-positivity	-	+	+	+	3

Abbreviations: TB, tuberculosis; QFT, QuantiFERON-TB Gold In-Tube.

*The number of positive for each factor or mean value for continuous variables among four active TB cases.

Table 3 Age-adjusted and multivariate odds ratios (OR) and 95% confidence intervals (95% CI) of QFT-positivity according to potential risk factors among homeless persons and caregivers, after excluding the subjects with active TB disease or indeterminate QFT results

		Homeless persons (n = 255)				Caregivers (n = 172)			
		No. of subjects	No.(%) of QFT-positivity	Age-adjusted OR (95% CI)	MultivariateOR (95% CI)†	No. of subjects	No.(%) of QFT-positivity	Age-adjusted OR (95% CI)	MultivariateOR (95% CI)†
Sex	Male	253	130 (51.4)	NA	NA	79	20 (25.3)	1.0	1.0
	Female	2	0 (0.0)	NA	NA	93	22 (23.7)	0.95 (0.46-1.93)	0.91 (0.39-2.11)
Current smoker	No	66	33 (50.0)	1.0	1.0	106	27 (25.5)	1.0	1.0
	Yes	185	93 (50.3)	1.19 (0.66-2.14)	1.23 (0.63-2.40)	63	13 (20.6)	0.81 (0.38-1.75)	0.72 (0.29-1.74)
Current drinker	No	121	56 (46.3)	1.0	1.0	114	26 (22.8)	1.0	1.0
	Yes	129	70 (54.3)	1.74 (1.02-2.96)*	1.84 (1.01-3.37)*	54	13 (24.1)	1.23 (0.56-2.69)	1.15 (0.48-2.74)
Length of time spent living and/or working in the Airin district, year	<10 years	107	38 (35.5)	1.0	1.0	105	18 (17.1)	1.0	1.0
	≥10 years	128	78 (60.9)	2.52 (1.46-4.35)*	2.53 (1.39-4.61)*	63	24 (38.1)	2.60 (1.24-5.42)*	2.32 (1.05-5.13)*
Past exposure to TB patients	No	196	96 (49.0)	1.0	1.0	138	27 (19.6)	1.0	1.0
	Yes	50	31 (62.0)	2.05 (1.05-3.99)*	1.51 (0.71-3.21)	32	14 (43.8)	3.63 (1.55-8.47)*	3.21 (1.30-7.91)*
Cough and/or sputum	No	194	100 (51.6)	1.0	1.0	166	42 (25.3)	NA	NA
	Yes	59	29 (49.2)	0.91 (0.50-1.67)	0.64 (0.32-1.32)	6	0 (0.0)	NA	NA
General fatigue	No	207	102 (49.3)	1.0	1.0	163	40 (24.5)	1.0	1.0
	Yes	46	27 (58.7)	1.66 (0.84-3.29)	1.47 (0.66-3.28)	9	2 (22.2)	0.96 (0.19-4.93)	1.06 (0.19-5.96)
Elevated body temperature	No	244	124 (50.8)	NA	NA	171	42 (24.6)	NA	NA
	Yes	9	5 (55.6)	NA	NA	1	0 (0.0)	NA	NA
Past history of TB	No	225	109 (48.4)	1.0	1.0	166	37 (22.3)	NA	NA
	Yes	30	21 (70.0)	2.23 (0.95-5.21)	1.51 (0.43-5.31)	6	5 (83.3)	NA	NA
Abnormal chest X-ray finding	No	193	86 (44.6)	1.0	1.0	165	37 (22.4)	NA	NA
	Yes	62	44 (71.0)	2.62 (1.39-4.93)*	1.90 (0.78-4.60)	7	5 (71.4)	NA	NA

*$p < 0.05$.

Abbreviations: TB, tuberculosis; NA, not applicable.

†Multivariate-adjusted for the listed factors and age.

to intensive population-based TB screening and DOTS [5,26].

Prevalence of latent TB infection

We found high prevalence of QFT-positivity for homeless persons (50.6%) and for caregivers (24.3%) at the ages of 30-74 years. When we restricted the sample to those of ages 40-69 years, the respective proportions were 50.4% for homeless persons and 30.8% for caregivers. These prevalences were far higher than that of the general Japanese population aged 40-69 years, which is estimated to be 7.1% [27].

Latent TB infection among vulnerable persons including homeless people

Garfein et al. investigated latent TB infection among 280 homeless persons in a Mexican city with the highest TB prevalence using an IGRA and found the prevalence of QFT-positivity was 51.8% [28], which was nearly equal to the prevalence in our study. In addition to homeless people, high-risk individuals for latent TB infection can be detected by the IGRA. For example, the prevalence of IGRA-positivity was reported to be 29.8% among immigrants, mostly from Latin America, in Italy [29], 33.6% among drug users in Houston, USA [30], and 53.9% among immigrants with close contact to sputum smear-positive TB patients in Netherlands [31].

Latent TB infection among caregivers

A study in Italy showed that the prevalence of latent TB infection (positive QFT) was 55.5% among caregivers working at a homeless shelter [11], which showed higher latent TB prevalence than the caregivers in our study. However, compared with healthcare workers for latent TB infection, the caregivers of our study had higher prevalence of latent TB infection. The prevalence of latent TB infection among healthcare workers in low- to intermediate-prevalence countries including Japan ranged from 1% to 19% [15,32-35], which was much lower than that of the caregivers in our study. Mirtskhulava et al. reported an extremely high prevalence of latent TB infection (60.0%) among healthcare workers, probably because they had frequent contact with TB patients and also high prevalence of TB in the community [36].

Putative risk factors for latent TB infection

Another aim of our study was to determine putative risk factors for latent TB infection. QFT-positivity was associated with past exposure to TB patients among caregivers, but not among homeless people. Caregivers usually know when and how they have been in contact with people with TB, whereas homeless persons often ignored or did not notice this [37]. Homeless people who drank almost every day had higher QFT-positivity than those who did not. Habitual drinkers may be more likely to have contact with other drinkers and had a higher risk of being infected [38]. The past history of TB was not significantly associated with a positive QFT result among homeless persons, which might be related to the waning of immune responses in the time course of TB infection [27]. The duration of living and/or working in Airin district was associated with QFT-positivity among both homeless people and caregivers, but this was the case only for ≥ 5 years among homeless people.

The risk of QFT-positivity was found to increase with increasing length of time spent in the Airin district, independently of acknowledgement of exposure to TB patients. Even caregivers without known exposure to TB patients in this study had approximately twofold higher QFT-positivity (19.6%, Table 3) than healthcare workers in Japan (9.9%) [16], indicating that they may be at high risk for TB infection. As mentioned above, caregivers at a homeless shelter had high latent TB prevalence even in a low-prevalence country [11]. Thus, anti-TB measures for caregivers should be strengthened to ensure their safety.

Limitations

This study has several potential limitations. The setting and selection of homeless persons in our study may limit the ability to generalize our results to the entire Airin district. Our sample of homeless persons may be at high risk to have been in contact with TB patients. The information on homelessness, past history of TB, past exposure to TB patients and length of time spent living and/or working in the Airin district was self-reported. Homeless people may be less likely to recall such information accurately because they are less health-conscious. The cross-sectional nature of the data limits the degree to which we can assign causality, especially with respect to temporality. However, it might be plausible that the exposure, such as past exposure to TB patients and length of time spent in the Airin district, may precede TB infection or disease. Information regarding TB-related factors such as HIV infection, drug abuse and history of incarceration was not included in the data collection. However, it is well known that TB/HIV co-infection is quite low in Japan [39]. In the present study, TST was not carried out because we wanted to avoid the refusal of study participation by homeless persons, and there is poor agreement between TST and QFT results caused by the effect of BCG vaccination in Japan [16].

Conclusions

We found that the prevalence of latent TB infection was approximately 50% for homeless people and 25% for

caregivers, and a long duration spent by both groups in the Airin district in Osaka, Japan, was associated with latent TB infection. Although no active TB was found for caregivers, one-quarter of them had latent TB infection. In addition to homeless persons, caregivers need examinations for latent TB infection as well as active TB and careful follow-up, especially when they have spent a long time in a high TB prevalence area and/or have been exposed to TB patients.

Acknowledgements
This study was supported by a grant-in-aid for social medical research (2007-2008) from Osaka socio-medical center hospital, without any involvement in the design, data collection, data analysis or data interpretation of this study. The authors wish to thank all personnel of the Osaka socio-medical center hospital and many NGOs for providing data used in this study. The authors are also grateful to Drs. S. Yamamoto, T. Ohira, K. Danno and Y. Toyoda for their assistance in the data collection and analyses.

Author details
[1]Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita-shi, Osaka, Japan. [2]Osaka Socio-Medical Center Hospital, 1-3-44 Haginochaya, Nishinari-ku, Osaka-shi, Osaka, Japan. [3]Osaka City Public Health Office, 1-2-7-1000, Asahimachi, Abeno-ku, Osaka-shi, Osaka, Japan. [4]Osaka Prefectural Hospital Organization Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3-7-1 Habikino, Habikino-shi, Osaka, Japan.

Authors' contributions
T. Takatorige, YH, NN, SH, AS, KF, HY and T. Takashima participated in the planning of the study. T. Takatorige and T. Takashima coordinated the study and took overall responsibility for the delivery of the work. T. Tabuchi, YH, KF and HY had responsibility for data collection. T. Tabuchi conducted the analysis, with statistical support from HI, YT, TN and TM. T. Tabuchi, HI and T. Takashima participated in writing the paper. All authors participated in the interpretation of the study and read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 15 July 2010 Accepted: 21 January 2011
Published: 21 January 2011

References

1. World Health Organization: *Global tuberculosis control: epidemiology, strategy, financing, WHO Report 2009* Geneva, Switzerland: WHO; 2009, WHO/HTM/TB/2009.411.
2. Shimao T: **Tuberculosis and its control-lessons from the past and future prospect.** *Kekkaku* 2005, **80(6)**:481-489.
3. Nakata N, Inori F, Nakamura F, Harihara S, Hirayama Y, Suzuki A, Shimouchi A, Takatorige T: **Significance of tuberculosis screening of outpatients in areas with high prevalence of tuberculosis.** *Kekkaku* 2007, **82**:455-458.
4. Takatorige T, Ohsaka T, Yamamoto S, Nishimori T, Fujikawa T, Kuroda K, Iso H: **Tuberculosis and its control measures for homeless people: implementation of chest X-ray examination for three successive years.** *Kekkaku* 2007, **82**:19-25.
5. Shimouchi A: **Fight against urban tuberculosis problems and program effects in Osaka City.** *Kekkaku* 2009, **84**:727-735.
6. Raviglione MC, Uplekar MW: **WHO's new Stop TB Strategy.** *Lancet* 2006, **367**:952-955.
7. Potter B, Rindfleisch K, Kraus CK: **Management of active tuberculosis.** *Am Fam Physician* 2005, **72**:2225-2232.
8. van Zyl Smit RN, Pai M, Yew WW, Leung CC, Zumla A, Bateman ED, Dheda K: **Global lung health: the colliding epidemics of tuberculosis, tobacco smoking, HIV and COPD.** *Eur Respir J* 2010, **35**:27-33.
9. Holtgrave DR, Crosby RA: **Social determinants of tuberculosis case rates in the United States.** *Am J Prev Med* 2004, **26**:159-162.
10. Lonnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M: **Drivers of tuberculosis epidemics: the role of risk factors and social determinants.** *Soc Sci Med* 2009, **68**:2240-2246.
11. Martini A, Tomao P, Di Renzi S, Vonesch N, Rubino L, Signorini S, Tomei F: **QuantiFERON-TB Gold test in homeless shelter staff: preliminary results.** *G Ital Med Lav Ergon* 2007, **29**:767-769.
12. de Vries G, van Hest RA: **From contact investigation to tuberculosis screening of drug addicts and homeless persons in Rotterdam.** *Eur J Public Health* 2006, **16**:133-136.
13. Dewan PK, Grinsdale J, Liska S, Wong E, Fallstad R, Kawamura LM: **Feasibility, acceptability, and cost of tuberculosis testing by whole-blood interferon-gamma assay.** *BMC Infect Dis* 2006, **6**:47.
14. Andersen P, Munk ME, Pollock JM, Doherty TM: **Specific immune-based diagnosis of tuberculosis.** *Lancet* 2000, **356**:1099-1104.
15. Harada N, Higuchi K, Yoshiyama T, Kawabe Y, Fujita A, Sasaki Y, Horiba M, Mitarai S, Yonemaru M, Ogata H, Ariga H, Kurashima A, Wada A, Takamori M, Yamagishi F, Suzuki K, Mori T, Ishikawa N: **Comparison of the sensitivity and specificity of two whole blood interferon-gamma assays for M. tuberculosis infection.** *J Infect* 2008, **56**:348-353.
16. Harada N, Nakajima Y, Higuchi K, Sekiya Y, Rothel J, Mori T: **Screening for tuberculosis infection using whole-blood interferon-gamma and Mantoux testing among Japanese healthcare workers.** *Infect Control Hosp Epidemiol* 2006, **27**:442-448.
17. Andreu J, Caceres J, Pallisa E, Martinez-Rodriguez M: **Radiological manifestations of pulmonary tuberculosis.** *Eur J Radiol* 2004, **51**:139-149.
18. **Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999.** *Am J Respir Crit Care Med* 2000, **161(4 Pt 1)**:1376-1395.
19. **QuantiFERON-TB Gold In-Tube package insert.** [http://www.cellestis.com/IRM/Company/ShowPage.aspx?CPID=1255].
20. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A: **Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States.** *MMWR Recomm Rep* 2005, **54**:49-55.
21. Katz MH: *Multivariable Analysis: a practical guide for clinicians.* Second edition. Cambridge University Press; 2006.
22. Osaka city public health center: *Tuberculosis of Osaka city* Osaka; 2008.
23. McAdam JM, Bucher SJ, Brickner PW, Vincent RL, Lascher S: **Latent tuberculosis and active tuberculosis disease rates among the homeless, New York, New York, USA, 1992-2006.** *Emerg Infect Dis* 2009, **15**:1109-1111.
24. Story A, Murad S, Roberts W, Verheyen M, Hayward AC: **Tuberculosis in London: the importance of homelessness, problem drug use and prison.** *Thorax* 2007, **62**:667-671.
25. Kameda K: **Thinking about tuberculosis in Osaka City.** *Kekkaku* 2000, **75**:717-724.
26. Bureau of Tuberculosis Control, New York City Department of Health and Mental Hygiene: *Clinical Policies and Protocols* , 4 2008.
27. Mori T, Harada N, Higuchi K, Sekiya Y, Uchimura K, Shimao T: **Waning of the specific interferon-gamma response after years of tuberculosis infection.** *Int J Tuberc Lung Dis* 2007, **11**:1021-1025.
28. Garfein RS, Laniado-Laborin R, Rodwell TC, Lozada R, Deiss R, Burgos JL, Cuevas-Mota J, Cerecer P, Moser K, Volker ML, Strathdee SA: **Latent tuberculosis among persons at risk for infection with HIV, Tijuana, Mexico.** *Emerg Infect Dis* 2010, **16**:757-763.
29. Orlando G, Merli S, Cordier L, Mazza F, Casazza G, Villa AM, Codecasa L, Negri E, Cargnel A, Ferrarese M, Rizzardini G: **Interferon-gamma releasing assay versus tuberculin skin testing for latent tuberculosis infection in targeted screening programs for high risk immigrants.** *Infection* 2010, **38(3)**:195-204.
30. Grimes CZ, Hwang LY, Williams ML, Austin CM, Graviss EA: **Tuberculosis infection in drug users: interferon-gamma release assay performance.** *Int J Tuberc Lung Dis* 2007, **11**:1183-1189.
31. Kik SV, Franken WP, Arend SM, Mensen M, Cobelens FG, Kamphorst M, van Dissel JT, Borgdorff MW, Verver S: **Interferon-gamma release assays in**

immigrant contacts and effect of remote exposure to Mycobacterium tuberculosis. *Int J Tuberc Lung Dis* 2009, **13**:820-828.
32. Alvarez-Leon EE, Espinosa-Vega E, Santana-Rodriguez E, Molina-Cabrillana JM, Perez-Arellano JL, Caminero JA, Serrano-Aguilar P: **Screening for tuberculosis infection in spanish healthcare workers: Comparison of the QuantiFERON-TB gold in-tube test with the tuberculin skin test.** *Infect Control Hosp Epidemiol* 2009, **30**:876-883.
33. Nienhaus A, Loddenkemper R, Hauer B, Wolf N, Diel R: **Latent tuberculosis infection in healthcare workers–evaluation of an Interferon-gamma release assay.** *Pneumologie* 2007, **61**:219-223.
34. Tripodi D, Brunet-Courtois B, Nael V, Audrain M, Chailleux E, Germaud P, Naudin F, Muller JY, Bourrut-Lacouture M, Durand-Perdriel MH, Gordeeff C, Guillaumin G, Houdebine M, Raffi F, Boutoille D, Biron C, Potel G, Roedlich C, Geraut C, Schablon A, Nienhaus A: **Evaluation of the tuberculin skin test and the interferon-gamma release assay for TB screening in French healthcare workers.** *J Occup Med Toxicol* 2009, **4**:30.
35. Soborg B, Andersen AB, Larsen HK, Weldingh K, Andersen P, Kofoed K, Ravn P: **Detecting a low prevalence of latent tuberculosis among health care workers in Denmark detected by M. tuberculosis specific IFN-gamma whole-blood test.** *Scand J Infect Dis* 2007, **39**:554-559.
36. Mirtskhulava V, Kempker R, Shields KL, Leonard MK, Tsertsvadze T, del Rio C, Salakaia A, Blumberg HM: **Prevalence and risk factors for latent tuberculosis infection among health care workers in Georgia.** *Int J Tuberc Lung Dis* 2008, **12**:513-519.
37. Marks SM, Taylor Z, Qualls NL, Shrestha-Kuwahara RJ, Wilce MA, Nguyen CH: **Outcomes of contact investigations of infectious tuberculosis patients.** *Am J Respir Crit Care Med* 2000, **162**:2033-2038.
38. Rehm J, Samokhvalov AV, Neuman MG, Room R, Parry C, Lonnroth K, Patra J, Poznyak V, Popova S: **The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review.** *BMC Public Health* 2009, **9**:450.
39. Yamada N, Nagai H: **The current and future situations of TB/HIV (co-infection of tuberculosis and HIV) in Japan.** *Kekkaku* 2009, **84**:203-211.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2334/11/22/prepub

doi:10.1186/1471-2334-11-22
Cite this article as: Tabuchi *et al.*: Tuberculosis infection among homeless persons and caregivers in a high-tuberculosis-prevalence area in Japan: a cross-sectional study. *BMC Infectious Diseases* 2011 **11**:22.

Jpn. J. Infect. Dis., 64, 341-344, 2011

Short Communication

Serotyping and Multilocus Sequence Typing of *Streptococcus pneumoniae* Isolates from the Blood and Posterior Nares of Japanese Children Prior to the Introduction of 7-Valent Pneumococcal Conjugate Vaccine

Tomohiro Oishi[1,2], Akihito Wada[3], Bin Chang[3], Shinichi Toyabe[4], and Makoto Uchiyama[1]

[1]*Department of Pediatrics and*
[4]*Crisis Management Office, Niigata University Medical and Dental Hospital, Niigata 951-8520;*
[2]*Department of Pediatrics, Niigata Prefectural Shibata Hospital, Niigata 957-8588; and*
[3]*Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo 162-8640, Japan*

(Received April 21, 2011. Accepted July 4, 2011)

SUMMARY: In Japan, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2010. To assess the effects of PCV7 on invasive pneumococcal infection in children, a population-based prospective survey has been conducted in 10 prefectures. As a part of the study, blood and nasopharyngeal isolates from children admitted to the Shibata Hospital, Niigata Prefecture, were analyzed for determining the serotypes, their susceptibilities to antimicrobial agents, and multilocus sequence types. Sixteen blood isolates were obtained from October 2007 to December 2009. Sixty-three nasopharyngeal isolates were obtained from the posterior nares of 118 children with pneumonia from April to September 2008. The coverage rates of the blood and nasopharyngeal isolates for PCV7 were 81.3% and 57.1%, respectively. Although none of these children had received PCV7, serotype 19A isolates were recovered from 12.5% (2/16) of the blood samples and 12.7% (8/63) of the nasopharyngeal samples. The sequence type of a nasopharyngeal isolate of serotype 19A was ST320, and the minimum inhibitory concentration of penicillin G was 4 μg/mL. In addition to the continuous prospective survey of pneumococcal infection, early introduction of the 13-valent conjugate vaccine, in which the 19A conjugate is included, will be necessary in Japan.

Streptococcus pneumoniae infection is a leading cause of childhood morbidity. In 2005, the World Health Organization estimated that pneumococcal diseases caused 1.6 million deaths, including 0.7–1 million deaths per year in children under 5 years of age (1). The 7-valent pneumococcal conjugate vaccine (PCV7) has been widely used in USA and other countries; this has resulted in a dramatic reduction of invasive pneumococcal disease (IPD) in both immunized children (2–10) and non-immunized adults (2–5)—the "herd immunity" effect. PCV7 has also reduced hospitalization due to all-cause pneumonia in children under the age of 2 years in USA (11). In Japan, PCV7 became available in February 2010. Several surveys on IPD had been conducted in Japan before the introduction of the vaccine (12–16). To characterize invasive pneumococcal infection and respiratory infection/colonization in the same population before the introduction of PCV7, we obtained blood and nasopharyngeal *S. pneumoniae* isolates from hospitalized children, and analyzed the serotypes, their susceptibility to antimicrobial agents, and multilocus sequence types.

All pneumococcal isolates were obtained from children hospitalized at the Department of Pediatrics, Niigata Prefectural Shibata Hospital. This hospital is the only one in the region that has inpatient wards for children. The population of Shibata City in 2011 is 102,758, of which 3,835 are children under 5 years of age. Blood isolates were obtained from October 2007 to December 2009. Posterior nares swabs were obtained from 118 children with pneumonia from April to September 2008. Pneumonia was diagnosed by fever, cough, sputum production, chest radiography examination, blood cell count, and/or elevation of C-reactive protein. The pneumococcal isolates were serotyped by the Quellung reaction with serotype-specific antisera (Statens Serum Institut, Copenhagen, Denmark) and factor serum for serotype 6C, which was made in our laboratory (17). Susceptibilities to antimicrobial agents were determined by the microbroth dilution method using Dry Plate (Eiken Chemical Co., Tokyo, Japan), according to the Clinical and Laboratory Standards Institute M100-S18 guidelines (18). Although the penicillin G susceptibility criteria have changed in M100-S18, the previous criteria in minimum inhibitory concentration (MIC) (penicillin-susceptible *S. pneumoniae* [PSSP], ≤ 0.06 μg/mL; penicillin-intermediate *S. pneumoniae* [PISP], 0.12–1 μg/mL; penicillin-resistant *S. pneumoniae* [PRSP], ≥ 2 μg/mL) were used in this study. To determine the sequence type (ST) of the isolates, multilocus sequence typing (MLST) was performed as described previously by Enright and Spratt (19). STs were determined by the Internet database search at http://spneumoniae.mlst.net/. Informed consent for this

*Corresponding author: Mailing address: Department of Pediatrics, Niigata University Medical and Dental Hospital, Asahimachidori 1-757, Chuo-ku, Niigata 951-8520, Japan. Tel & Fax: +81-25-227-2222/+81-25-227-0778, E-mail: oo0612@hotmail.com

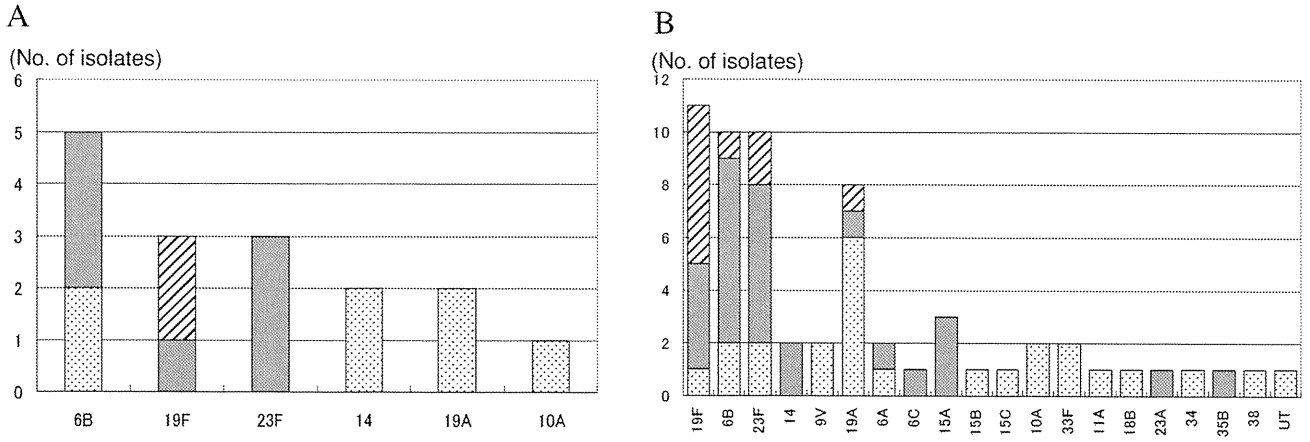

Fig. 1. Relationship between serotype and penicillin G resistance categories for *S. pneumoniae* isolates from (A) blood and (B) posterior nares. Shaded bar, gray bar, and dotted bar represent PRSP (penicillin G MIC ≥ 2 μg/mL), PISP (penicillin G MIC 0.12–1 μg/mL), and PSSP (penicillin G MIC ≤ 0.06 μg/mL), respectively. One serotype 14 isolate, in which MIC could not be determined, is not included in panel B.

study was obtained from parents or guardians, in accordance with the Helsinki Declaration. None of the children had received PCV7 or the 23-valent polysaccharide vaccine.

Sixteen blood isolates were obtained from 16 patients with bacteremia (age, 4 months to 3 years). No meningitis or sepsis was observed in the study period. In addition, a total of 63 nasopharyngeal isolates were obtained. Out of the 63 isolates, 58 (2 isolates of different serotypes were obtained from 2 patients) were from 56 hospitalized patients (age, 1 month to 15 years) who had pneumonia without bacteremia, and 5 isolates were from 5 patients with pneumonia and bacteremia. One-year-old children were most frequently affected by invasive and respiratory infections; these children account for 68.8% and 38.1% of those with bacteremia and pneumonia, respectively. The serotypes of the 16 blood isolates were as follows: type 6B (5 isolates, 31.3%), types 19F and 23F (3 isolates each, 18.8%), types 14 and 19A (2 isolates each, 12.5%), and type 10A (1 isolate, 6.3%) (Fig. 1A). The coverage rates of PCV7 and the 13-valent conjugate vaccine (PCV13) for the blood isolates were 81.3 and 93.8%, respectively. The serotypes of the 63 nasopharyngeal isolates were as follows: type 19F (11 isolates, 17.5%); types 6B and 23F (10 isolates each, 15.9%); type 19A (8 isolates, 12.7%); types 14 and 15A (3 isolates each, 4.8%); types 9V, 6A, 10A, and 33F (2 isolates each, 3.2%); other serotypes (9 serotypes, including 6C, with 1 isolate of each type, 1.6%); and untypeable (1 isolate, 1.6%) (Fig. 1B). Isolates of serotypes 19F and 33F were obtained from 1 patient and those of serotypes 6B and 14 from another patient. Some isolates obtained from both blood and nasopharyngeal samples of a single patient showed the same serotypes: 6B (1 patient), 19F (2 patients), and 23F (2 patients). The coverage rates of PCV7 and PCV13 for the nasopharyngeal isolates were 57.1% and 73.0%, respectively. The blood isolates were tested for susceptibility to penicillin G, and the results were as follows: 7 isolates (43.8%), PSSP; 7 isolates (43.8%), PISP; and 2 isolates (12.5%), PRSP. The relationship between serotypes and penicillin G susceptibility in the blood isolates is shown in Figure 1A. Isolates of serotypes 19F and 23F were PISP or PRSP, while those of serotypes 14, 19A, and 10A were PSSP. The 63 nasopharyngeal isolates were examined for susceptibility to penicillin G also, and 62 isolates showed the following results: 25 isolates (40.3%), PSSP; 27 isolates (43.6%), PISP; and 10 isolates (16.1%), PRSP. Isolates of serotypes 6B, 19F, 23F, and 19A were PRSP (Fig. 1B). The antimicrobial susceptibility of a serotype 14 isolate could not be determined because it did not show growth in Mueller Hinton broth.

The results of the MLST analysis of the blood and nasopharyngeal isolates are shown in Table 1. Blood isolates of serotype 6B comprised ST90 and ST2983, and those of serotype 19F comprised ST236 and ST115. ST2983 and ST115 are double-locus variants (DLVs) of ST90 and ST236, respectively. Isolates of serotypes 23F, 14, and 19A showed had only 1 ST (ST1437, ST13, and ST3111, respectively). Nasopharyngeal isolates showed more variation in STs than blood isolates. For example, isolates of serotype 6B comprised 4 STs: ST90, ST2983, ST902, and ST5864. ST2983 is a DLV of ST90, but ST90, ST902, and ST5864 are not related to each other. Another example was serotype 19A with 3 STs—ST320, ST3111, and ST5842—that are not related to each other. The isolates of serotype 23F comprised 5 STs, but these STs could be grouped under 2 STs (ST242 and ST1437). Two blood isolates and 6 nasopharyngeal isolates of serotype 19F showed resistance to penicillin G at MIC of 2–4 μg/mL; all the isolates were ST236 or ST115. The MIC of penicillin G for a nasopharyngeal serotype 19F isolate with ST257 was 0.03 μg/mL. This was the only PSSP found among isolates of serotype 19F. The MIC of penicillin G for a nasopharyngeal isolate of serotype 19A with ST320 was 4 μg/mL, whereas other serotype 19A isolates with ST3111 or ST5842 were PSSP or PISP (Fig. 1B). The MIC of cefotaxime, meropenem, and panipenem for the isolate of serotype 19A with ST320 was 2 μg/mL, 0.5 μg/mL, and 0.12 μg/mL, respectively.

A population-based survey has been conducted across 10 prefectures of Japan to assess the effect of PCV7 on invasive pneumococcal infection in children. This study was started in 2007, 3 years before the introduction of

Table 1. Serotype and sequence type of blood and posterior nares isolates

Serotype	Blood		Nasopharynx		Remarks[1]
	Sequence type	No. of isolates	Sequence type	No. of isolates	
6B	ST90, ST2983	3, 2	ST90, ST2983	6, 2	ST2983, DLV of ST90 (*xpt, ddl*)
			ST902	1	
			ST5846[2]	1	
19F	ST236, ST115	2, 1	ST236, ST257	10, 1	ST115, DLV of ST236 (*spi, ddl*) ST257, DLV of ST236 (*aroE, ddl*)
23F	ST1437	3	ST1437, ST5845[2]	2, 1	ST5845, SLV of ST1437 (*gdh, gki*)
			ST242, ST5841[2], ST5844[2]	5, 1, 1	ST5841, DLV of ST242 (*gdh, recP*) ST5844, SLV of ST242 (*xpt*)
14	ST13	2	ST13, ST2922	1, 1	ST2922, SLV of ST13 (*xpt*)
			ST5240[3]	1	
19A	ST3111	2	ST3111	6	
			ST320	1	
			ST5842[2]	1	
10A	ST6412[2]	1	ST1263	1	
			ST5236	1	
6A			ST3787	2	
6C			ST5241	1	
9V			ST280	2	
15A			ST63	3	
15B			ST199	1	
15C			ST5843[2]	1	ST5843, SLV of ST199 (*spi*)
33F			ST5840[2]	2	
11A			ST99	1	
18B			ST3594	1	
23A			ST5246	1	
34			ST3116	1	
35B			ST558	1	
38			ST393	1	
Untypeable			ST1106	1	

[1]: SLV/DLV, single-/double-locus variant.
[2]: newly identified sequence type in this study.
[3]: MICs could not be determined (see text).

PCV7. Shibata Hospital participated in this surveillance study from the beginning, and the period of this study corresponds to the period just prior to the introduction of PCV7. The children in Shibata City and the surrounding area did not receive PCV7 in this period. In spite of this, *S. pneumoniae* serotype 19A—well recognized as a major replacement serotype following PCV7 introduction in USA and other countries (2,3,5,7,20)—was isolated in 12.5% and 12.7% of the blood and nasopharyngeal cultures, respectively. These rates are much higher than those observed before PCV7 introduction in USA (2,3,20) and Canada (5) and are comparable to those that had been observed in France (7). In a country-wide survey in Japan, Chiba et al. reported 12 pediatric invasive cases by serotype 19A *S. pneumoniae* (6.2%) out of a total of 193 cases from 2006 to 2007 (15). MLST analysis showed that the ST of a nasopharyngeal isolate of serotype 19A was ST320. *S. pneumoniae* with this serotype and ST has been isolated from multiple regions in Japan (unpublished data) and from many other countries, including USA, Venezuela, Spain, Italy, China, and South Korea (http://spneumoniae.mlst.net/sql/burstspadvanced.asp). The penicillin G MIC of all the ST320 isolates obtained from patients in Japan was 2–4 μg/mL. However, the 2 blood isolates of serotype 19A obtained in this study showed a penicillin G MIC of 0.03 μg/mL and belonged to ST3111. At all of the 7 loci [*aroE, ghd, gki, recP, spi, xpt,* and *ddl*] in the pneumococcal MLST analysis, the alleles differed between ST320 and ST3111 (ST320 [4, 16, 19, 15, 6, 20, 1] and ST3111 [61, 60, 67, 16, 10, 104, 14]). In addition, ST5842 [10, 16, 150, 1, 13, 1, 29], isolated from a swab sample, had allele numbers that were different from those of both ST320 and ST3111. These findings suggest that multiple serotype 19A variants have already spread in children in the Shibata City region; these variants may cause respiratory infections and would cause invasive infections. The invasive infection surveillance in 10 prefectures showed that various STs, including ST320 and ST3111, have been observed in serotype 19A isolates (unpublished data). In Japan, routine immunization with PCV7 has been recently initiated in 2011, and the reduction in the number of invasive and respiratory infection cases caused by the vaccine-serotype *S. pneumoniae* is anticipated, as has been observed in USA and other countries (2–11). PCV13, however, is not yet available in Japan. The domestic phase III study is still on-going in 2011. This situation raises concern about the rapid replacement of the PCV7 serotypte by non-PCV7 serotypes, as has been observed in USA (2,3,20). Replacement by serotype 19A (ST320), in particular, would be serious because of its high

resistance to penicillin and non-susceptible phenotype to meropenem. The prospective survey of pneumococcal infection in both children and adults, together with intensive laboratory analysis, will be necessary for detecting the very early stage of the replacement. We do anticipate the early introduction of PCV13 in Japan.

Acknowledgments This work is supported by grants from the Ministry of Health, Labour and Welfare of Japan (H21-Shinkou-ippan-002, H21-Shinkou-ippan-008, and H22-Iyaku-shitei-028).

Conflict of interest None to declare.

REFERENCES

1. World Health Organization (2007): Pneumococcal conjugate vaccine for childhood immunization—WHO position paper. Wkly. Epidemiol. Rec., 82, 93–104.
2. Hicks, L.A., Harrison, L.H., Flannery, B., et al. (2007): Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. J. Infect. Dis., 196, 1346–1354.
3. Pilishvili, T., Lexau, C., Farley, M.M., et al. (2010): Sustained reductions in invasive pneumococcal disease in era of conjugate vaccine. J. Infect. Dis., 201, 32–41.
4. Roche, P.W., Krause, V., Cook, H., et al. (2008): Invasive pneumococcal disease in Australia, 2006. Commun. Dis. Intell., 32, 18–30.
5. Kellner, J.D., Vanderkooi, O.G., MacDonald, J., et al. (2009): Changing epidemiology of invasive pneumococcal disease in Canada, 1998–2007: update from Calgary-area *Streptococcus pneumoniae* research (CASPER) study. Clin. Infect. Dis., 49, 205–212.
6. Hsu, H.E., Shutt, K.A., Moore, M.R., et al. (2009): Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. N. Engl. J. Med., 360, 244–256.
7. Lepoutre, A., Varon, E., Georges, S., et al. (2008): Impact of infant pneumococcal vaccination on invasive pneumococcal diseases in France, 2001–2006. Euro Surveill., 13(35):pii = 18962.
8. Dubos, F., Marechal, I., Husson, M.O., et al. (2007): Decline in pneumococcal meningitis after the introduction of the heptavalent-pneumococcal conjugate vaccine in northern France. Arch. Dis. Child., 92, 1009–1012.
9. Verstrheim, D.F., Løvoll, Ø., Aaberge, I.S., et al. (2008): Effectiveness of a 2 + 1 dose schedule pneumococcal conjugate vaccination programme on invasive pneumococcal disease among children in Norway. Vaccine, 26, 3277–3281.
10. Rückinger, S., van der Linden, M., Reinert, R.R., et al. (2009): Reduction in the incidence of invasive pneumococcal disease after general vaccination with 7-valent pneumococcal conjugate vaccine in Germany. Vaccine, 27, 4136–4141.
11. Grijalva, C.G, Nuorti, J.P., Arbogast, P.G, et al. (2007): Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. Lancet, 369, 1179–1186.
12. Sakata, H. (2005): Study of *Streptococcus pneumoniae* bacteremia in children. J. Jpn. Assoc. Infect. Dis., 79, 1–6 (in Japanese).
13. Nishimura, T., Fukuzawa, M., Yoshida, H., et al. (2008): Clinical epidemiological study of occult pneumococcal bacteremia in a pediatric outpatient setting. J. Jpn. Pediatr. Soc., 112, 973–980 (in Japanese).
14. Sakata, H. (2005): A study of bacterial meningitis in Hokkaido between 1999 and 2003. J. Jpn. Assoc. Infect. Dis., 79, 680–687 (in Japanese).
15. Chiba, N., Morozumi, M., Sunaoshi, K., et al. (2010): Serotype and antibiotic resistance of isolates from patients with invasive pneumococcal disease in Japan. Epidemiol. Infect., 138, 61–68.
16. Sakai, F., Chiba, N., Ono, A., et al. (2010): Molecular epidemiologic characteristics of *Streptococcus pneumoniae* isolates from children with meningitis in Japan from 2007 through 2009. J. Infect. Chemother. DOI: 10.1007/s10156-010-0180-3.
17. Chang, B., Otsuka, T., Iwaya, A., et al. (2010): Isolation of *Streptococcus pneumoniae* serotypes 6C and 6D from the nasopharyngeal mucosa of healthy Japanese children. Jpn. J. Infect. Dis., 63, 381–383.
18. Clinical and Laboratory Standards Institute (2008): Performance standards for antimicrobial susceptibility testing; 18th Informational Supplement. Document M100-S18. Clinical and Laboratory Standards Institute, Wayne, Pa., USA.
19. Enright, M.C. and Spratt, B.G. (2008): A mutilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology, 144, 3049–3060.
20. Jacobs, M.R., Good, C.E., Bajaksouzian, S., et al. (2008): Emergence of *Streptococcus pneumoniae* serotypes 19A, 6C, and 22F and serogroup 15 in Cleaveland, Ohio, in relation to introduction of the protein-conjugated pneumococcal vaccine. Clin. Infect. Dis., 47, 1388–1395.