

Fig. 3. Vaccination rates against varicella and its prevalence according to age (17,742 children aged 0–12 years in 20 cities, towns, and villages), in 2005 in Japan.

measles within 1 year before the survey, which means, however, that, more than 30% of children remained unvaccinated until they were 2–6 years old. It is important to strongly prompt parents to vaccinate their children with the MR vaccine as soon as they reach 1 year of age and to complete the vaccination before the age of two.

From the results on the vaccination rate against rubella and its prevalence, the vaccination rate (cumulative) against rubella did not exceed 80% until after 3 years of age, and remained at 85% or lower at all age levels. As a result, children who had contracted rubella were observed at all age levels. The percentage of children vaccinated against rubella within 1 year before the survey was highest, at 30.9% in 1-year-olds. Most children were vaccinated against this disease after the age of 2 years, and some were first vaccinated in elementary school. Parents should be strongly encouraged to vaccinate their children with the MR vaccine after the age of one, and before the age of 2 years.

From the results on the vaccination rate (cumulative) with routine DPT (1 time, 3 or more times) and the incidence rate (cumulative) of pertussis, the vaccination rate with DPT (1 time) was very high, reaching 95% or higher at the age of 2–3 years or above. The percentage of children who had received a DPT-3-or more times reached roughly 80% between the ages of 4–12 years. Consequently, the incidence rate of pertussis in these children, aged 4–12 years was lower (0.0–0.1%) than that (0.2–0.6%) in those aged 0–3 years, and was also lower than that (1.0–1.1%) in those aged 0–12 years who had received DPT one time only.

As shown in Fig. 3, the vaccination rate (cumulative) against varicella (voluntary) was very low, with only 1 of every 4–5 children having been vaccinated before entering elementary school. As a result, outbreaks of varicella recur every year at nursery schools (daycare centers), kindergartens, and elementary schools. Early implementation of free vaccination (routine) is recommended. A peak in prevalence of varicella within 1 year before the survey was observed at the age of 1–2 years. During this period, however, the vaccination rate (9.9% and 8.5% in 2-year-olds and 1-year-olds, respectively) was too low to prevent outbreaks of varicella. It is

important to guide parents in having their children vaccinated as early as possible after the age of 1 year, so as to immunize younger children prior to exposure to epidemic varicella.

From the results on the vaccination rate against mumps and its prevalence, the vaccination rate (cumulative) against mumps was low (<25% among children 3–5 years of age), and only 1 of every 4–5 children had been vaccinated upon admission to elementary school. For this reason, outbreaks of mumps recur every year at nursery schools (daycare centers), kindergartens, and elementary schools. Early implementation of routine vaccination is recommended. The vaccination rate within 1 year before the survey was highest in 2-year-olds, at 9.1%. Since a peak in prevalence is observed between the ages of 3–6 years, routine vaccination of children at the lower ages of 1–2 years old is desirable as a measure to control mumps outbreaks in nursery schools (daycare centers) and kindergartens.

From the results on the vaccination rate against influenza and its prevalence in 2005 (within 1 year), was not low (50% among children 3–6 years of age), compared with that (36.9%) among elementary school children aged 7–12 years old, even though vaccination was being performed voluntarily. However, the incidence rate was higher (31%) in the younger children than in elementary school children (25%). The development of effective vaccines is urgently anticipated.

Regarding the vaccination attitude against measles, rubella, mumps, varicella, and influenza during the past 1 year before the survey, the frequency order of diseases against which 1-year-old children had been vaccinated was, measles (64.2% in Fig. 2), rubella (30.9%), influenza (25.3%), varicella (8.5% in Fig. 3), and mumps (7.4%). Two-year-old children were vaccinated in the frequency order: influenza (47.8%), rubella (23.1%), measles (18.0% in Fig. 2), varicella (9.9% in Fig. 3), and mumps (9.1%). The order of diseases against which children aged 3–6 years were vaccinated was: influenza (45.9–53.6%), rubella (1.3–5.6%), mumps (1.2–5.6%), varicella (0.5–4.6% in Fig. 3), and measles (0.3–3.1% in Fig. 2). The age at which the highest percentage of children were vaccinated was 1 year for measles (64.2% in Fig. 2), and rubella (30.9%), 2 years for

varicella (9.9% in Fig. 3), and mumps (9.1%), and 3 years for influenza (53.6%). The prevalence of the diseases in children aged 0–12 years during the past 1 year ranged in the order of influenza (27.2%), varicella (8.2% in Fig. 3), and mumps (8.0%), but was low for measles (0.1% in Fig. 2), and rubella (0.1%).

In order to achieve prevention of infectious diseases via elevation of the vaccination rate, the (1) diseases that show repeated outbreaks, and high-risk age groups, (2) effectiveness and safety of vaccines against the diseases, and (3) vaccination attitude of the susceptible population should be analyzed, and the obtained results should be used for (1) reevaluation of the vaccination strategy and (2) improvement in cooperation between the local government and physicians who perform vaccinations. In routine vaccinations, people who wish to be vaccinated visit their family doctors during the period stipulated by the law. The MR vaccine was newly introduced on April 1, 2006 due to an amendment of the preventive vaccination law. This amendment requires efforts to vaccinate 1-year-old children before reaching 2 years old with the intention of eradicating measles and rubella, by having most children ($\geq 95\%$) vaccinated against these diseases while 1 year old. However in this questionnaire survey, the vaccination rate during the past 1 year was 64.2% for measles (Fig. 2) and 30.9% for rubella, both of which are low. In the campaign to elevate these vaccination rates to 95% or above by the introduction of the MR vaccine, there may be regional differences in the results depending on the promotional abilities of local governments, which are responsible for organizing vaccination activities. It is our wish that the valuable results of this survey, which could only be performed due to the understanding and voluntary cooperation of parents and local government officials, be used again for infectious disease control with the cooperation of physicians. Further attention to the vaccination rate of 1-year-old children with the MR vaccine in the years ahead is a focus of interest.

This report is an excerpt from the Report of the Preventive Vaccination Study Group, Ministry of Health, Labor and Welfare (March, 2006).

Acknowledgements

The authors wish to express their gratitude to K. Yamanishi for advice and helpful comments, and to C. Sato and P.L. Ogra for editorial review.

Appendix A. Web sites that contain information about vaccines and vaccine preventable diseases

1. National Immunization Program (NIP) (www.cdc.gov/nip)
2. National Vaccine Program Office (NVPO) (www.cdc.gov/od/nvpo)
3. Children's Vaccine Program at PATH (www.childrensvaccine.org)
4. WHO's Vaccine Preventable Diseases Monitoring System (www.who.int/immunization_monitoring/en/globalsummary/countryprofileselect.cfm)
5. American Academy of Pediatrics (AAP) (www.cispimmunize.org)
6. National Foundation for Infectious Diseases (NFID) (www.nfid.org)
7. Varicella-Zoster Virus Research Foundation (VZVRF) (www.vzvrfoundation.org)
8. National Institute of Infectious Diseases (NIID) (www.nih.go.jp)
9. Osaka Prefectural Institute of Public Health (www.iph.pref.osaka.jp)
10. The Research Foundation for Microbial Diseases of Osaka University (www.biken.or.jp/about/outline.html)



Incidence of childhood pneumonia and serotype and sequence-type distribution in *Streptococcus pneumoniae* isolates in Japan

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(Accepted 28 July 2011)

SUMMARY

The 7-valent pneumococcal conjugate vaccine (PCV7) is reported to decrease the incidence of community-acquired pneumonia (CAP) in children. To determine the annual incidence of CAP before the introduction of PCV7, we counted the number of children hospitalized with CAP between 2008 and 2009 in Chiba City, Japan. We investigated serotype and multilocus sequence typing (MLST) for *Streptococcus pneumoniae* isolates in CAP cases. The annual incidence of hospitalized CAP in children aged < 5 years was 17.6 episodes/1000 child-years. In 626 episodes, *S. pneumoniae* was dominant in 14.7% and 0.8% of sputum and blood samples, respectively. The most common serotypes were 6B, 23F and 19F. The coverage rates of PCV7 were 66.7% and 80% in sputum samples and blood samples, respectively. MLST analysis revealed 37 sequence types. Furthermore, 54.1% of the sputum isolates and 40% of the blood isolate were related to international multidrug-resistant clones.

Key words: Antibiotic resistance, community-acquired pneumonia, immunization (vaccination), incidence, *Streptococcus pneumoniae* (pneumococcus).

INTRODUCTION

Streptococcus pneumoniae is a frequent aetiological cause of community-acquired pneumonia (CAP) in children. The 7-valent pneumococcal conjugate vaccine (PCV7), introduced in the USA and Europe, has reduced the incidence of invasive pneumococcal disease (IPD) caused by vaccine serotypes (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) and of carriage of these serotypes [1–4]. Several reports indicate that PCVs

are effective against pneumonia [5–7]. Black *et al.* [5] reported that PCV7 reduces the incidence of first episode of clinically diagnosed pneumonia by 6.0%. The rate of all-cause pneumonia hospitalizations in children in the USA aged < 2 years decreased by about 35% after the vaccine was licensed [6]. In these studies, bacteraemic pneumococcal pneumonia constituted a minority of the total amount of observed clinical pneumonia. These results indicate that PCV not only prevents invasive pneumococcal pneumonia but also reduces the incidence of all-cause pneumonia. However, little is known about the rate of pneumonia attributable to *S. pneumoniae* and their serotypes.

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PCV7 was introduced in Japan in February 2010. Surveillance of the population-based incidence of CAP and molecular characterization of isolates causing CAP are fundamental to understanding the impact of PCV7 and to assessing whether the genetic structure of the pneumococcal population changes after implementation of an immunization programme. However population-based studies of CAP in Japanese children are rare.

To estimate bacterial pathogens and to better manage lower respiratory tract infections in children, we examined microbiological specimens of washed sputum according to the Japanese Guidelines for the Management of Respiratory Infectious Diseases [8–13]. Here, we surveyed the incidence of CAP in hospitalized children to obtain baseline data before the introduction of PCV7. We also determined the isolation rate of *S. pneumoniae* in children with CAP using washed sputum and blood samples to estimate the effect of PCV7 on CAP. The isolates were characterized by serotyping and by multilocus sequence typing (MLST).

METHODS

Incidence of CAP and of CAP with pneumococcal bacteraemia in Chiba City

We determined the annual incidence of hospitalized CAP and CAP with pneumococcal bacteraemia in children aged < 16 years in Chiba City as follows. We retrospectively counted the total number of patients admitted to 18 hospitals with paediatric wards in and around Chiba City serving the catchment population between 1 April 2008 and 31 March 2009. A questionnaire was sent to 18 hospitals and information was obtained from the clinical records of all of them. We defined CAP as pneumonia that occurred in patients who had not been hospitalized within the past 2 weeks. Acute lower respiratory infection was diagnosed by clinicians at each hospital based on clinical symptoms of one or more of: fever, rapid or difficult breathing, cough and crackle in lung fields on auscultation. Radiographs were taken before admission and the diagnosis of CAP was confirmed by clinicians based on positive radiograph findings at the time of occurrence. Patients with CAP who did not require hospitalization were excluded from this study. The catchment area comprised 944 557 inhabitants, including 140 345 and 42 606 children aged < 16 and < 5 years, respectively [14].

Rate of *S. pneumoniae* isolated from sputum and blood

We surveyed children who were admitted to six major hospitals in Chiba City. These six hospitals covered 75% of hospitalized children who were diagnosed with CAP within the city during 2005. [15]. Written informed consent was obtained from the parents or guardians of the patients before collecting samples, in accordance with the guidelines of the Institutional Review Board of Chiba University. Demographic and clinical data were collected by paediatricians. Upon admission, blood samples were collected and sputum samples were obtained using a tongue depressor with a light as follows. The tongue was depressed to induce the cough reflex and then sputum was collected using a swab or aspirated into a 1-ml disposable syringe. Sputum samples were washed three times in sterilized saline as described previously [9]. A small portion of washed sputum was homogenized and smeared onto glass slides for Gram staining. Stained smears were judged valid according to Geckler's classification based on the number of leucocytes or alveolar macrophages and squamous or ciliated epithelial cells per low-power field (100x). Smears with Geckler's groups of 4–5 containing > 25 leucocytes or macrophages and < 25 squamous or ciliated epithelial cells in the low-power microscopic field (100x) were considered adequate. Washed sputum and blood samples were cultured at the microbiology laboratory of each hospital. Pathogens accounting for > 50% of the colonies in culture or presenting > 1×10^7 c.f.u./ml of washed sputum were regarded as 'dominant'. *S. pneumoniae* isolates dominant in sputum samples and/or isolates from blood samples were initially stored at -80°C at each hospital and then sent to Chiba University Hospital and the Department of Bacteriology of the National Institute of Infectious Diseases.

Antimicrobial susceptibility

Antimicrobial susceptibility was tested *in vitro* using broth dilution according to the Clinical and Laboratory Standards Institute guidelines (CLSI M100-S18). Although the CLSI published new breakpoints for penicillin therapy in 2008 (CLSI M100-S18), we used the previously published breakpoints. *S. pneumoniae* was interpreted as susceptible (PSSP), intermediate (PISP), and resistant (PRSP) if the minimum inhibitory concentration (MIC) of penicillin G was ≤ 0.06 , 0.12–1 and ≥ 2 $\mu\text{g/ml}$, respectively.

Serotyping

Serotypes were determined by the Quellung reaction using antiserum purchased from Statens Serum Institut, Copenhagen, Denmark. We serotyped 6C and 6D using an in-house antiserum and confirmed the results by genetic characterization as described previously [16].

MLST

We performed MLST as described previously [17]. Briefly, internal fragments of each of the seven housekeeping genes, *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt* and *ddl* were amplified by Polymerase chain reaction (PCR) and their sequence types (STs) were determined by reference to the MLST database (<http://spneumoniae.mlst.net/>). New alleles and allelic profiles were submitted to the database for assignment. The relatedness of isolates and known similar strains in the database were determined by constructing a neighbour-joining tree using the online program, Draw Tree Using Own MLST Data. Relationships among the isolates were determined using eBURST v. 3 software and strains were assigned to clonal complexes (CCs) using the definition of a stringent group, in which all STs share six of seven identical alleles with at least one of the other STs within the group [single-locus variants (SLV)]. We compared STs with those of 43 pneumococcal clones in the Pneumococcal Molecular Epidemiology Network (PMEN; <http://www.sph.emory.edu/PMEN/>).

Statistical analysis

Data were analysed using PASW Statistics 18 (SPSS Japan Inc., Japan). Associations between underlying diseases and the number of hospitalizations or the results of sputum culture were tested using Fisher's exact test. Correlations between age and isolation rates of pneumococcus and coverage rates of PCV7 were analysed using Pearson's χ^2 test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Annual incidence of CAP

During the study period, CAP caused 860 episodes of children being hospitalized. The incidences of CAP in children aged <16 and <5 years were 6.13 and 17.6/1000 child-years, respectively.

Annual incidence of CAP with pneumococcal bacteraemia

Five patients were diagnosed with pneumococcal bacteraemia combined with CAP. The incidences of CAP with pneumococcal bacteraemia in children aged <16 and <5 years were 3.56 and 11.7 episodes/100 000 child-years, respectively.

Isolation of *S. pneumoniae* from sputum and blood culture

A total of 579 children with 626 episodes were admitted to six major hospitals with a diagnosis of CAP. This corresponded to 73% of all CAP episodes occurring in Chiba City during the study period. We obtained sputum and blood samples representing 502 (80.2%) and 544 (86.9%) of the 626 episodes. *S. pneumoniae* was identified and culture-dominant in 175 (27.9%) and 92 (14.7%) sputum samples, respectively. Of five patients with blood samples that were positive for *S. pneumoniae*, one also had a positive sputum culture. The serotypes and STs of the blood and sputum isolates from this patient were identical (serotype 6B, ST90).

Figure 1 shows the age distribution of children with CAP and the results of *S. pneumoniae* identified in sputum and blood cultures. The median age of children with CAP episodes was 1 year. Patients with CAP included 331 (52.9%) children aged <2 years and 230 (36.7%) aged 2–4 years. The median age of children with pneumococcus-positive episodes was also 1 year. *S. pneumoniae* was isolated from the blood of one 2-year-old and four 1-year-old patients. The detection rate of pneumococcus from sputum was the highest in children aged 2–4 years (18.7%), followed by those aged <2 (11.5%) and 5–15 (16.9%) years [statistically not significant, $\chi^2(2)=5.92$, *P*=0.052].

Of the 579 patients, 215 had one or more underlying diseases, 174 had bronchial asthma, 24 were premature, ten had congenital heart diseases, seven had chromosome anomalies, five had cerebral palsy, and 18 had other diseases. Thirty-seven patients were hospitalized more than once. Multiple hospitalizations were significantly associated with bronchial asthma (*P*<0.001), congenital heart disease (*P*=0.021) and cerebral palsy (*P*=0.035) (Table 1). Underlying diseases and the detection of *S. pneumoniae* from sputum were not significantly related. No one had sequelae with CAP episodes.

Table 1. Risk of multiple hospitalizations with community-acquired pneumonia based on underlying diseases

Underlying disease	No. of multiple hospitalizations/ no. with factors (%)	No. of multiple hospitalization/ no. without factors (%)	<i>P</i> value*	Relative risk (95% CI)
Bronchial asthma	21/174 (12%)	16/405 (4%)	<0.001	3.06 (1.63–5.71)
Premature birth				
<37 weeks†	3/24 (13%)	34/555 (6%)	0.193	1.07 (0.92–1.25)
<30 weeks	1/8 (13%)	36/571 (6%)	0.412	1.98 (0.31–12.74)
Congenital heart disease	3/10 (30%)	34/569 (6%)	0.021	5.02 (1.85–13.67)
Chromosomal anomaly	1/7 (14%)	36/572 (6%)	0.372	2.27 (0.36–14.32)
Cerebral palsy	2/5 (40%)	35/574 (6%)	0.035	6.56 (2.14–20.12)
Other‡	1/18 (6%)	36/561 (6%)	1.000	0.87 (0.13–5.97)

CI, Confidence interval.

* Fisher's exact test.

† Including preterm birth <30 weeks.

‡ Epilepsy (2), neutropenia (2), achondroplasia (1), acute myeloid leukaemia (during consolidation therapy) (1), bronchiectasis (1), congenital diaphragmatic hernia (1), cretinism (1), gastro-oesophageal reflux disease (1), Kawasaki disease (1), mitochondrial diseases (1), periodic fever syndrome (1), polycystic kidney disease (1), post-cleft lip and palate repair (1), Sotos syndrome (1), tracheal stenosis (1), malnutrition (1).

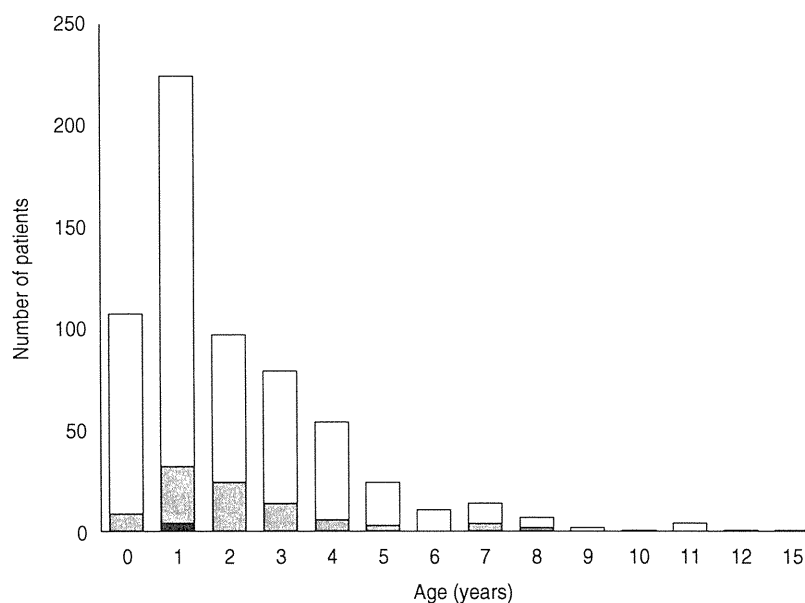


Fig. 1. Age distribution and identification of *Streptococcus pneumoniae* in children hospitalized with community-acquired pneumonia. □, *S. pneumoniae* dominantly isolated from sputum; ■, *S. pneumoniae* isolated from blood ($n=626$).

Antimicrobial susceptibility and serotype distribution

Antimicrobial susceptibility and serotypes were tested against 63/92 cultured sputum isolates in which *S. pneumoniae* was the dominant organism and against five blood isolates. Table 2 shows the susceptibility of the *S. pneumoniae* isolates to penicillin G and their serotype distribution. The rates of PISP and PRSP in sputum isolates were 54% and 22%, respectively, and 40% and 40%, respectively, in the blood

isolates. Resistance rates in sputum isolates against cefotaxime (MIC $\geq 2 \mu\text{g/ml}$), erythromycin (MIC $\geq 1 \mu\text{g/ml}$) and clindamycin (MIC $\geq 1 \mu\text{g/ml}$) were 4.8%, 92% and 60%, respectively, and 20%, 100% and 60%, respectively, in blood isolates. All isolates were susceptible to meropenem and vancomycin.

Of the 17 identified serotypes, the most frequent in the sputum isolates were 6B (28.6%), 23F (17.5%), and 19F (15.9%) and those in the blood isolates were 6B (60%), 19F (20%), and 19A (20%). Serotype 6C

Table 2. Serotype distribution and susceptibility of *Streptococcus pneumoniae* isolated from samples obtained from children with community-acquired pneumonia in Japan

Sample	Coverage rate	Serotype	No. of isolates			
			PSSP	PISP	PRSP	All
Sputum	7-valent (66.7%)	6B	3	9	6	18
		23F		9	2	11
		19F		5	5	10
		14		2		2
		9V	1			1
	10-valent (71.4%)	1	2			2
		7F	1			1
	13-valent (81.0%)	6A		3	1	4
		3	1			1
		19A	1			1
		6C	1	2		3
	Others	23A			2	2
		35B			2	2
		38	2			2
		15B	1			1
		22F	1			1
		24B	1			1
Total		15	34	14	63	
Blood		7-valent (80%)	6B		2	1
	19F				1	1
	13-valent (100%)	19A	1			1
		Total	1	2	2	5

PSSP, Penicillin-susceptible *S. pneumoniae*; PISP, penicillin-intermediate *S. pneumoniae*; PRSP, penicillin-resistant *S. pneumoniae*.

was identified in three sputum isolates. Serotype 6D was not found. The coverage rates of PCV7 were 66.7% and 80.0% in sputum and blood isolates, respectively. The coverage rates in sputum isolates based on age were 65.2%, 73.5% (70.2% in those aged <5 years) and 33.3% in children aged <2, 2-4 and 5-15 years [statistically not significant, $\chi^2(2)=3.74$, $p=0.154$]. Ten-valent (PCV7 plus additional serotypes 1, 5, 7F), 13-valent (PCV7 plus additional serotypes 1, 3, 5, 6A, 7F, 19A) and investigational 15-valent (PCV7 plus additional serotypes 1, 3, 5, 6A, 7F, 19A, 22F, 33F) PCVs would potentially increase the coverage rates of sputum isolates by 4.7%, 14.3% and 15.8%, respectively. The 13-valent and investigational 15-valent PCVs covered all of the blood isolates.

The serotypes of PSSP in sputum isolates widely varied whereas the 14 PRSP sputum isolates fell into only the following serotypes: 6B (42.9%), 19F (35.7%), 23F (14.3%) and 6A (7.1%). The PISP sputum isolates were represented by eight serotypes

with 6B (26.5%), 23F (26.5%), 19F (14.7%) and 6A (8.8%) being the most prevalent. The serotypes of PRSP in blood isolates were also 6B (50%) and 19F (50%), whereas that of PISP isolates was only 6B (100%). The PCV7 and PCV13 coverage rates for PRSP were 92.9% and 100% in sputum, respectively, and 100% in blood isolates.

MLST

MLST was performed on 61/92 sputum isolates in which *S. pneumoniae* was the dominant organism and on five blood isolates. Of the 66 isolates, 37 STs were found including nine new STs (ST5830-5834 and ST5494-5497) with four new alleles. A dendrogram was constructed (Fig. 2) and eBURST analysis revealed six CCs and 25 singletons containing 23 and 43 isolates, respectively. Furthermore, 54.1% and 40% of the sputum and blood isolates had STs identical to 11 international PMEN clones or their SLVs. Eight multidrug-resistant PMEN clones

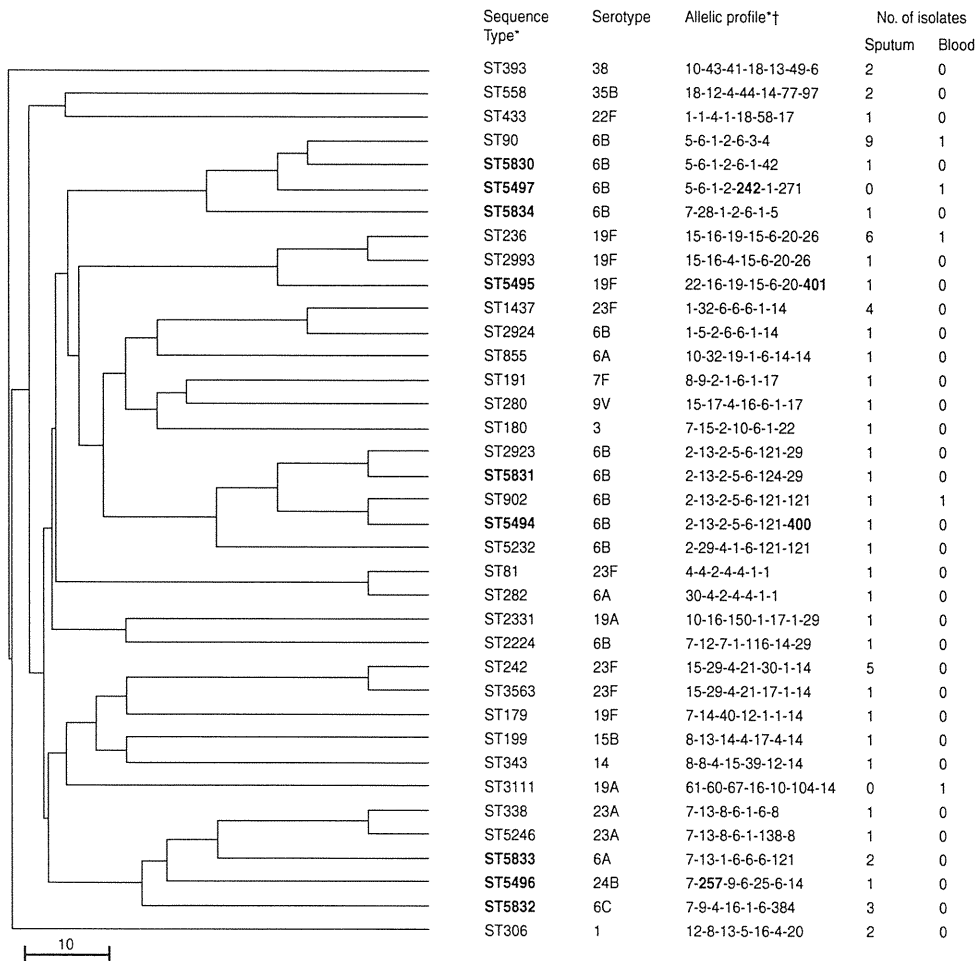


Fig. 2. Genetic relatedness, multilocus sequence-typing profile, and serotypes in 37 sequence types of 66 *Streptococcus pneumoniae* isolates from children with community-acquired pneumonia in Japan. Scale bar indicates genetic linkage distance. PMEN, Pneumococcal Molecular Epidemiology Network. * New sequence types and alleles in bold. † In the order: *aroE-gdh-gki-recP-spi-xpt-ddl*.

comprised Spain^{6B}-2, Taiwan^{19F}-14, Taiwan^{23F}-15, Spain^{23F}-1, Utah^{35B}-24, Colombia^{23F}-26, Portugal^{19F}-21, Greece^{6B}-22 and three susceptible PMEN clones comprised Sweden¹-28, Netherlands^{15B}-37 and Netherlands^{7F}-39. Isolates related to the eight multidrug-resistant PMEN clones comprised 49.1% and 40% of sputum and blood isolates, respectively. Table 3 shows the numbers and antimicrobial susceptibility of isolates with STs identical to multidrug-resistant PMEN clones or their SLVs. Sputum isolates related to multidrug-resistant PMEN clones comprised 62.5% and 71.4% of PISP and PRSP clones, respectively.

DISCUSSION

The annual incidence of CAP in children aged <5 years who were hospitalized with this condition

was 17.6 episodes/1000 child-years and that of CAP with pneumococcal bacteraemia in those aged <5 years was 11.7 episodes/100 000 child-years. In 626 episodes, *S. pneumoniae* was dominant in 14.7% and 0.8% of sputum and blood samples, respectively.

Our findings of the incidence of pneumococcal bacteraemia in children hospitalized with CAP were equivalent to those of our previous survey [15, 18]. The incidence of pneumonia in children aged <5 years that required hospitalization before the introduction of PCV7 in the USA and European countries was 2.25–6.55/1000 child-years [19–21]. The annual incidence of paediatric CAP was higher in the present study than in these reports and similar to that of a study in Germany that included outpatients (13.7–16.9/1000 child-years in children aged <5 years) [22]. The reasons for the variation in incidence

Table 3. Antimicrobial susceptibility of isolates with sequence types identical to multidrug-resistant PMEN clones or their single locus variants

Sequence type	Sero-type	Related PMEN clone	No.	MIC ₅₀ (μg/ml)/(MIC range)								
				PcG	ABPC	CDTR	CTX	MEPM	PAPM	EM	CLDM	VCM
ST90	6B	Spain6B-2 (ST90)	10	1.0 (0.25–2.0)	1.0 (0.25–2.0)	0.50 (0.12–0.50)	0.50 (0.12–0.50)	0.50 (0.50)	0.03 (≤0.008–0.06)	≥8 (≥8)	≥8 (≥8)	0.25 (0.25–0.50)
ST236, ST2993	19F	Taiwan19F-14 (ST236)	8	2.0 (1.0–2.0)	2.0 (1.0–4.0)	0.50 (0.25–2.0)	0.50 (0.25–4.0)	0.25 (0.25)	0.06 (0.03–0.06)	4 (2–≥8)	≤0.12 (≤0.12)	0.25 (0.25–0.50)
ST242, ST3563	23F	Taiwan23F-15 (ST242)	6	1.00 (1.0–2.0)	2.0 (1.00–2.0)	0.50 (0.50–2.0)	0.50 (0.25–2.00)	0.25 (0.12–0.25)	0.03 (0.03–0.06)	≥8 (4–≥8)	≥8 (4–≥8)	0.25 (0.25–0.50)
ST81, ST282	23F 6A	Spain23F-1 (ST81)	2	2.0 (2.0)	2.0 (2.0–4.0)	0.50 (0.50)	0.5 (0.5–1.0)	0.50 (0.50)	0.06 (0.06–0.12)	2 (2–≥8)	≤0.12 (≤0.12–≥8)	0.25 (0.25–0.50)
ST558	35B	Utah35B-24 (ST377)	2	1.0 (1.0)	2.0 (2.0–4.0)	0.25 (0.25–0.50)	0.50 (0.50)	0.25 (0.25)	0.03 (0.03–0.06)	≤0.12 (≤0.12–≥8)	≤0.12 (≤0.12)	0.50 (0.25–0.50)
ST338, ST5246	23A	Colombia23F-26 (ST338)	2	0.25 (0.25–0.5)	0.5 (0.5–1.0)	0.25 (0.25)	0.25 (0.25–0.50)	0.03 (0.03)	≤0.008 (≤0.008)	4 (4)	0.5 (0.5–≥8)	0.25 (0.25–0.50)
ST179	19F	Portugal19F-21 (ST177)	1	1.0	2.0	0.5	0.5	0.03	0.25	≥8	≥8	0.25
ST5830	6B	Greece6B-22 (ST273)	1	1.0	1.0	0.5	0.5	0.06	0.03	≥8	≤0.12	0.5
Other			34	0.12 (≤0.015–2.0)	0.50 (≤0.03–4.0)	0.25 (≤0.03–0.50)	0.25 (≤0.03–0.50)	0.06 (≤0.008–0.25)	≤0.008 (≤0.008–0.06)	≥8 (≤0.12–≥8)	≥8 (≤0.12–≥8)	0.25 (0.25–0.50)

ABPC, Ampicillin; CDTR, cefditoren; CLDM, clindamycin; CTX, cefotaxime; EM, erythromycin; MEPM, meropenem; MIC, minimum inhibitory concentration; PAPM, panipenem; PcG, penicillin G; PMEN, Pneumococcal Molecular Epidemiology Network; VCM, vancomycin.

rates in countries might include differences in access to healthcare, willingness to hospitalize patients and the costs of admission. Free access to any hospital or clinic is guaranteed in Japan, and the costs of almost all medical care for children up to age 6 years in Chiba City are compensated by local government. Thus, most children with CAP in Chiba City, and infants in particular, were treated in hospital.

Another possible reason for the higher incidence is differences in the definition of pneumonia. Pneumonia is usually diagnosed in Japan based on clinical signs and chest radiographic findings confirmed by clinicians, not radiologists. A World Health Organization (WHO) working group developed a method for standardizing the interpretation of chest X-rays of children for epidemiological purposes [23]. Pneumonia was diagnosed by clinicians at each hospital in the present study and included not only WHO-confirmed end-point pneumonia but also other radiographic findings. The incidence of CAP with end-point pneumonia according to WHO standards has yet to be determined.

Identifying the aetiology of childhood CAP is difficult because of the lack of accurate, non-invasive tests. The diagnostic yields of sputum culture are limited by potential contamination from the upper respiratory tract. However, the reliability of microbiological sputum tests can be improved by washing. Bartlett & Finegold [24] showed that washing sputum decreases the number of contaminants by 100- to 1000-fold and does not result in a qualitative and quantitative loss of bacteria recovered in percutaneous transtracheal aspirates. Combining quantitative culture with washing sputum specimens enhances the value of findings. We combine a washing technique with semi-quantitative culture to evaluate pathogenic bacteria [8, 9]. We applied this method with serology to determine the aetiology of CAP in 596 hospitalized children between 1990 and 1991 [25] and identified pathogens in 64.4% of them. Evidence of bacterial, *Mycoplasma pneumoniae* and viral (mostly respiratory syncytial virus) infection was found in 28.8%, 14.9% and 29.9%, respectively, of these children. Two major bacterial pathogens were *Haemophilus influenzae* (19.6%) and *S. pneumoniae* (8.6%). The major pathogens defined in this study were consistent with a study of 1700 Japanese paediatric patients with CAP using real-time reverse transcription-PCR [26]. Moreover, the clinical responses to antibiotics administered based on the results of sputum culture are good [10, 11].

Of the 626 episodes in six hospitals examined in the present study, *S. pneumoniae* was identified as the causative pathogen in 96 (15.3%) episodes. Five and 92 were identified from blood and sputum cultures, respectively, including one that tested positive in both cultures. The rate of infection with pneumococcus (15.3%) was similar to that described in a study from Turkey (17.1%) that used washing and quantitative sputum cultures [27], and with a study from Italy (17.8%) that used serological assays with paired sera [28]. However, the findings were relatively lower than those in a study from the USA using pneumolysin-based PCR (44%) [29], even when all *S. pneumoniae* isolates identified in sputum (27.9%) were taken into account. One of the limitations of the present study is the absence of information about previous antibiotic use. The low rate of *S. pneumoniae* detection herein compared with PCR might be related to previous use of antibiotics, which is frequent in Japan.

We could not identify a relationship between underlying disease and the detection of *S. pneumoniae*. However, patients with asthma, congenital heart disease, and cerebral palsy had multiple hospitalizations for CAP. The only vaccines for the prevention of bacterial pneumonia (excluding pertussis) are *H. influenzae* type b and pneumococcal vaccines. Therefore, such patients should be recommended for immunization with these vaccines, both of which are elective in Japan.

The incidence of *S. pneumoniae* that is not susceptible to penicillin has rapidly increased in Japan since around 1990 [30]. The rate of PRSP in the present study was as high as that in previous studies of IPD [18] and acute otitis media (AOM) [31] in Japanese children. The frequency of STs related to multi-resistant PMEN clones was also high in the present study. The spread of these clones might be responsible for the high rate of resistant strains developing in Japanese children.

The most prevalent serotype in sputum isolates of children with CAP was 6B, followed by 19F and 23F. The high prevalence of these serotypes was the same as that in a report describing IPD in Japanese children [32]. The overall PCV7 coverage rates in sputum and blood were 66.7% and 80%, respectively. These rates in children aged <5 years were 70.2% and 80%, respectively. PCV7 coverage of bacteraemic pneumonia was equal to that of IPD in the USA and Europe before the introduction of PCVs [33]. However, serotype 14, which is the most common serotype in the USA

and Europe preceding PCVs [34], and serotype 1, which has been predominant in complicating pneumonia before and after PCVs became available [35], were undetectable in our blood culture. Information about non-bacteraemic pneumonia serotypes is limited. The coverage rates of PCV7 in patients aged <5 years with respiratory infections determined using oropharyngeal swab samples in Vietnam, hypopharyngeal aspirates in China and nasopharyngeal isolates in Switzerland were 88.7% [36], 76.3% [37], and about 70% (<2 years) [38], respectively, with serotype 19F being the most frequent.

PCV7 coverage rates for PRSP were 92.9% and 100% in sputum and blood isolates, respectively. We hope that PCV7 will reduce the incidence of respiratory tract infections, especially those caused by strains that are less susceptible to penicillin.

Serotypes have changed in countries where PCV7 has been introduced as routine immunization and the emergence of serotype 19A with multidrug resistance has become a problem [1, 2, 39–41]. ST199 and ST320 are the major STs found in these countries. Here, we found only serotype 19A *S. pneumoniae* with ST2331. One isolate had ST199 but its serotype was 15B.

The incidence of serotype 6C, which was distinguished from serotype 6A in 2007 [42], also increased after the introduction of PCV7 [43]. Serotype 6C was isolated from <2% of children with IPD and from 9.5% of samples from the nasopharyngeal mucosa of healthy children in Japan [16]. We identified three 6C isolates from sputum (4.8%) with the new sequence type ST5832 in our patients with CAP.

Some limitations of this study should be considered. This study covered only a 1-year period and therefore does not account for annual variations in either the incidence of disease or the detected serotypes. In addition, information about previous antibiotic administration was not available.

PCV7 was introduced as an elective vaccine in Japan in February 2010. New PCVs, especially 13-valent and the investigational 15-valent types, would potentially increase the coverage rate of sputum isolates. Switching to these new PCVs should be considered with the increase of non-vaccine serotype. Continued surveillance to detect changes in the incidence of CAP caused by pneumococci, their antimicrobial resistance, serotypes and genotypes are crucial for evaluating the impact of PCV7 and to effectively prevent pneumococcal infections.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the participants in the Chiba Paediatric Infectious Disease Meeting, namely Fumie Nagai, Masanaga Arima, Katuaki Abe, Hisae Kazukawa, Chie Fukasawa, Tadashi Hoshino, Jiro Aizawa, Hiroko Oshima, Nobuyasu Ishikawa and Yoshio Kori in the planning and implementation of this study. The authors are also grateful to the paediatricians in and around Chiba City who contributed toward this study. This study was financially supported by the Japanese Ministry of Health, Labour and Welfare grants for Research on the Mechanism, Epidemiology, Prevention and Control of Acute Respiratory Infections (H22-iyaku-shitei-028), Research on Regulatory Science of Pharmaceuticals and Medical Devices (H22-kokui-shitei-009) and the Research Project for Emerging and Re-emerging Infectious Disease (H21-shinkou-ippan-008 to A.W.).

DECLARATION OF INTEREST

None.

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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

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(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

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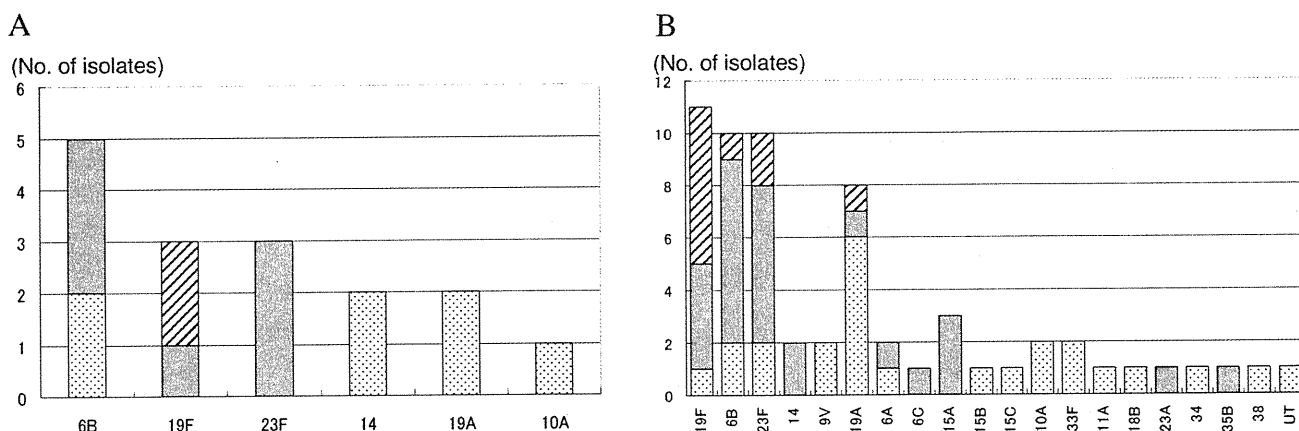


Fig. 1. Relationship between serotype and penicillin G resistance categories for *S. pneumoniae* isolates from (A) blood and (B) posterior nares. Shaded bar, gray bar, and dotted bar represent PRSP (penicillin G MIC ≥ 2 $\mu\text{g}/\text{mL}$), PISP (penicillin G MIC 0.12–1 $\mu\text{g}/\text{mL}$), and PSSP (penicillin G MIC ≤ 0.06 $\mu\text{g}/\text{mL}$), 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 $\mu\text{g}/\text{mL}$; all the isolates were ST236 or ST115. The MIC of penicillin G for a nasopharyngeal serotype 19F isolate with ST257 was 0.03 $\mu\text{g}/\text{mL}$. 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 $\mu\text{g}/\text{mL}$, 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 $\mu\text{g}/\text{mL}$, 0.5 $\mu\text{g}/\text{mL}$, and 0.12 $\mu\text{g}/\text{mL}$, 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 ST902 ST5846 ²⁾	6, 2 1 1	ST2983, DLV of ST90 (<i>xpt</i> , <i>ddl</i>)
19F	ST236, ST115	2, 1	ST236, ST257	10, 1	ST115, DLV of ST236 (<i>spi</i> , <i>ddl</i>) ST257, DLV of ST236 (<i>aroE</i> , <i>ddl</i>)
23F	ST1437	3	ST1437, ST5845 ²⁾ ST242, ST5841 ²⁾ , ST5844 ²⁾	2, 1 5, 1, 1	ST5845, SLV of ST1437 (<i>gdh</i> , <i>gki</i>) ST5841, DLV of ST242 (<i>gdh</i> , <i>recP</i>) ST5844, SLV of ST242 (<i>xpt</i>)
14	ST13	2	ST13, ST2922 ST5240 ³⁾	1, 1 1	ST2922, SLV of ST13 (<i>xpt</i>)
19A	ST3111	2	ST3111 ST320 ST5842 ²⁾	6 1 1	
10A	ST6412 ²⁾	1	ST1263 ST5236 ST3787	1 1 2	
6A			ST5241	1	
6C			ST280	2	
9V			ST63	3	
15A			ST199	1	
15B			ST5843 ²⁾	1	ST5843, SLV of ST199 (<i>spi</i>)
15C			ST5840 ²⁾	2	
33F			ST99	1	
11A			ST3594	1	
18B			ST5246	1	
23A			ST3116	1	
34			ST558	1	
35B			ST393	1	
38			ST1106	1	
Untypeable					

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 serotype 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

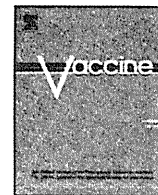
resistance to penicillin and non-susceptible phenotype to meropenem. The prospective survey of pneumococcal infection in both children and adults, together with intensive laboratory analysis, will be necessary for detecting the very early stage of the replacement. We do anticipate the early introduction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

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My continuing efforts toward the eradication of the vaccine-preventable diseases from Japan

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ARTICLE INFO

Article history:

Received 14 April 2011

Received in revised form 20 May 2011

Accepted 20 May 2011

Available online 28 July 2011

Keywords:

Measles

Monoclonal antibody

Influenza

Encephalopathy

Haemophilus influenzae

Streptococcus pneumoniae

ABSTRACT

When compared to the Western countries, unfortunately, the progress toward the eradication of vaccine-preventable diseases in Japan has been relatively slow and hampered by some problems inherent to Japan. Concerning this, I have been working on the vaccine-preventable diseases, that is, measles, influenza, *Haemophilus influenzae*, and *Streptococcus pneumoniae* infections. My first work was to produce monoclonal antibodies against measles virus proteins, and by using these antibodies, I established the immunofluorescent measles virus detection system in tissue samples. I constructed the 'Hokkaido measles-zero strategy' to eliminate measles from Hokkaido prefecture, the northernmost island of Japan, within 5 years since 2001 with very kind efforts of pediatricians in Hokkaido and those who are engaged in political activities. Coworkers and I established the disease entity of influenza-associated encephalopathy. And finally, I worked as a member of some groups that accumulated the basic data on the occurrence of bacterial meningitis in Japan and urged the government to introduce the *H. influenzae* type b conjugate vaccine and the pediatric 7-valent pneumococcal conjugate vaccine in Japan. I would like to express many thanks to all of my colleagues for their contributions to these achievements.

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1. Introduction

When compared to the Western countries, unfortunately, the progress toward the eradication of vaccine-preventable diseases in Japan has been relatively slow and hampered by some problems inherent to Japan. Concerning this, I have been working on the vaccine-preventable diseases, that is, measles, influenza, *Haemophilus influenzae*, and *Streptococcus pneumoniae* infections. In this opportunity I summarize my works on these diseases and hope that this article provides readers with some useful information.

2. Measles

2.1. Production of monoclonal antibodies against virus proteins by use of the mouse hybridoma technique

My first work was to produce monoclonal antibodies against measles virus proteins under the instruction of professor Erling Norrby of Karolinska Institute [1]. Briefly, mouse hybridoma cell lines were produced by fusion of P3x63 Ag8 myeloma cells with spleen cells collected from BALB/c mice immunized with

purified measles virions. Selected measles antibody producing hybridoma cell lines were passaged intraperitoneally in mice, and ascites fluids were collected. Seventeen lines produced high titers of hemagglutination inhibiting and hemolysis-inhibition antibodies. Homogenous immunoglobulin products carrying measles antibody activity were demonstrated by imprint immunoelectrophoresis of ascites materials. A representative result using hemagglutinin-highly specific clones 02 and 03 is shown in Fig. 1.

2.2. Immunofluorescent staining of lung tissues from patients with measles pneumonia using monoclonal antibodies

By use of the monoclonal antibodies which are mentioned above, I established the immune fluorescent measles virus detection system using tissue samples [2]. A representative result is presented in Fig. 2, which shows a successful detection of measles virus antigens in a lung tissue from a patient with a primary immunodeficiency syndrome who died of giant cell pneumonia. In this context, I found that the measles antigens cannot be detected in a lung tissue of an immunocompetent patient with interstitial, but not giant cell, pneumonia (Fig. 3, data unpublished). This result clearly demonstrated that the measles pneumonia in an immunocompetent host, unlike giant cell pneumonia in an immunocompromised host, is not a result of direct invasion by the virus itself.

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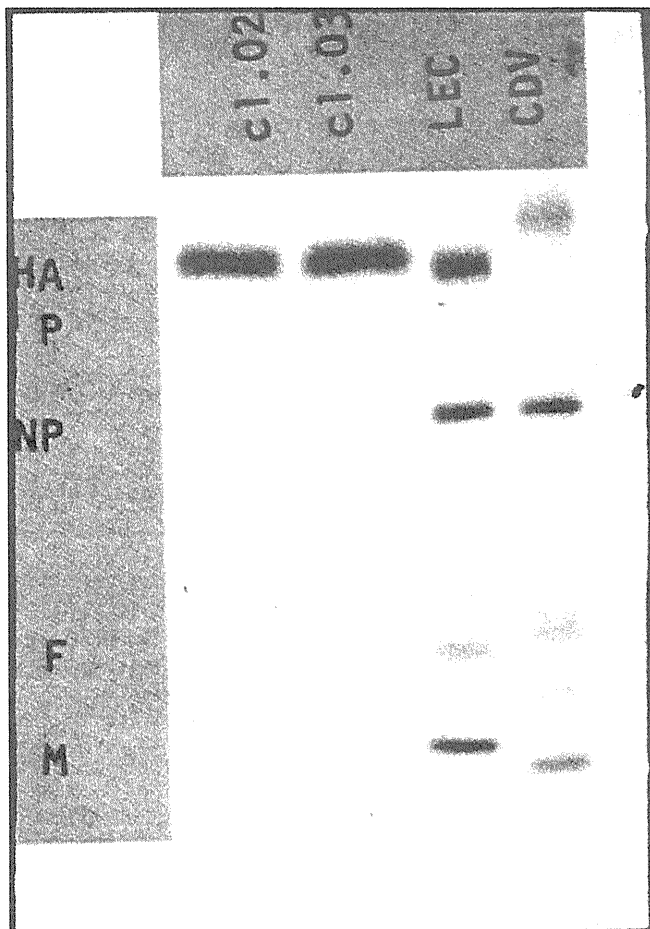


Fig. 1. Detection of HA antigen of measles virus by immunoprecipitation with using monoclonal antibody produced in hybridoma clones 02 and 03. HA; hemagglutinin, NP; nucleoprotein, F: fusion, M; matrix. LEC; measles virus reference strain. CDV; canine distemper virus.

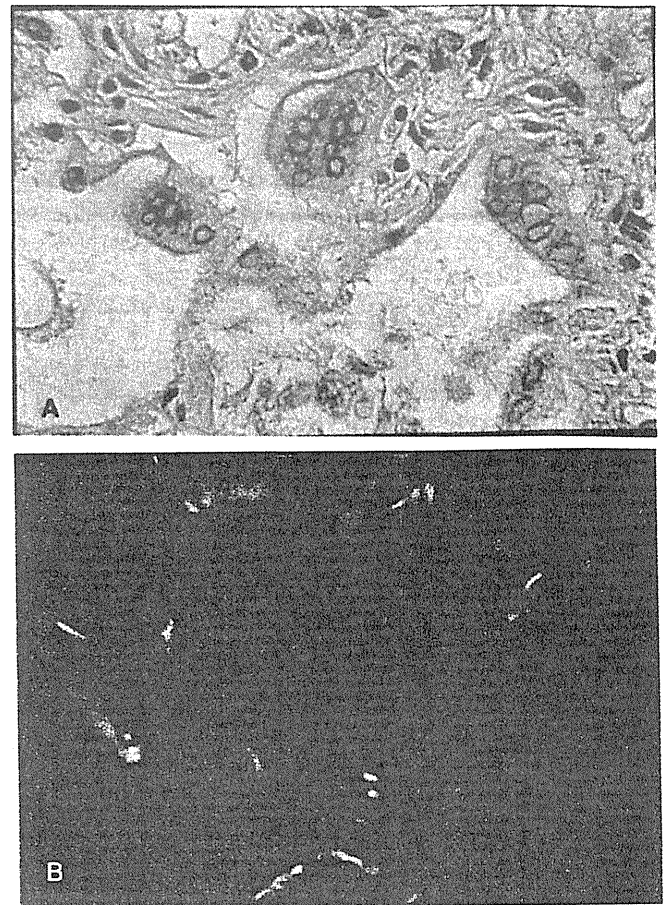


Fig. 2. Histological analysis of the lung tissue section from giant cell pneumonia. A; Hematoxylin–eosin staining. B; Immunofluorescent staining using monoclonal antibody against hemagglutinin of measles virus (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.3. 'Hokkaido measles-zero strategy'

Unfortunately it has been well known that Japan is nowadays one of the disgraceful countries, which 'export' measles. In this point, I decided to accomplish to eliminate measles firstly from Hokkaido prefecture, where I was living, and created a plan, the 'Hokkaido measles-zero strategy', to eliminate measles from Hokkaido within 5 years in 2001 with very kind efforts of pediatricians in Hokkaido and those who were engaged in political and administrative activities. The result is shown in Fig. 4. The attempt, although initially looked successful until 2006, to my great regret, resulted in failure because of the fact that measles, which was transported from Tokyo to Hokkaido by a patient with measles during an incubation period, subsequently prevailed in Hokkaido in 2007. Secondary vaccine failure was a dominant reason for the outbreak. Since 2006 multiple (two) dose administration policy with combined measles-rubella vaccine has started in Japan. It is highly expected that measles will be eliminated from Japan in the near future.

3. Establishing the disease entity of influenza-associated encephalopathy

I encountered a peculiar case of encephalopathy, which occurred in a healthy 4-year-old boy with a fatal outcome in 32 h from the onset of fever to his death, during an influenza epidemic of 1994. I myself saw the patient at the Sapporo City General

Hospital and felt instinctively at that time that the case was a distinct entity from previously known Reye syndrome or febrile convulsion (Fig. 5), and coworkers and I started a survey. We sent inquiries to 94 hospitals and institutes, in which pediatric age patients were treated in Hokkaido prefecture, asking if there were any hospitalized cases of acute onset brain dysfunction of unknown etiology during the current winter season (1994/95), irrespective of whether influenza was initially suspected or not. As a result, a total of 12 such cases were reported. As shown in Fig. 6, the peak incidence of the acute onset brain dysfunction was fairly coincident with the peak isolation of influenza virus from outpatients in the Sapporo City General Hospital, as well as the peak of cases of influenza-like illness reported weekly in Hokkaido. Since a very similar study result was again obtained in the 1995/96 winter season, we submitted the first report concerning 'influenza-associated encephalopathy' in 1996 to a scientific medical journal. However, unfortunately, the paper had not been accepted for publication by any journals published outside Japan. The paper was finally accepted for publication in 2000 in the journal "Pediatrics International" which is published by the Japan Pediatric Society. The survey on the 5 successive seasons (1994/95–1998/99) were described in the paper [3]. Subsequently, coworkers and I have reported that the vasogenic brain edema with generalized impairment of vascular endothelial cells caused by highly activated cytokines (i.e., 'cytokine storm') plays a central role in the pathophysiology of the disease entity (Fig. 7), through the journals which are published outside Japan [4,5]. Influenza-associated encephalopathy, for

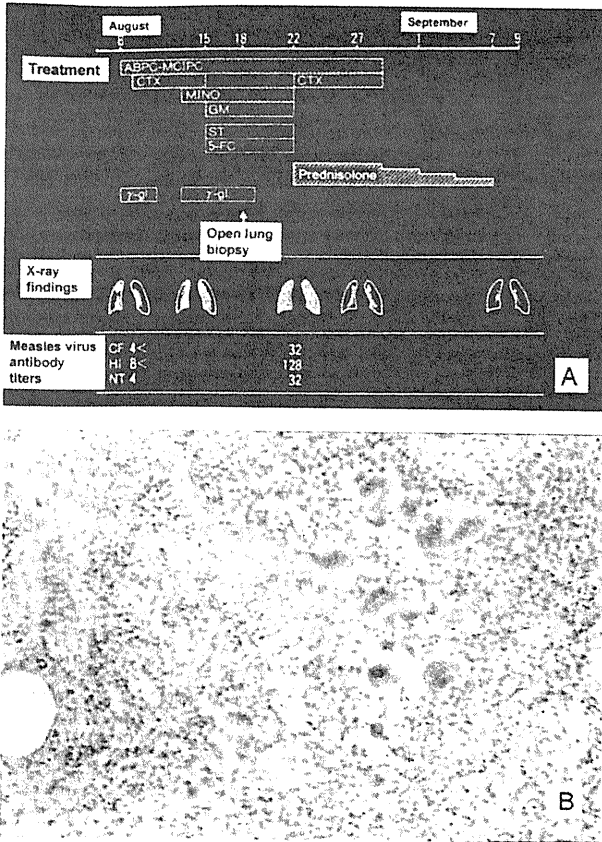


Fig. 3. Clinical course of a patient with measles pneumonia without immunodeficiency (A). Lung tissue of this patient (hematoxylin–eosin staining, B). No measles virus antigens were detected by immunofluorescent staining using the monoclonal antibodies in this case.

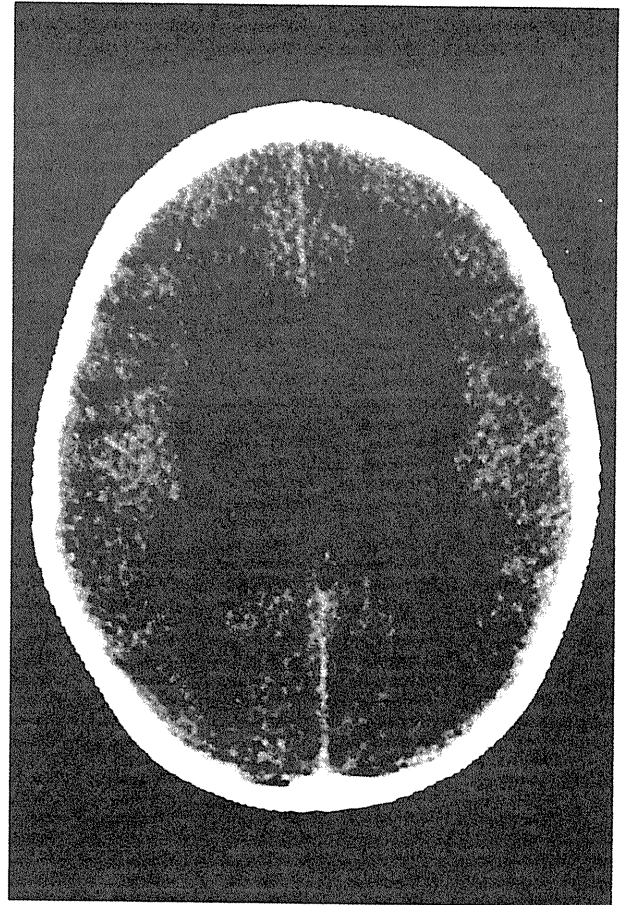


Fig. 5. A representative imaging by computerized tomography scanning of a case with influenza-associated encephalopathy which shows symmetrical low density areas in the bilateral thalami.

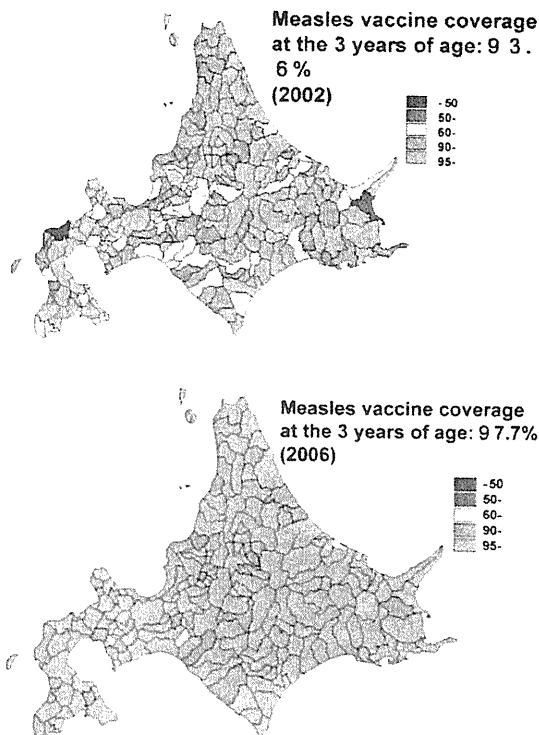


Fig. 4. Attempt to increase the measles vaccine coverage in Hokkaido ('Hokkaido measles-zero strategy'). The vaccine coverage increased from 93.6% in 2002 (upper panel) to 97.7% in 2006 (lower panel).

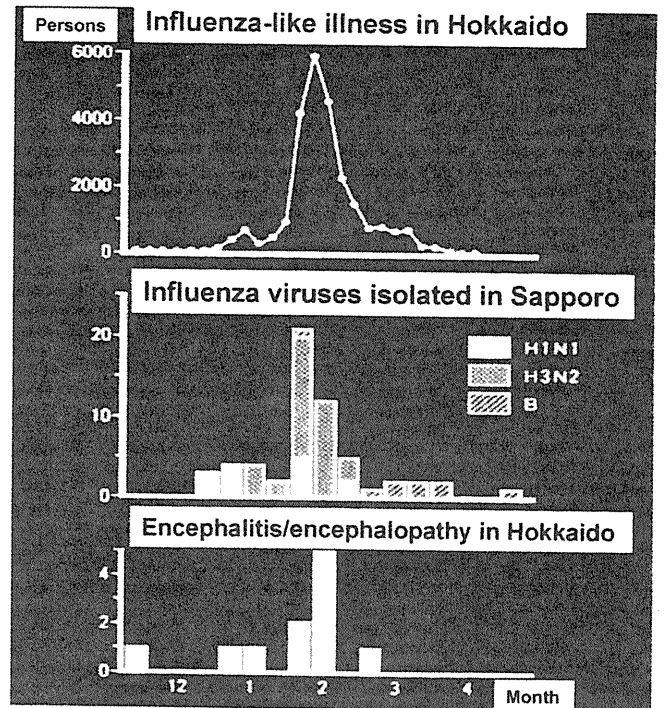


Fig. 6. Influenza epidemic, virus isolation, and encephalopathy during the 1994/95 season in Sapporo city, Hokkaido prefecture. Sapporo city is a Prefectural capital of Hokkaido prefecture.