TABLE 2. SENSITIVITES AND SPECIFICITIES OF REAL-TIME PCR COMPARED WITH THOSE CONVENTIONAL PCR

Genotype	*Real-time PCR*	*Conventional PCR (%)*		*Total no. of samples*
		Positive	*Negative*	
gPSSP	Positive	67 (98.5)[a]	0 (0.0)	67
	Negative	1 (1.5)	232 (100.0)[b]	233
	Total	68	232	300
gPISP (*pbp2x*)	Positive	75 (96.2)	1 (0.5)	76
	Negative	3 (3.8)	221 (99.5)	224
	Total	78	222	300
gPISP (*pbp2b*)	Positive	22 (100.0)	0 (0.0)	22
	Negative	0 (0.0)	278 (100.0)	278
	Total	22	278	300
gPISP (*pbp1a+2x*)	Positive	31 (100.0)	3 (1.1)	34
	Negative	0 (0.0)	266 (98.9)	266
	Total	31	269	300
gPISP (*pbp2x+2b*)	Positive	14 (73.7)	0 (0.0)	14
	Negative	5 (26.3)	281 (100.0)	286
	Total	19	281	300
gPRSP (*pbp1a+2x+2b*)	Positive	82 (100.0)	5 (2.3)	87
	Negative	0 (0.0)	213 (97.7)	213
	Total	82	218	300

[a]Sensitivity.
[b]Specificity.

vaccine (PPV23)[5] began in the early 1980s in the United States, and this vaccine was introduced in Japan in 1988. In Japan, 7-valent pneumococcal conjugate vaccine (PCV7) has just been approved on a voluntary basis to prevent IPD among children with immunologic immaturity.

In countries where PCV7 has been introduced into the vaccine schedule, incidence of pediatric IPD caused by vaccine-type strains has decreased significantly,[4,6,22] while a related decrease of IPD among adults also has been reported.[17] However, prevalence of IPD caused by serotypes 19A and 6A (nonvaccine types) has increased, accompanied by a shift from PEN-susceptible to PEN-resistant strains.[7,11,21] Some investigators also have reported that overall incidence of IPD is little changed.[26]

In Japan, great clinical attention has been paid to the increase of PRSP and PISP among *S. pneumoniae* isolates from IPD,[8] which strongly reflects the difference in use of oral antibiotics between pediatricians and internists. Specifically, in pediatric practice, oral cephalosporins are favored over penicillins for outpatients, although a recent shift back toward amoxicillin and AMP has been noted. On the other hand, in internal medicine, ML and fluoroquinolone agents rather than β-lactam antibiotics are preferred. This might contribute significantly to rates of

TABLE 3. DETAILS OF 9 STRAINS SHOWING A DISCREPANCY IN RESULTS IN BETWEEN REAL-TIME PCR AND CONVENTIONAL PCR

No of strain	*Genotype*		*MIC (mg/L)*					*Serotype*	*ST*	*CC*
	Conventional PCR	*Real-time PCR*	*PEN*	*AMP*	*CTX*	*MEM*	*PAM*			
Ref R6	gPSSP	gPSSP	0.016	0.016	0.016	0.008	0.004	—	—	—
RS-009	gPISP(*pbp2x*)	gPISP(*pbp1a+2x*)	0.125	0.5	1	0.031	0.008	14	13	15
RS-027	gPISP(*pbp2x*)	gPISP(*pbp1a+2x*)	0.125	0.5	1	0.031	0.008	6B	385	156
RS-083	gPISP(*pbp2x*)	gPISP(*pbp1a+2x*)	0.125	0.5	1	0.031	0.004	6B	2983	156
RS-046	gPISP(*pbp2x+2b*)	gPRSP	0.5	1	2	0.063	0.016	14	343	554
RS-101	gPISP(*pbp2x+2b*)	gPRSP	0.5	2	2	0.063	0.016	14	343	554
RS-193	gPISP(*pbp2x+2b*)	gPRSP	0.5	1	0.5	0.063	0.016	14	343	554
RS-311	gPISP(*pbp2x+2b*)	gPRSP	0.5	1	2	0.125	0.016	14	343	554
RS-065	gPISP(*pbp2x+2b*)	gPRSP	1	4	1	0.25	0.031	6B	6939	None[a]
RS-208	gPSSP	gPISP(*pbp2x*)	0.063	0.125	0.125	0.016	0.004	6A	4542	156

When DNA amplification occurred, the corresponding *pbp* gene showed the same sequences as the susceptible strain; for example, a strain showing amplification of *pbp1a* and *pbp2b* genes was designation gPISP(pbp2x).
[a]ST6939 is not present in any group of clonal complexes.
MIC, minimum inhibitory concentration; PEN, penicillin; AMP, ampicillin; CTX, cefetaxime; MEM, meropenem; PAM, panipenem; ST, sequence type; CC, clonal complex; gPSSP, genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP, genotypic penicillin-intermediate *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*.

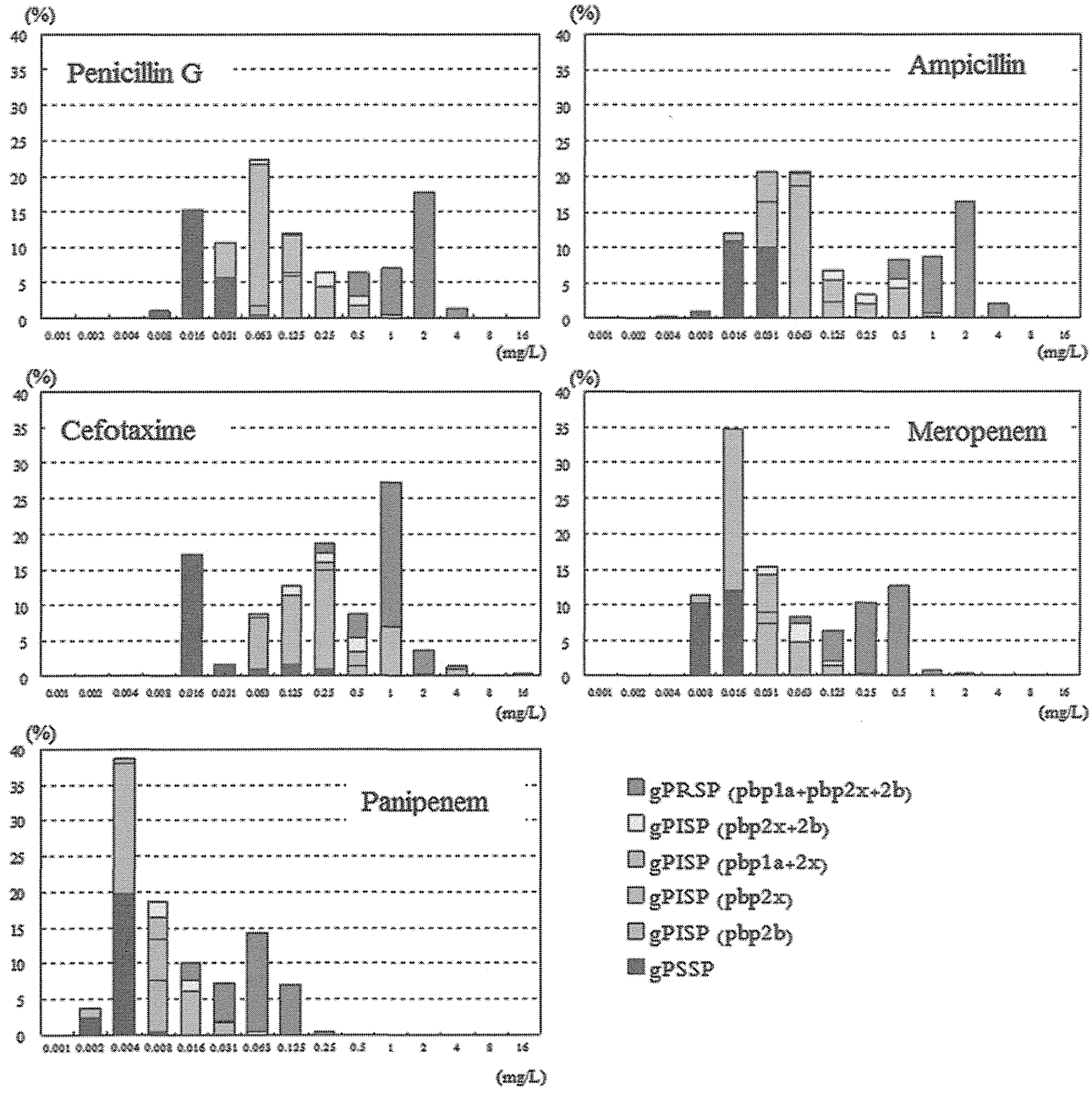

FIG. 2. Correlation between minimal inhibitory concentrations (MICs) of five β-lactam antibiotics and penicillin-binding protein (PBP) gene alterations for 300 *Streptococcus pneumoniae* isolates from invasive infections.

gPRSP isolated from pediatric patients and gPISP isolated from adult patients.

These situations concerning antibiotic resistance, in addition to the present state of pneumococcal vaccination, show that a need for rapid and accurate determination of resistance in clinical isolates is necessary for appropriate selection of chemotherapeutic agents in pneumococcal infections.

We initially identified species and antibiotic resistance using colony samples likely to be *S. pneumoniae* from blood agar plate using a conventional PCR method completed within 2.5 hr using gel electrophoresis.[30] Intrinsically, three primer sets designed on *pbp1a*, *pbp2x*, and *pbp2b* genes detect the most important amino acid substitutions affecting β-lactam susceptibilities, all positioned within or adjacent to conserved amino acid motifs in each PBP—substitutions from STMK to SAMK or SSMK in PBP1A, substitutions from STMK to SAMK or SAFK and from (L)KSG to (V)KSG in PBP2X, and substitution from SSN(T) to SSN(A) or SSN(S) in PBP2B. The genotypic resistance pattern based on the *pbp* gene analysis was divided into six categories: gPSSP, gPISP(*pbp2x*), gPISP(*pbp2b*), gPISP(*pbp1a*+*pbp2x*), gPISP (*pbp2x*+*pbp2b*), and gPRSP (*pbp1a*+*pbp2x*+*pbp2b*).

This was not shown in the results, but each class of resistance genes was not of a single clone. For example, gPRSP was divided into 11 serotypes with various clonal complexes (CCs). The major serotypes and CCs were serotype 6B with

TABLE 4. ESTIMATED MIC_{50s} AND FITTING RANGES OF 90% OF β-LACTAM ANTIBIOTICS FOR 6 PBP GENOTYPE CLASSES

Genotype	n	Estimated MIC (mg/L)				
		PEN	*AMP*	*CTX*	*MEM*	*PAM*
gPSSP	67	0.016 (0.016–0.031)	0.016 (0.016–0.031)	0.016 (0.016–0.125)	0.016 (0.008–0.016)	0.004 (0.002–0.004)
gPISP (*pbp2b*)	22	0.125 (0.063–0.125)	0.031 (0.016–0.031)	0.063 (0.063)	0.031 (0.031)	0.008 (0.008)
gPISP (*pbp2x*)	76	0.063 (0.031–0.063)	0.063 (0.031–0.063)	0.25 (0.125–0.25)	0.016 (0.016–0.031)	0.004 (0.002–0.008)
gPISP (*pbp1a+2x*)	34	0.25 (0.125–0.5)	0.25 (0.063–0.5)	1 (0.25–2)	0.063 (0.031–0.125)	0.016 (0.008–0.031)
gPISP (*pbp2x+2b*)	14	0.25 (0.063–0.5)	0.25 (0.063–0.5)	0.25 (0.125–0.5)	0.063 (0.031–0.125)	0.016 (0.008–0.031)
gPRSP (*pbp1a+pbp2x+2b*)	87	2 (0.5–2)	2 (0.5–2)	1 (0.5–2)	0.5 (0.125–0.5)	0.063 (0.031–0.125)

MIC, minimum inhibitory concentration; PEN, penicillin; AMP, ampicillin; CTX, cefetaxime; MEM, meropenem; PAM, panipenem; gPSSP, genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP, genotypic penicillin-intermediate *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*.

CC156 and CC490, serotype 19F with CC320, serotype 23F with CC156, CC242 and CC1437, serotype 6A with CC3115, CC3787 and CC81, and serotype 14 with CC320 and CC554.

As stated in the Results section, real-time PCR yielded satisfactory sensitivity and specificity compared with conventional PCR. Accurate estimation of MICs of each β-lactam antibiotic on the basis of genotypic patterns is highly important. Our novel real-time PCR assay also can be completed within 90 min after selection of colony samples, with elimination of gel electrophoresis, saving both time and labor.

Another merit of this assay is possible direct testing of usually sterile specimens (such as cerebrospinal fluid, joint fluid, and pleural fluid) from IPD patients, because primers and MB probes for amplification of the *lytA* gene are included in the real-time PCR. In the future, simultaneous performance of speciation and identification of resistance gene(s) by real-time PCR should optimize cost and benefit in clinical settings.

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

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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 $>1\times10^7$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 $\leqslant0{\cdot}06$, $0{\cdot}12$–1 and $\geqslant2\,\mu$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 $<0{\cdot}05$ 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, $\chi^2(2)=5{\cdot}92$, $P=0{\cdot}052$].

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 ($P<0{\cdot}001$), congenital heart disease ($P=0{\cdot}021$) and cerebral palsy ($P=0{\cdot}035$) (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 (%)	*P* 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).

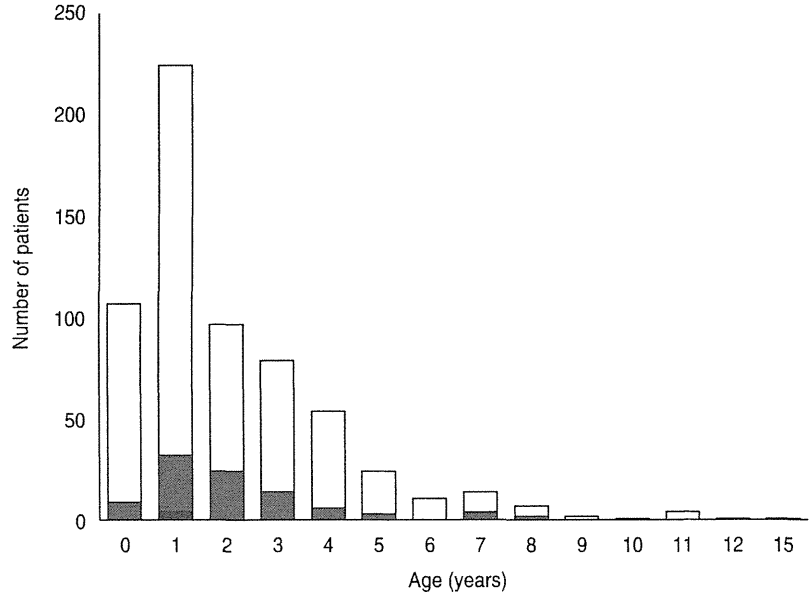


Fig. 1. Age distribution and identification of *Streptococcus pneumoniae* in children hospitalized with community-acquired pneumonia. ■, *S. pneumoniae* dominantly isolated from sputum; ■, *S. pneumoniae* isolated from blood ($n=626$).

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 $\geqslant 2\,\mu g/ml$), erythromycin (MIC $\geqslant 1\,\mu g/ml$) and clindamycin (MIC $\geqslant 1\,\mu g/ml$) 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*

			No. of isolates			
Sample	Coverage rate	Serotype	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
	Others	6C	1	2		3
		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	3
		19F			1	1
	13-valent (100%)	19A	1			1
		Total	1	2	2	5

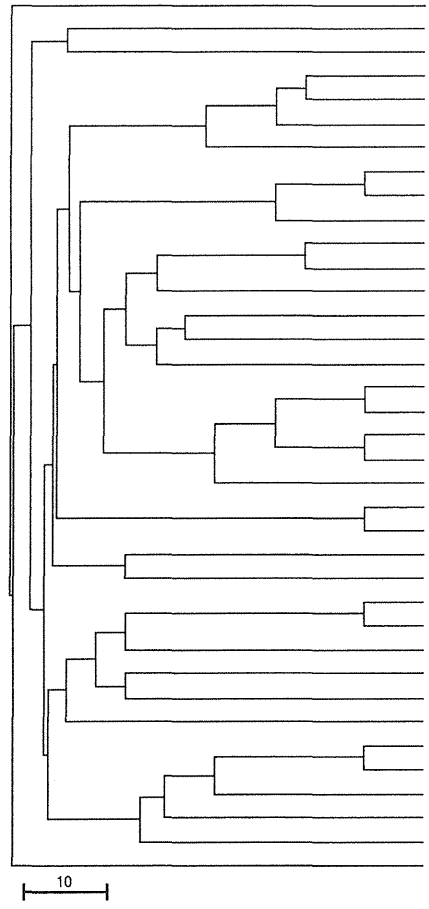
PSSP, Penicillin-susceptible *S. pneumoniae*; PISP, penicillin-intermediate *S. pneumoniae*; PRSP, penicillin-resistant *S. pneumoniae*.

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, $\chi^2(2)=3{\cdot}74$, $p=0{\cdot}154$]. 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



Sequence Type*	Serotype	Allelic profile*†	No. of isolates	
			Sputum	Blood
ST393	38	10-43-41-18-13-49-6	2	0
ST558	35B	18-12-4-44-14-77-97	2	0
ST433	22F	1-1-4-1-18-58-17	1	0
ST90	6B	5-6-1-2-6-3-4	9	1
ST5830	6B	5-6-1-2-6-1-42	1	0
ST5497	6B	5-6-1-2-**242**-1-271	0	1
ST5834	6B	7-28-1-2-6-1-5	1	0
ST236	19F	15-16-19-15-6-20-26	6	1
ST2993	19F	15-16-4-15-6-20-26	1	0
ST5495	19F	22-16-19-15-6-20-**401**	1	0
ST1437	23F	1-32-6-6-6-1-14	4	0
ST2924	6B	1-5-2-6-6-1-14	1	0
ST855	6A	10-32-19-1-6-14-14	1	0
ST191	7F	8-9-2-1-6-1-17	1	0
ST280	9V	15-17-4-16-6-1-17	1	0
ST180	3	7-15-2-10-6-1-22	1	0
ST2923	6B	2-13-2-5-6-121-29	1	0
ST5831	6B	2-13-2-5-6-124-29	1	0
ST902	6B	2-13-2-5-6-121-121	1	1
ST5494	6B	2-13-2-5-6-121-**400**	1	0
ST5232	6B	2-29-4-1-6-121-121	1	0
ST81	23F	4-4-2-4-4-1-1	1	0
ST282	6A	30-4-2-4-4-1-1	1	0
ST2331	19A	10-16-150-1-17-1-29	1	0
ST2224	6B	7-12-7-1-116-14-29	1	0
ST242	23F	15-29-4-21-30-1-14	5	0
ST3563	23F	15-29-4-21-17-1-14	1	0
ST179	19F	7-14-40-12-1-1-14	1	0
ST199	15B	8-13-14-4-17-4-14	1	0
ST343	14	8-8-4-15-39-12-14	1	0
ST3111	19A	61-60-67-16-10-104-14	0	1
ST338	23A	7-13-8-6-1-6-8	1	0
ST5246	23A	7-13-8-6-1-138-8	1	0
ST5833	6A	7-13-1-6-6-6-121	2	0
ST5496	24B	7-**257**-9-6-25-6-14	1	0
ST5832	6C	7-9-4-16-1-6-384	3	0
ST306	1	12-8-13-5-16-4-20	2	0

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 Sweden^{1}-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. pneumonia* (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 multiresistant 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.

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DECLARATION OF INTEREST

None.

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原著

同一血清型の肺炎球菌性髄膜炎を反復した1例

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要旨　生後11カ月時と1歳2カ月時に血清型6Bの肺炎球菌性髄膜炎を反復した女児．分離菌のmultilocus sequence typing解析より，同一株による再発と判明した．初発，再発後に7価肺炎球菌結合型ワクチンを接種したが，6Bに対する抗体価が上昇しなかった．一部の血清型の肺炎球菌上咽頭常在例や，侵襲性肺炎球菌感染症罹患後には，ワクチン接種後の抗体価上昇が不良な場合があるとされており，注意を要する．このような例を防ぐためにも，早急なPCV7の定期接種化が望まれる．

はじめに

細菌性髄膜炎の再発はまれで約5～6%とされ，小児に関しては1%程度と報告される[1)]．今回われわれは，血清型6B同一株による肺炎球菌性髄膜炎反復例を経験した．経過中に7価肺炎球菌結合型ワクチン（7-valent pneumococcal conjugate vaccine：PCV7）の接種を行うとともに，PCV7含有血清型別特異抗体価を測定したので，その結果と併せ報告する．

Ⅰ．症　例

症例：当院初診時1歳2カ月の女児．

家族歴，既往歴：特記すべきことなし．

現病歴：生後11カ月時発症の肺炎球菌性髄膜炎に対し，panipenem/betamipron（PAPM/BP），ampicillin（ABPC）による治療を12日間行い，後遺症なく治癒し，退院1週間後にPCV7初回接種を行った．退院から6週間後（PCV7接種から5週間後）に40℃台の発熱を認め，近医でclarithromycinの処方を受けた．第2病日に全身性強直性けいれんを発症し，前医へ緊急入院となった．入院時，髄膜刺激徴候はみられなかったが，血液検査でWBC 21,100/μl，CRP 15.2 mg/dlと炎症反応は上昇していた．また，髄液検査で細胞数76/3，蛋白25 mg/dl，糖70 mg/dlと細胞数が軽度増加しており，塗抹検査でグラム陽性球菌を認め，ラテックス凝集法による肺炎球菌抗原が陽性であった．以上より肺炎球菌性髄膜炎と診断され，PAPM/BP 160 mg/kg/day，cefotaxime 300 mg/kg/day，dexamethasone 0.15 mg/kg×4/dayが開始となった．入院時の頭部CT検査で右前頭部に硬膜下血

Key words：肺炎球菌性髄膜炎，7価肺炎球菌結合型ワクチン，血清型特異抗体価
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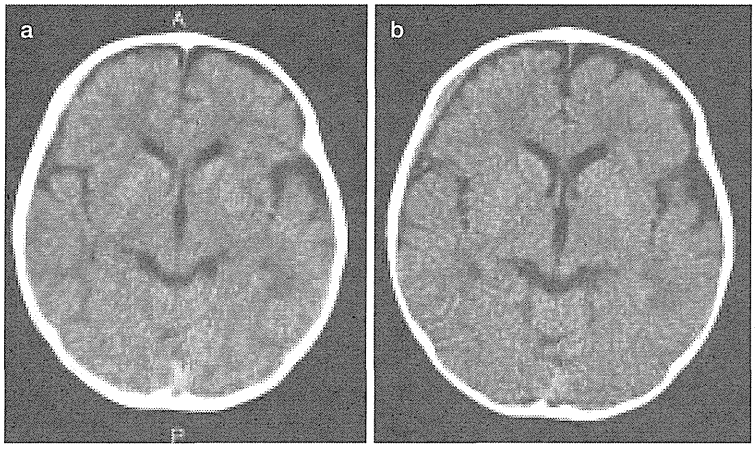

図 1 頭部 CT
a：第 2 病日
b：第 5 病日
右前頭部に少量の硬膜下血腫を認めた.

腫が認められ（**図 1** a），脳外科的処置の要否の判断のために第 3 病日に当院へ転院となった.

転院時現症：体温 37.0℃，血圧 90/45 mmHg，心拍数 120/min，呼吸数 30/min，咽頭発赤なし．意識は清明で，髄膜刺激徴候はみられなかったが，膝蓋腱反射は両側で軽度亢進していた．鼓膜所見は正常であった.

転院時検査所見：血液検査で WBC 16,600/μl（Stab 1.0%，Seg 82.0%），CRP 19.5 mg/dl と炎症反応は高値であったが，電解質，生化学所見に異常はみられなかった.

転院後経過：前医で施行された血液，髄液培養で肺炎球菌が分離されたため，PAPM/BP 160 mg/kg/day 単剤とし治療を継続した．抗菌薬感受性検査の結果，原因菌は penicillin-susceptible *Streptococcus pneumoniae*（PSSP）と判明し，第 5 病日に抗菌薬を ABPC 300 mg/kg/day に変更した．また，同日施行した髄液検査で細菌は検出されなかった．転院後は発熱を認めず，血清 CRP 値も順調に低下し，第 17 病日に 0.73 mg/dl となり抗菌薬を中止した．また，第 5 病日に頭部 CT 検査を行ったが，右前頭部の硬膜下血腫は増大しておらず（図 1 b），第 12 病日の頭部 MRI 検査で血腫は消失していたため，脳外科的処置は要さなかった．第 25 病日に合併症なく退院となり，第 29 病日に PCV7 追加接種を行った.

各種検査：国立感染症研究所に依頼し，初発時の髄液，血液，再発時の髄液，血液，喀痰より分離された肺炎球菌に対し，微量液体希釈法による抗菌薬感受性試験，膨潤法による莢膜血清型判定試験，multilocus sequence typing（MLST）法による遺伝子解析[2]を行った結果，分離された菌すべてが血清型 6B の PSSP で，シークエンスタイプも同一であることが判明した（**表 1**）.

肺炎球菌性髄膜炎を反復した原因については，免疫不全症や解剖学的異常を考慮し，精査を行った．免疫グロブリン，IgG サブクラス分画，血清補体価，好中球殺菌能，貪食能はいずれも正常であった（**表 2**）．また，肺炎球菌，黄色ブドウ球菌などの細菌感染症を繰り返す免疫不全症として知られている interleukin-1 receptor associated kinase 4（IRAK4）欠損症[3]の検索を九州大学で行ったが，否定的であった．解剖学的異常に関しては，頭部 CT，MRI 検査を施行したが，骨折や頭蓋骨の奇形，Mondini 奇形などの内耳異常はみられなかった[4].

さらに，大阪大学に依頼し，再発 60 日前（初発時），49 日前（初回治癒後），第 1 病日，第 18 病日，第 39 病日の患児の凍結保存血清を用いて，PCV7 含有血清型別特異 IgG 抗体価を測定した．なお，PCV7 は再発から 39 日前に初回接種を，第 24 病日に 2 回目接種を行った．PCV7 初回接種後に 6B 以外の血清型に対する抗体価は明らかに上昇したが，6B に対する抗体価のみほとんど上昇を認めなかった（**図 2**）.

II．考　察

髄膜炎再発の厳密な定義はないが，一般的には

表 1　分離された肺炎球菌の抗菌薬感受性試験，莢膜血清型判定試験，multilocus sequence typing 法による遺伝子シークエンスタイピング（ST）

		MIC（μg/ml）				血清型	ST
		PCG	ABPC	CTX	PAPM/BP		
初発時	髄液	0.03	＜0.03	0.25	＜0.008	6B	2983
	血液	0.06	＜0.03	0.5	＜0.008	6B	2983
再発時	髄液	0.03	＜0.03	0.25	＜0.008	6B	2983
	血液	0.06	0.06	0.25	＜0.008	6B	2983
	喀痰	0.06	0.06	0.25	＜0.008	6B	2983

分離された菌すべての ST は 2983（*aroE* 5, *gdh* 6, *gki* 1, *recP* 2, *spi* 6, *xpt* 1, *ddl* 271）で同一であった．

表 2　免疫機能検査所見

IgA	65 mg/dl	IgG1	381 mg/dl
IgM	87 mg/dl	IgG2	124 mg/dl
IgG	711 mg/dl	IgG3	27.2 mg/dl
C3	174.5 mg/dl	IgG4	5.0 mg/dl
C4	54.9 mg/dl	好中球貪食能	73%
CH50	83.6 U/ml	好中球殺菌能	95%

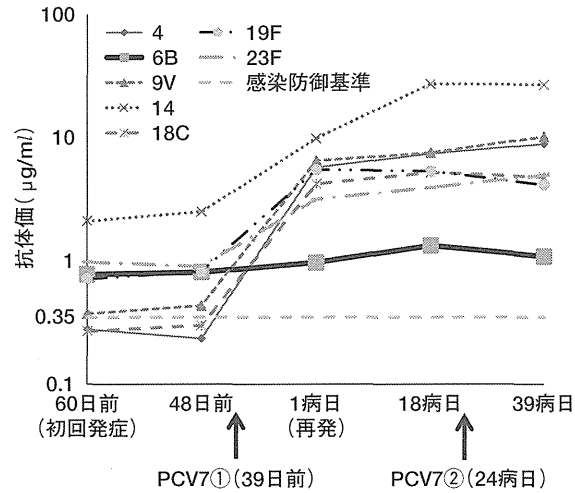

図 2　PCV7 含有血清型別 IgG 抗体価の推移

原因菌が異なる場合，もしくは前回感染の治療完了から 3 週間以上経過している場合とされる[1,5]．本症例では，初回の髄膜炎治療終了から再罹患するまで 6 週間が経過していたが，MLST 解析により同一株による髄膜炎反復と判明したこと，再罹患時に硬膜下血腫を認めたことから，局所の残存菌による再燃も考えられた．しかし，再発 53 日前の頭部 MRI では血腫や水腫は認めておらず，初発時と同様に再罹患時の血液培養からも肺炎球菌が検出されていた．これらを考慮すると，上咽頭に常在する同一菌株による再発であった可能性が高い．Tebruegge らによると，細菌性髄膜炎再発の原因は外傷や手術による頭蓋底の損傷，内耳奇形，骨欠損，髄膜瘤などの解剖学的異常が 59%で最も多く，免疫不全症候群が 36%，中耳炎，副鼻腔炎，骨髄炎などの慢性感染症が 5%であった[6]．本症例においても，各種免疫不全，解剖学的異常に関する精査を行ったが，検索し得た限り異常は認められなかった．

患児は，6B の肺炎球菌による髄膜炎を反復し，さらに初回発症後と再発後に計 2 回の PCV7 の接種を行っていたが，6B に対する抗体価の上昇は認められなかった．Dagan らは，PCV7 接種以前に 6B，19F，23F いずれかの肺炎球菌が上咽頭に常在している場合，PCV7 を 2 回，ないし 3 回接種した後でも，常在する血清型の肺炎球菌に対する抗体価の上昇が得られなかったと報告している[7]．本症例では，再発時の喀痰から 6B の肺炎球菌が分離されており，血清型を未確認ではあるものの初発時の喀痰からも肺炎球菌が検出されていた．したがって，髄膜炎発症以前から 6B の肺炎球菌が上咽頭に常在していた可能性があり，そのため 6B に対する抗体価だけが上昇しなかったとも推測される．また，侵襲性肺炎球菌感染症（inva-

sive pneumococcal disease：IPD）発症後にワクチンによる抗体産生が不応となることが報告されており[8]，IPD に繰り返し罹患したことにより抗体価の上昇がみられなかった可能性も考えられる．

なお，本症例では 6B に対する抗体価は 0.35 μg/ml とされる感染予防基準値[9]を超えていたにもかかわらず，6B の肺炎球菌による髄膜炎を再発した．この点に関しては，オプソニン活性の測定を検討している．いずれにせよ，髄膜炎再発の原因に関してはまだ不明な点も残っており，今後も抗体価の測定や，ワクチンの追加接種を行うなど慎重な経過観察を要する．

PCV7 が IPD の予防に有用であることは明らかであるが，呈示したような例を防ぐためには，肺炎球菌に曝露される機会が少ない時期，すなわち，乳児期早期から PCV7 を接種開始する必要がある．また，PCV7 の接種率が高まれば，IPD が減少するとともに，集団免疫効果による未接種者への予防効果も期待される[10,11]．早急な PCV7 の定期接種化が望まれる．

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A case of recurrent meningitis of *Streptococcus pneumoniae* serotype-6B

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We reported on a case of a girl who had recurrent meningitis from *Streptococcus pneumoniae* serotype-6B at the age of 11 months and 14 months. Multilocus sequence typing analysis revealed the same strain caused each episode of meningitis. Seven-valent pneumococcal conjugate vaccines (PCV7) were administered after recovery from each episode of meningitis. Serotype-specific immunoglobulin G level to 6-B did not rise in spite of the vaccinations. It is suggested that the nasopharyngeal carriage of *Streptococcus pneumoniae* or invasive pneumococcal disease (IPD) caused serotype-specific hyporesponsiveness to PCV7. Routine immunization of PCV7 should be estimated immediately.

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