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).
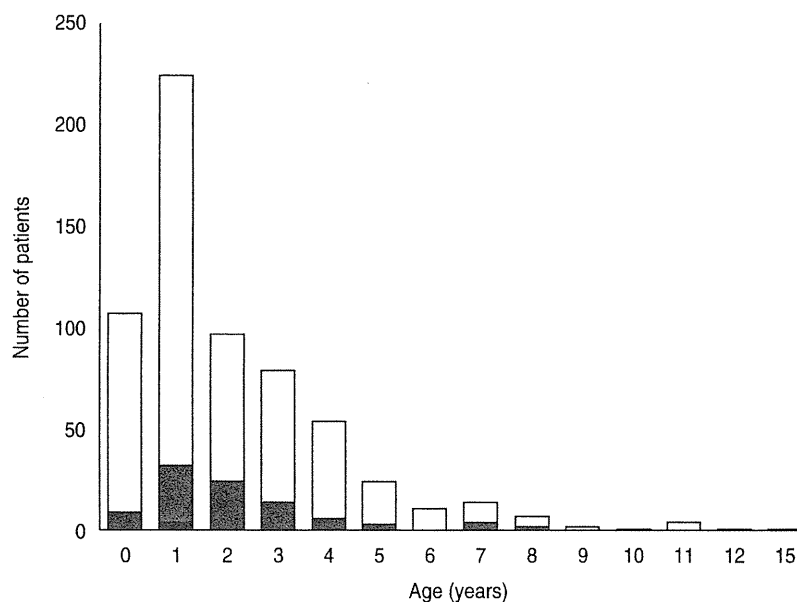

Fig. 1. Age distribution and identification of *Streptococcus pneumoniae* in children hospitalized with community-acquired pneumonia. ▨, *S. pneumoniae* dominantly isolated from sputum; ▦, *S. pneumoniae* isolated from blood ($n = 626$).

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*

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
	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, $\chi^2(2) = 3\cdot74$, $p = 0\cdot154$]. 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
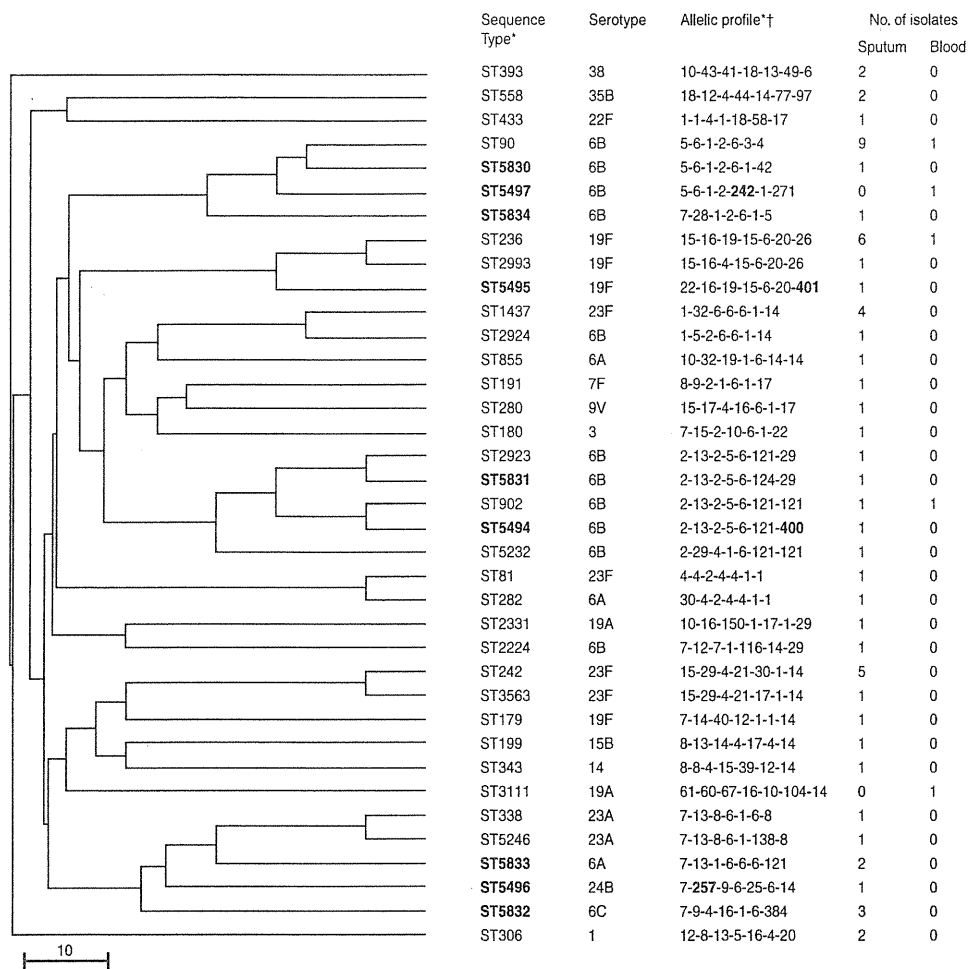
Sequence Type*	Serotype	Allelic profile*†	No. of isolates Sputum	No. of isolates Blood
ST393	38	10-43-41-18-13-49-6	2	0
ST558	35B	18-12-4-44-14-77-97	2	0
ST433	22F	1-1-4-1-18-58-17	1	0
ST90	6B	5-6-1-2-6-3-4	9	1
ST5830	6B	5-6-1-2-6-1-42	1	0
ST5497	6B	5-6-1-2-242-1-271	0	1
ST5834	6B	7-28-1-2-6-1-5	1	0
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 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.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Pilishvili T, et al.** Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *Journal of Infectious Diseases* 2010; **201**: 32–41.
2. **Isaacman DJ, McIntosh ED, Reinert RR.** Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *International Journal of Infectious Diseases* 2010; **14**: e197–209.
3. **Grivea IN, et al.** Impact of heptavalent pneumococcal conjugate vaccine on nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* among day-care center attendees in central Greece. *Pediatric Infectious Disease Journal* 2008; **27**: 519–525.
4. **Bettinger JA, et al.** The effect of routine vaccination on invasive pneumococcal infections in Canadian children, Immunization Monitoring Program, Active 2000–2007. *Vaccine* 2009; **28**: 2130–2136.
5. **Black SB, et al.** Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. *Pediatric Infectious Disease Journal* 2002; **21**: 810–815.
6. **Grijalva CG, et al.** Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. *Lancet* 2007; **369**: 1179–1186.

7. Madhi SA, Klugman KP, Group TVT. A role for *Streptococcus pneumoniae* in virus-associated pneumonia. *Nature Medicine* 2004; **10**: 811–813.

8. Kubo S, *et al.* Clinical aspects of 'asthmatic bronchitis' and chronic bronchitis in infants and children. *Journal of Asthma Research* 1978; **15**: 99–132.

9. Uehara S. A method of bacteriological examination of washed sputum in infants and children. *Acta Paediatrica Japonica* 1988; **30**: 253–260.

10. Cao LD, *et al.* Value of washed sputum gram stain smear and culture for management of lower respiratory tract infections in children. *Journal of Infection and Chemotherapy* 2004; **10**: 31–36.

11. Takeda N, *et al.* Evaluation of ampicillin for the initial treatment of pneumonia in pediatric inpatients. *Journal of the Japan Pediatric Society* 2008; **112**: 1081–1087.

12. Hishiki H, *et al.* Incidence of bacterial coinfection with respiratory syncytial virus bronchopulmonary infection in pediatric inpatients. *Journal of Infection and Chemotherapy* 2011; **17**: 87–90.

13. Uehara S, *et al.* Japanese guidelines for the management of respiratory infectious diseases in children 2007 with focus on pneumonia. *Pediatrics International* 2011; **53**: 264–276.

14. Japanese Census. Japan census data for Chiba city (http://www.city.chiba.jp/sogoseisaku/sogoseisaku/tokei/jinkou.html).

15. Ogita J, *et al.* Incidence of community-acquired pneumonia and pneumococcal pneumonia, among children in Chiba city, Japan. *Kansenshogaku Zasshi* 2008; **82**: 624–627.

16. Chang B, *et al.* Isolation of Streptococcus pneumoniae serotypes 6C and 6D from the nasopharyngeal mucosa of healthy Japanese children. *Japanese Journal of Infectious Diseases* 2010; **63**: 381–383.

17. Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiology* 1998; **144**: 3049–3060.

18. Ishiwada N, *et al.* The incidence of pediatric invasive pneumococcal disease in Chiba prefecture, Japan (2003–2005). *Journal of Infection* 2008; **57**: 455–458.

19. Clark JE, *et al.* Epidemiology of community-acquired pneumonia in children seen in hospital. *Epidemiology and Infection* 2007; **135**: 262–269.

20. Djuretic T, *et al.* Hospital admissions in children due to pneumococcal pneumonia in England. *Journal of Infection* 1998; **37**: 54–58.

21. Henrickson KJ, *et al.* National disease burden of respiratory viruses detected in children by polymerase chain reaction. *Pediatric Infectious Disease Journal* 2004; **23**: S11–18.

22. Weigl JA, *et al.* Population-based burden of pneumonia before school entry in Schleswig-Holstein, Germany. *European Journal of Pediatrics* 2003; **162**: 309–316.

23. Cherian T, *et al.* Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. *Bulletin of the World Health Organization* 2005; **83**: 353–359.

24. Bartlett JG, Finegold SM. Bacteriology of expectorated sputum with quantitative culture and wash technique compared to transtracheal aspirates. *American Review of Respiratory Disease* 1978; **117**: 1019–1027.

25. Ishiwada N, *et al.* Etiology of pediatric inpatients with pneumonia. *Kansenshogaku Zasshi* 1993; **67**: 642–647.

26. Hamano-Hasegawa K, *et al.* Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. *Journal of Infection and Chemotherapy* 2008; **14**: 424–432.

27. Ziyade N, Aksu B, Yagci A. Value of washed sputum samples in children with lower respiratory tract infections. *Pediatrics International* 2009; **51**: 438–440.

28. Don M, *et al.* Aetiology of community-acquired pneumonia: serological results of a paediatric survey. *Scandinavian Journal of Infectious Diseases* 2005; **37**: 806–812.

29. Michelow IC, *et al.* Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 2004; **113**: 701–707.

30. Yoshida R, *et al.* Trends in antimicrobial resistance of *Streptococcus pneumoniae* in Japan. *Antimicrobial Agents and Chemotherapy* 1995; **39**: 1196–1198.

31. Hotomi M, *et al.* Serotype distribution and penicillin resistance of *Streptococcus pneumoniae* isolates from middle ear fluids of pediatric patients with acute otitis media in Japan. *Journal of Clinical Microbiology* 2008; **46**: 3808–3810.

32. Chiba N, *et al.* Serotype and antibiotic resistance of isolates from patients with invasive pneumococcal disease in Japan. *Epidemiology and Infection* 2010; **138**: 61–68.

33. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. *Lancet Infectious Diseases* 2005; **5**: 83–93.

34. Hausdorff WP, *et al.* Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clinical Infectious Diseases* 2000; **30**: 100–121.

35. Hausdorff WP, Dagan R. Serotypes and pathogens in paediatric pneumonia. *Vaccine* 2008; **26** (Suppl. 2): B19-23.

36. Watanabe K, *et al.* Drug-resistant pneumococci in children with acute lower respiratory infections in Vietnam. *Pediatrics International* 2008; **50**: 514–518.

37. Yao KH, *et al.* Pneumococcal serotype distribution and antimicrobial resistance in Chinese children hospitalized for pneumonia. *Vaccine* 2011; **29**: 2296–2301.

38. Muhlemann K, *et al.* Nationwide surveillance of nasopharyngeal Streptococcus pneumoniae isolates from children with respiratory infection, Switzerland, 1998–1999. *Journal of Infectious Diseases* 2003; **187**: 589–596.

39. Moore MR, *et al.* Population snapshot of emergent *Streptococcus pneumoniae* serotype 19A in the United States, 2005. *Journal of Infectious Diseases* 2008; **197**: 1016–1027.

40. **Ardanuy C,** *et al.* Emergence of a multidrug-resistant clone (ST320) among invasive serotype 19A pneumococci in Spain. *Journal of Antimicrobial Chemotherapy* 2009; **64**: 507–510.

41. **Choi EH,** *et al. Streptococcus pneumoniae* serotype 19A in children, South Korea. *Emerging Infectious Diseases* 2008; **14**: 275–281.

42. **Park IH,** *et al.* Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae. Journal of Clinical Microbiology* 2007; **45**: 1225–1233.

43. **Tocheva AS,** *et al.* Increase in serotype 6C pneumococcal carriage, United Kingdom. *Emerging Infectious Diseases* 2010; **16**: 154–155.

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 $> 1 \times 10^7$ 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 μg/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 (%)	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).
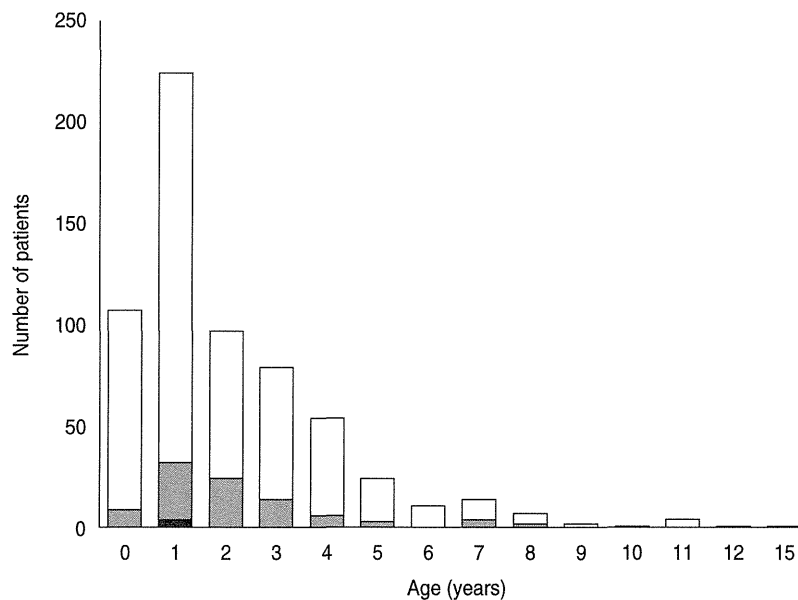

Fig. 1. Age distribution and identification of *Streptococcus pneumoniae* in children hospitalized with community-acquired pneumonia. ▣, *S. pneumoniae* dominantly isolated from sputum; ▪, *S. pneumoniae* isolated from blood (n = 626).

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*

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
	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, $\chi^2(2) = 3·74$, $p = 0·154$]. 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
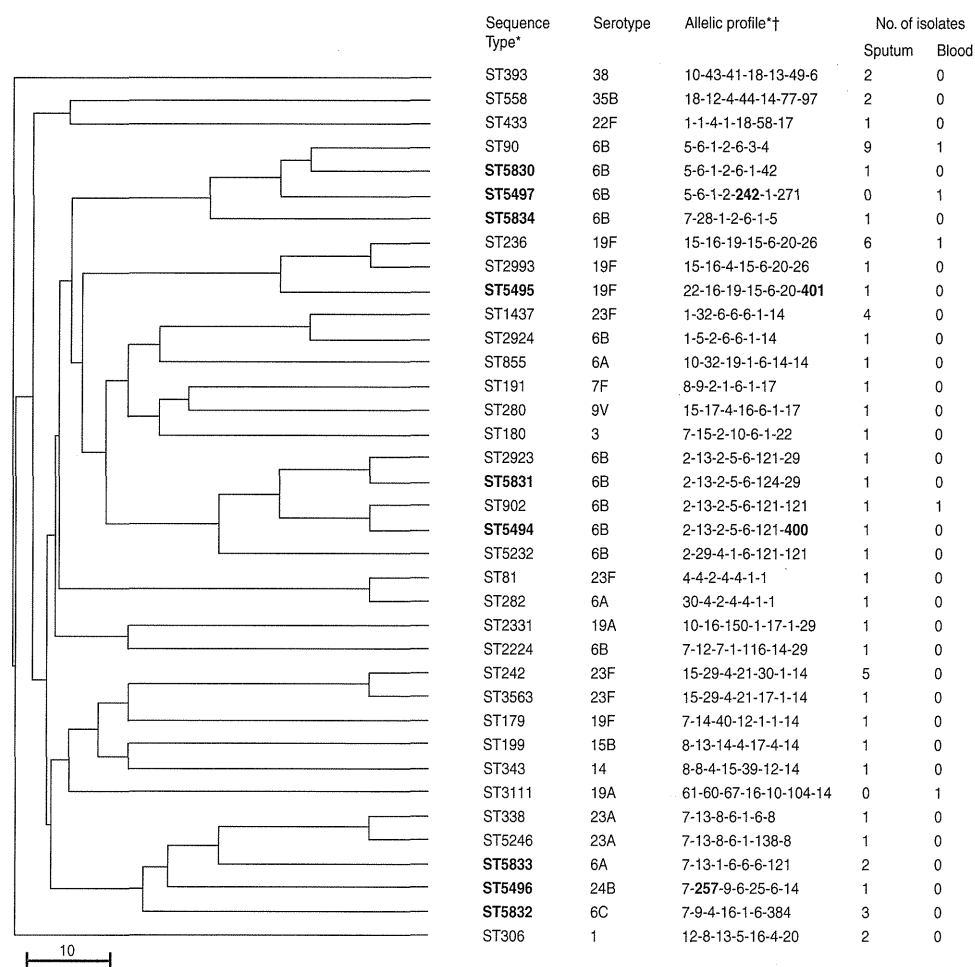
Sequence Type*	Serotype	Allelic profile*†	Sputum	Blood
ST393	38	10-43-41-18-13-49-6	2	0
ST558	35B	18-12-4-44-14-77-97	2	0
ST433	22F	1-1-4-1-18-58-17	1	0
ST90	6B	5-6-1-2-6-3-4	9	1
ST5830	6B	5-6-1-2-6-1-42	1	0
ST5497	6B	5-6-1-2-**242**-1-271	0	1
ST5834	6B	7-28-1-2-6-1-5	1	0
ST236	19F	15-16-19-15-6-20-26	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/100000 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 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.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Pilishvili T, et al.** Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *Journal of Infectious Diseases* 2010; **201**: 32–41.
2. **Isaacman DJ, McIntosh ED, Reinert RR.** Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *International Journal of Infectious Diseases* 2010; **14**: e197–209.
3. **Grivea IN, et al.** Impact of heptavalent pneumococcal conjugate vaccine on nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* among day-care center attendees in central Greece. *Pediatric Infectious Disease Journal* 2008; **27**: 519–525.
4. **Bettinger JA, et al.** The effect of routine vaccination on invasive pneumococcal infections in Canadian children, Immunization Monitoring Program, Active 2000–2007. *Vaccine* 2009; **28**: 2130–2136.
5. **Black SB, et al.** Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. *Pediatric Infectious Disease Journal* 2002; **21**: 810–815.
6. **Grijalva CG, et al.** Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. *Lancet* 2007; **369**: 1179–1186.

7. **Madhi SA, Klugman KP, Group TVT.** A role for *Streptococcus pneumoniae* in virus-associated pneumonia. *Nature Medicine* 2004; **10**: 811–813.

8. **Kubo S, et al.** Clinical aspects of 'asthmatic bronchitis' and chronic bronchitis in infants and children. *Journal of Asthma Research* 1978; **15**: 99–132.

9. **Uehara S.** A method of bacteriological examination of washed sputum in infants and children. *Acta Paediatrica Japonica* 1988; **30**: 253–260.

10. **Cao LD, et al.** Value of washed sputum gram stain smear and culture for management of lower respiratory tract infections in children. *Journal of Infection and Chemotherapy* 2004; **10**: 31–36.

11. **Takeda N, et al.** Evaluation of ampicillin for the initial treatment of pneumonia in pediatric inpatients. *Journal of the Japan Pediatric Society* 2008; **112**: 1081–1087.

12. **Hishiki H, et al.** Incidence of bacterial coinfection with respiratory syncytial virus bronchopulmonary infection in pediatric inpatients. *Journal of Infection and Chemotherapy* 2011; **17**: 87–90.

13. **Uehara S, et al.** Japanese guidelines for the management of respiratory infectious diseases in children 2007 with focus on pneumonia. *Pediatrics International* 2011; **53**: 264–276.

14. **Japanese Census.** Japan census data for Chiba city (http://www.city.chiba.jp/sogoseisaku/sogoseisaku/tokei/jinkou.html).

15. **Ogita J, et al.** Incidence of community-acquired pneumonia and pneumococcal pneumonia, among children in Chiba city, Japan. *Kansenshogaku Zasshi* 2008; **82**: 624–627.

16. **Chang B, et al.** Isolation of Streptococcus pneumoniae serotypes 6C and 6D from the nasopharyngeal mucosa of healthy Japanese children. *Japanese Journal of Infectious Diseases* 2010; **63**: 381–383.

17. **Enright MC, Spratt BG.** A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiology* 1998; **144**: 3049–3060.

18. **Ishiwada N, et al.** The incidence of pediatric invasive pneumococcal disease in Chiba prefecture, Japan (2003–2005). *Journal of Infection* 2008; **57**: 455–458.

19. **Clark JE, et al.** Epidemiology of community-acquired pneumonia in children seen in hospital. *Epidemiology and Infection* 2007; **135**: 262–269.

20. **Djuretic T, et al.** Hospital admissions in children due to pneumococcal pneumonia in England. *Journal of Infection* 1998; **37**: 54–58.

21. **Henrickson KJ, et al.** National disease burden of respiratory viruses detected in children by polymerase chain reaction. *Pediatric Infectious Disease Journal* 2004; **23**: S11–18.

22. **Weigl JA, et al.** Population-based burden of pneumonia before school entry in Schleswig-Holstein, Germany. *European Journal of Pediatrics* 2003; **162**: 309–316.

23. **Cherian T, et al.** Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. *Bulletin of the World Health Organization* 2005; **83**: 353–359.

24. **Bartlett JG, Finegold SM.** Bacteriology of expectorated sputum with quantitative culture and wash technique compared to transtracheal aspirates. *American Review of Respiratory Disease* 1978; **117**: 1019–1027.

25. **Ishiwada N, et al.** Etiology of pediatric inpatients with pneumonia. *Kansenshogaku Zasshi* 1993; **67**: 642–647.

26. **Hamano-Hasegawa K, et al.** Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. *Journal of Infection and Chemotherapy* 2008; **14**: 424–432.

27. **Ziyade N, Aksu B, Yagci A.** Value of washed sputum samples in children with lower respiratory tract infections. *Pediatrics International* 2009; **51**: 438–440.

28. **Don M, et al.** Aetiology of community-acquired pneumonia: serological results of a paediatric survey. *Scandinavian Journal of Infectious Diseases* 2005; **37**: 806–812.

29. **Michelow IC, et al.** Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 2004; **113**: 701–707.

30. **Yoshida R, et al.** Trends in antimicrobial resistance of *Streptococcus pneumoniae* in Japan. *Antimicrobial Agents and Chemotherapy* 1995; **39**: 1196–1198.

31. **Hotomi M, et al.** Serotype distribution and penicillin resistance of *Streptococcus pneumoniae* isolates from middle ear fluids of pediatric patients with acute otitis media in Japan. *Journal of Clinical Microbiology* 2008; **46**: 3808–3810.

32. **Chiba N, et al.** Serotype and antibiotic resistance of isolates from patients with invasive pneumococcal disease in Japan. *Epidemiology and Infection* 2010; **138**: 61–68.

33. **Hausdorff WP, Feikin DR, Klugman KP.** Epidemiological differences among pneumococcal serotypes. *Lancet Infectious Diseases* 2005; **5**: 83–93.

34. **Hausdorff WP, et al.** Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clinical Infectious Diseases* 2000; **30**: 100–121.

35. **Hausdorff WP, Dagan R.** Serotypes and pathogens in paediatric pneumonia. *Vaccine* 2008; **26** (Suppl. 2): B19-23.

36. **Watanabe K, et al.** Drug-resistant pneumococci in children with acute lower respiratory infections in Vietnam. *Pediatrics International* 2008; **50**: 514–518.

37. **Yao KH, et al.** Pneumococcal serotype distribution and antimicrobial resistance in Chinese children hospitalized for pneumonia. *Vaccine* 2011; **29**: 2296–2301.

38. **Muhlemann K, et al.** Nationwide surveillance of nasopharyngeal Streptococcus pneumoniae isolates from children with respiratory infection, Switzerland, 1998–1999. *Journal of Infectious Diseases* 2003; **187**: 589–596.

39. **Moore MR, et al.** Population snapshot of emergent *Streptococcus pneumoniae* serotype 19A in the United States, 2005. *Journal of Infectious Diseases* 2008; **197**: 1016–1027.

40. **Ardanuy C,** *et al.* Emergence of a multidrug-resistant clone (ST320) among invasive serotype 19A pneumococci in Spain. *Journal of Antimicrobial Chemotherapy* 2009; **64**: 507–510.

41. **Choi EH,** *et al.* Streptococcus pneumoniae serotype 19A in children, South Korea. *Emerging Infectious Diseases* 2008; **14**: 275–281.

42. **Park IH,** *et al.* Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae. Journal of Clinical Microbiology* 2007; **45**: 1225–1233.

43. **Tocheva AS,** *et al.* Increase in serotype 6C pneumococcal carriage, United Kingdom. *Emerging Infectious Diseases* 2010; **16**: 154–155.

Original Article

Prevalence and Risk Factors of Nasopharyngeal Carriage of *Streptococcus pneumoniae* in Healthy Children in Japan

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SUMMARY: The findings of this study revealed a 22% prevalence of *Streptococcus pneumoniae* among healthy children in Japan; the prevalence tended to increase with age. We identified attendance at day-care facilities and the presence of older siblings as the major risk factors associated with pneumococcal carriage. A significant decrease in pneumococcal colonization was observed among children during the summer; however, a seasonal variation was independent of various factors. Our findings suggest that prior immunization with heptavalent pneumococcal conjugate vaccine within the current recommended schedule is required for children exposed to these risk factors.

INTRODUCTION

Streptococcus pneumoniae is a clinically important pathogen that causes invasive pneumococcal diseases (IPDs) such as sepsis, meningitis, and pneumonia in children. *S. pneumoniae* is part of the commensal flora of the nasopharynx (1), but only a few reports have been published in Japan on the prevalence of nasopharyngeal carriage of *S. pneumoniae* in children (2,3). The risk factors cited for nasopharyngeal colonization with pneumococci in children in the United States and Europe are ethnicity, attendance at day-care centers, the presence of older siblings, recent antibiotic use, and respiratory tract infections (1,4–7). In Japan, a report based on routine public medical checkups (2), suggests that the presence of older siblings and day-care center attendance are the major risk factors for pneumococcal carriage in healthy children. To the best of our knowledge, no reports on seasonal variations in pneumococcal colonization among healthy children have yet been published in Japan. The aims of this study were to estimate the prevalence of nasopharyngeal carriage of *S. pneumoniae* in healthy children in Japan, and to examine the potential risk factors for the infection.

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MATERIALS AND METHODS

Study population: During the study period (July 2010 through March 2012), 229 healthy children were enrolled. Three institutions in Saitama Prefecture and Tokyo were involved. Parents were informed about this study when they brought their children for their first routine heptavalent pneumococcal conjugate vaccine (PCV7) immunizations. Written informed consent was obtained from the parents who agreed to let their children participate in the study. All the participants were between 2 months and 6 years of age; none had any prior history of immunization with a pneumococcal vaccine, and none had any obvious infection. The Ethical Review Board of Toho University approved this study.

Specimen collection and testing: Pediatricians took nasopharyngeal swabs (Amies charcoal transport medium) of the children during their first routine PCV7 immunizations. The swabs were plated onto a medium containing 5% sheep blood. The plates were incubated overnight at 35°C in 5% CO_2. Pneumococci were isolated and identified on the basis of colony morphology and susceptibility to optochin. Colonies that were difficult to identify with the optochin susceptibility test were identified by means of *lytA* gene polymerase chain reaction (8).

Potential risk factors: Information on exposure to potential risk factors was collected from the parents or caregivers of each participating child, examination of medical records, and/or via a questionnaire. The following factors were examined: age (<1, 1–2, 3–6 years), sex, day-care attendance, presence of older/younger siblings, vaccination history (acellular pertussis vaccine