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The impact of heptavalent pneumococcal conjugate vaccine on the incidence of childhood community-acquired pneumonia and bacteriologically confirmed pneumococcal pneumonia in Japan

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SUMMARY

Heptavalent pneumococcal conjugate vaccine (PCV7) was introduced to Japan in 2010. We investigated the impact of PCV7 on childhood community-acquired pneumonia (CAP) and pneumococcal pneumonia (PP). Children aged <5 years living in Chiba city, Japan, who were admitted to hospitals were enrolled to estimate the incidence of CAP based on the mid-year population. PP was determined by the presence of *Streptococcus pneumoniae* in cultured blood and/or sputum samples of CAP patients. The incidence of CAP and *S. pneumoniae* isolated from PP patients was compared before (April 2008–March 2009) and after (April 2012–March 2013) the introduction of PCV7 immunization. The annual incidence of CAP was reduced [incidence rate ratio 0·81, 95% confidence interval (CI) 0·73–0·90]. When comparing post-vaccine with pre-vaccine periods, the odds ratio for PP incidence was 0·60 (95% CI 0·39–0·93, $P = 0·024$). PCV7-covered serotypes markedly decreased (66·6% in pre-vaccine *vs.* 15·6% in post-vaccine, $P < 0·01$), and serotypes 6C, 15A, 15C and 19A increased. Multidrug-resistant international clones in the pre-vaccine period (Spain^{6B}-2/ST90, Taiwan^{19F}-14/ST236) decreased, while Sweden^{15A}-25/ST63 was the dominant clone in the post-vaccine period. A significant reduction in the incidence of both CAP hospitalizations and culture-confirmed PP of vaccine serotypes was observed at 2 years after PCV7 vaccination.

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

INTRODUCTION

Community-acquired pneumonia (CAP) is a serious cause of morbidity and one of the leading causes of hospital admission in children in developed countries. *Streptococcus pneumoniae* is considered to be the most important pathogen identified from children aged <5 years with bacterial pneumonia [1].

Introduction of the heptavalent pneumococcal polysaccharide conjugate vaccine (PCV7) has been shown to provide significant protection against childhood CAP in European and American countries [2–4]. However, publications detailing the aetiology of CAP are scarce in Asian countries. PCV7 has led

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to marked decreases in invasive and complicated pneumococcal pneumonia (PP) of vaccine serotypes [5–7]. Changes in serotype distribution after the introduction of PCV7 are also known to affect the antimicrobial susceptibility of *S. pneumoniae* isolated from invasive pneumococcal infections [8]. Most of the *S. pneumoniae* isolates in studies that investigated the effect of PCV7 in other countries were derived from blood, pleural fluid samples, or nasal carriage; however, due to the low prevalence of bacteraemic pneumonia in these regions, the impact of PCV7 on PP in children is difficult to assess [9]. Examination of washed sputum is used to investigate microbial pathogens identified from pneumonia patients, and thus to estimate the bacterial cause of non-invasive CAP in children [10, 11]; this enables us to investigate the effect of PCV7 on the incidence of non-invasive PP.

PCV7 was introduced in Japan in February 2010 as a voluntary vaccine for children aged <5 years. It was recommended for children at ages 2, 3, and 4 months with a booster dose at 12–15 months as a 3 + 1 dosing schedule. When it was first introduced, the vaccination rate was under 10%; however, the vaccination rate quickly rose in Chiba city after February 2011, when the Provisional Special Fund for the Urgent Promotion of Vaccination started to cover the vaccination fee [12]. Two years before PCV7 introduction in Chiba city, Tanaka *et al.* reported the annual incidence of CAP in children aged <5 years to be 17·6 episodes/1000 child-years [13]. The primary objective of this study was to investigate the incidence of CAP 2 years after the introduction of PCV7 in Chiba city.

The secondary aim of our study was to reveal trends associated with CAP and PP after the introduction of PCV7, and to determine whether the incidence of CAP due to other bacterial causes had changed after the introduction of PCV7. Of the PP patients, we further investigated changes in serotype, sequence-type (ST) distribution, and antimicrobial susceptibility of the *S. pneumoniae* isolated from blood and sputum.

To our knowledge, this is the first population-based study in Asia both to show the effect of PCV7 on CAP, and to further investigate the serotype/ST distribution of *S. pneumoniae* isolated from invasive and non-invasive PP. The results from our study enable us to monitor the effect of the current vaccine, and could also affect the decision to introduce higher valency pneumococcal vaccines in Japan in the future.

MATERIALS AND METHODS

Incidence of CAP and CAP with pneumococcal bacteraemia in Chiba city

The incidence of CAP and bacteraemic PP was calculated based on an observational retrospective population-based study in 18 hospitals in and around Chiba city.

A questionnaire was sent to 18 hospitals, and the numbers of hospital admissions due to pneumonia and of blood culture-positive pneumonia patients were obtained from the clinical records of all hospitals, which were estimated to cover all of the ~40 000 inhabitants of Chiba city aged <5 years. The number of inhabitants was calculated from Japanese census data [14]. Person-years were based on mid-year population estimates [15].

The two study periods were from April 2008 to March 2009 (2008), and from April 2012 to March 2013 (2012). Children aged 1 month to 5 years who lived in Chiba city and were admitted to hospital with CAP were included in this study. Pneumonia was diagnosed based on abnormal findings in chest radiographs and on clinical findings with at least one of the following symptoms: fever, cough, rapid breathing, difficulty breathing, or crackles upon auscultation of the lungs. The same doctors in each hospital, who were not directly related to our study team, read the chest radiographs and diagnosed pneumonia in both studies.

Incidence of PP

The incidence of PP was surveyed during the same periods in five of the above hospitals for the same target population (children aged <5 years living in Chiba city who were admitted to hospital with pneumonia). These five hospitals covered 53% of the hospitalized pneumonia patients in the CAP study [13].

Blood cultures and sputum samples were collected upon admission. Bacterial pneumonia was diagnosed based on a positive blood culture or the isolation of microorganisms from sputum samples. Sputum samples were collected from children as described previously [13]. Briefly, the tongue was depressed to induce the cough reflex, and sputum was collected directly from the throat using a 1-ml disposable syringe. Sputum samples were washed three times with sterilized saline, and a small purulent portion of the washed sputum was smeared onto glass slides. Gram-stained smears were considered to be valid for

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sputum culture samples according to Geckler's classification 4 or 5. This effectively isolated pathogenic bacteria (e.g. *S. pneumoniae, Haemophilus influenza, Moraxella catarrhalis*) and reduced the contamination by oral flora. Washed sputum samples were cultured in each hospital, and pathogens accounting for >50% of the colonies in culture or presenting as $>1 \times 10^7$ c.f.u./ml were regarded as pathogenic.

Patients' backgrounds and clinical information were collected and recorded on a standard case report sheet. Administration of PCV7 was documented in the patient's medical record or in the maternity health record book used to document children's vaccination history in Japan. We reviewed the case report sheets to collect details on patients' backgrounds, and then statistically analysed the confounders, which may have influenced the disease burden of PP along with the introduction of the vaccine.

Laboratory testing for *S. pneumoniae*

Serotype and multi-locus sequence typing (MLST) and antibiotic resistance were determined for *S. pneumoniae* isolates from blood and sputum samples if the isolates were stocked in each hospital and sent to Chiba University Hospital for further testing.

Serotype was determined by the Quellung reaction using antiserum (Staten Serum Institut, Denmark); serotyping was performed at the Chiba University Hospital and the Department of Bacteriology I of the National Institute of Infectious Diseases.

MLST was performed as described previously [16]. Briefly, internal fragments of the seven housekeeping genes (*aroE, gdh, gki, recP, spi, xpt, ddl*) were amplified by polymerase chain reaction, and both strands of each fragment then were sequenced. STs were determined by comparing the derived sequences of each locus to all known alleles by reference to the MLST database (http://spneumoniae.mlst.net).

The STs were compared with 43 pneumococcal clones, which included 26 multidrug-resistant (MDR) clones, in the Pneumococcal Molecular Epidemiology Network (PMEN; http://www.sph.emory.edu/PMEN/).

Relationships of the isolates were determined by eBURST v. 3 software (http://eburst.mlst.net). Strains were assigned to one clonal complex (CC) when six of the seven alleles were identical to those of another ST in the group (single locus variants).

Antimicrobial susceptibilities for penicillin, amoxicillin, cefditoren, cefotaxime, meropenem, panipenem, tebipenem, erythromycin, clindamycin, tosfuloxacin (TFLX) and vancomycin were analysed using a broth microdilution method according to the protocol of the Clinical and Laboratory Standards Institute (CLSI M100-S18). Minimal inhibitory concentration (MIC) breakpoints were defined according to CLSI criteria (CLSI, 2008).

Statistical analysis

All statistical analyses were performed using SAS software v. 9.3 (SAS Institute Inc., USA). A Poisson regression model was used to estimate the incidence rates, the incidence rate ratios, and the confidence intervals of CAP and PP. Between-group differences in patients' characteristics were analysed with the Wilcoxon rank-sum test and Fisher's exact test for continuous and categorical variables, respectively. Fisher's exact test was used to compare the coverage rate of PCV7 serotypes of *S. pneumoniae* isolated from PP patients before and after the introduction of PCV7. A logistic regression model adjusted by potential confounders was used to estimate the odds ratio for the incidence of PP as the effect of PCV7 vaccine, based on a comparison of pre- and post-vaccine incidences. All point estimates, Wald-type 95% confidence intervals (CIs), and *P* values in the logistic regression models were performed based on Firth's penalized likelihood estimation [17]. The potential confounders of multivariable analysis were chosen from the patients' backgrounds (two study periods, quinolone antibiotics, and bronchial asthma), which had been shown to be relevant to pneumonia admissions in previous studies [18], and statistically significant in the univariable analysis of our results. All *P* values represented two-tailed tests, with $P<0{\cdot}05$ considered as statistically significant.

Ethical issues

This study was approved by the Chiba University Ethics Committee (no. 1301). For the CAP study performed in 18 hospitals in Chiba city, patients' records and information were anonymized prior to analysis. For the PP study performed in five hospitals, written informed consent was obtained from the parents of children with CAP at the time of admission, in accordance with the guidelines of the Institutional Review Board of Chiba University.

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Table 1. *Changes in all-cause pneumonia and bacteraemic pneumococcal pneumonia incidence before and after the introduction of PCV7*

	April 2008–March 2009				April 2012–March 2013				Total		
	No. of events	Person-years*	Incidence†	(95% CI)†	No. of events	Person-years*	Incidence†	(95% CI)†	IRR†	(95% CI)†	*P* value
CAP	752	42 606	17·65‡	(16·43–18·96)	588	41 102	14·31‡	(13·20–15·51)	0·811	(0·728–0·903)	<0·001
IPP	5	42 606	0·117‡	(0·0489–0·282)	4	41 102	0·0973‡	(0·0365–0·259)	0·829	(0·223–3·088)	0·780

CAP, Community-acquired pneumonia (hospitalization); IPP, invasive (bacteraemic) pneumococcal pneumonia; CI, Confidence interval; IRR, incidence rate ratio.
* Person-years are based on mid-year population estimates.
‡ Cases/1000 population per year.
† A Poisson regression model was used to estimate the incidence rates, the incidence rate ratios, and the confidence intervals of CAP and IPP.

RESULTS

Incidence of CAP and bacteraemial pneumococcal pneumonia before and after the introduction of PCV7

Overall, 752 and 588 children were hospitalized with CAP in Chiba City in 2008 and 2012, respectively (Table 1). A decrease in the annual incidence of pneumonia admissions of children aged <5 years was observed, from 17·6 episodes/1000 child-years in 2008 to 14·3 episodes/1000 child-years in 2012.

The incidence of CAP with pneumococcal bacteraemia in children aged <5 years was 0·117 and 0·0973 episodes/1000 child-years in 2008 and 2012, respectively. Of the 18 hospitals included in this survey, all of the blood culture-positive pneumonia cases were from the five major hospitals.

Incidence of PP before and after the introduction of PCV7

PP was investigated in the five major hospitals. The numbers of samples obtained from patients were 83·2% (341/410) in 2008 *vs.* 85·6% (308/360) in 2012 for blood samples ($P = 0·374$), and 82·9% (340/410) in 2008 *vs.* 89·4% (322/360) in 2012 for sputum samples ($P = 0·009$).

Microorganisms isolated from blood samples were five and four *S. pneumoniae* in 2008 and 2012, respectively, and one *M. catarrhalis* in 2012. There were 66/410 (16·1%) cases of PP reported in 2008, and 34/360 (9·4%) cases of PP reported in 2012. One patient in each study period had *S. pneumoniae* isolated from both blood and sputum. The numbers of *S. pneumoniae* isolated from sputum markedly decreased, while the detection rate of *H. influenzae* and *M. catarrhalis* remained almost unchanged between the two study periods (Table 2). One patient with an underlying disease (myotubular myopathy) died from invasive PP due to complications of septic shock in 2012. All of the other patients with bacterial pneumonia recovered without any complications. Based on chest radiograph findings, no patients showed obvious empyema.

The characteristics of CAP patients in the five major hospitals are given in Table 3*a–c*. The total numbers of episodes of pneumonia were 410 and 360 in 2008 and 2012, respectively. These values corresponded to 54·5% and 61·2% of all CAP admissions that occurred in Chiba city in 2008 and 2012, respectively. Because some patients were admitted more than once, the actual numbers of patients admitted with

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Table 2. *Microorganisms isolated from blood and sputum samples in community-acquired pneumonia patients in five hospitals*

	April 2008–March 2009 ($N = 410$)		April 2012–March 2013 ($N = 360$)		
	n	(%)	*n*	(%)	*P* value*
Sputum					
Streptococcus pneumoniae†	62	(15·1)	31	(8·6)	0·006
Haemophilus influenzae	44	(10·7)	49	(13·6)	0·225
Moraxella catarrhalis	11	(2·7)	12	(3·3)	0·674
Blood					
Streptococcus pneumoniae	5	(1·2)	4	(1·1)	1·000
Moraxella catarrhalis	0	(0·0)	1	(0·3)	0·468

* Proportions were analysed with Fisher's exact test.
† *S. pneumoniae* was isolated from both blood and sputum from one patient in each study period.

pneumonia in the five major hospitals were 382 and 337 in 2008 and 2012, respectively. Except for antimicrobial pre-treatment, past medical history of asthma, and PCV7 immunization, patients' characteristics were similar between both periods (Table 3*a*). Of the children aged <5 years with reliable immunization histories ($n = 322$), 80·4% had more than one vaccination with PCV7 in 2012. The use of antibiotics prior to admission was similar regarding penicillin and cephalosporins, but an increase in use was noted for macrolides (19·5% in 2008 *vs.* 25·3% in 2012, $P = 0·056$) and quinolones (0·0% in 2008 *vs.* 5·3% in 2012, $P < 0·001$) (Table 3*b*). After adjusting for confounders, the odds ratio (comparing 2012 with 2008) for PP incidence in children aged <5 years was 0·60 (95% CI 0·39–0·94, $P = 0·024$) (Table 3*c*).

Serotype distribution of the *S. pneumoniae* isolates

Serotypes were determined for 46/62 (in 2008) and 28/31 (in 2012) of the *S. pneumoniae* isolated from sputum samples, and against five (in 2008) and four (in 2012) of the *S. pneumoniae* isolated from blood samples. The prevalence of serotypes dominant in 2008 (6B, 23 F, 19 F) markedly declined by 2012 (Fig. 1). This led to a decrease in the PCV7 coverage rate of *S. pneumoniae*, from 66·6% (34/51) in 2008 to 15·6% (5/32) in 2012 ($P < 0·01$). A decrease was also seen in the PCV13 coverage rate, from 80·4% (41/51) in 2008 to 37·5% (12/32) in 2012 ($P < 0·01$), where the percentage of serotypes included in PCV13, but not in PCV7 (serotypes 1, 3, 5, 6A, 7 F. 19A), increased from 13·7% (7/51) to 21·9% (7/32) ($P = 0·376$). All four patients with invasive PP in 2012 had received more than two PCV7 immunizations, and none of the infecting *S. pnuemoniae* isolates corresponded to PCV7 serotypes. Of the five patients with PCV7 serotype isolated from sputum, three had received PCV7 immunization, and the *S. pneumoniae* isolates were all serotype 6B. None of these patients had completed the recommended 3 + 1 dosing schedule of PCV7 vaccination (one patient had one dose, while two patients had two doses).

MLST of *S. pneumoniae* isolates

MLST was performed for 46/62 (in 2008) and 28/31 (in 2012) of the *S. pneumoniae* isolated from sputum, and for five (in 2008) and four (in 2012) of the *S. pneumoniae* isolated from blood (Table 4). The most common STs in 2008 were ST90 (seven cases, 13·7%), ST236 (six cases, 11·8%), and ST242 (four cases, 7·8%), which were serotypes 6B, 19 F, and 23 F, respectively. Including all of these clones (Spain^{6B}-2/ST90, Taiwan^{19F}-14/ST236, Taiwan^{23F}-15/ST242), a total of 17/51 (33·3%) clones were registered as MDR PMEN clones. The most common STs in 2012 were ST63 (seven cases, 21·9%), ST199 (two cases, 6·3%), and ST3111 (three cases, 9·4%), which were serotypes 15A, 15C, and 19A, respectively. With the inclusion of Sweden^{15A}-25/ST63 (seven cases), Spain^{6B}-2/ST90 (one case), and Taiwan^{19F}-14/ST236 (one case), 9/32 (28·1%) clones were registered as MDR PMEN clones in 2012. Most of the clones isolated in 2008 carried the same serotypes in 2012, except for ST199 (15B and 15C) and ST2942 (6B and 6C).

The eBURST analysis of all the clones isolated in 2008 and 2012 revealed six CCs and 28 singletons containing 20 and 63 of the isolates, respectively (Fig. 2).

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Table 3*a*. *Backgrounds of patients with pneumonia in the five major hospitals – hospitalization and patients' characteristics*

Characteristics	April 2008–March 2009	April 2012–March 2013	*P* value*
Hospitalization characteristics†	**(*N* = 410)**	**(*N* = 360)**	
Age, years			0·513
<2	235 (57·3)	197 (54·7)	
2–4	175 (42·7)	163 (45·3)	
Sex			0·715
Male	232 (56·6)	209 (58·1)	
Female	178 (43·4)	151 (41·9)	
No. of antimicrobial pretreatments			0·590
0	199 (48·5)	162 (45·0)	
1	165 (40·2)	157 (43·6)	
⩾2	46 (11·2)	41 (11·4)	
PCV7 immunization			<0·001
None	410 (100)	63 (17·5)	
Ongoing	0 (0·0)	90 (25·0)	
Complete	0 (0·0)	169 (46·9)	
Unknown	0 (0·0)	38 (10·6)	
Daycare centres			0·610
No	72 (17·6)	73 (20·3)	
Yes	211 (51·5)	176 (48·9)	
Unknown	127 (31·0)	111 (30·8)	
Patients' characteristics‡	**(*N* = 382)**	**(*N* = 337)**	
Bronchial asthma			0·038
No	287 (75·1)	275 (81·6)	
Yes	95 (24·9)	62 (18·4)	
Premature birth			0·444
Normal	355 (92·9)	321 (95·3)	
30–36 weeks	21 (5·5)	13 (3·9)	
<30 weeks	6 (1·6)	3 (0·9)	
Low-birth-weight infant			0·209
Normal	346 (90·6)	309 (91·7)	
2000–2500 g	19 (5·0)	16 (4·7)	
1500–2000 g	7 (1·8)	8 (2·4)	
1000–1500 g	6 (1·6)	0 (0·0)	
<1000 g	4 (1·0)	4 (1·2)	
Congenital heart disease			0·806
No	374 (97·9)	329 (97·6)	
Yes	8 (2·1)	8 (2·4)	
Chromosome abnormality			0·336
Normal	377 (98·7)	328 (97·3)	
Trisomy 21	3 (0·8)	7 (2·1)	
Other	2 (0·5)	2 (0·6)	
Cerebral palsy			1·000
No	380 (99·5)	336 (99·7)	
Yes	2 (0·5)	1 (0·3)	
Siblings			0·699
No	140 (36·6)	129 (38·3)	
Yes	242 (63·4)	208 (61·7)	

Values given are *n* (%).

* Proportions were analysed with Fisher's exact test.

† Hospitalization characteristics: numbers of pneumonia admission cases.

‡ Patient characteristics: numbers of patients with pneumonia admitted to hospitals.

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Table 3*b*. *Backgrounds of patients with pneumonia in the five major hospitals – antimicrobial pretreatment*

Characteristics	April 2008–March 2009 ($N = 410$)	April 2012–March 2013 ($N = 360$)	*P* value*
Penicillin			0·482
No	279 (68·0)	254 (70·6)	
Yes	131 (32·0)	106 (29·4)	
Cephalosporins			0·444
No	363 (88·5)	312 (86·7)	
Yes	47 (11·5)	48 (13·3)	
Macrolides			0·056
No	330 (80·5)	269 (74·7)	
Yes	80 (19·5)	91 (25·3)	
Quinolones			<0·001
No	410 (100)	341 (94·7)	
Yes	0 (0·0)	19 (5·3)	
Carbapenems			0·047
No	410 (100)	356 (98·9)	
Yes	0 (0·0)	4 (1·1)	
Others			0·100
No	384 (93·7)	347 (96·4)	
Yes	26 (6·3)	13 (3·6)	

Values given are *n* (%).
*Proportions were analysed with Fisher's exact test.

Table 3*c*. *Backgrounds of patients with pneumonia in the five major hospitals – logistic analysis of the confounders of the diagnosis of pneumococcal pneumonia (<5 years of age, hospitalization based)*

	Univariate analysis ($N = 770$)			Multivariate analysis ($N = 770$)		
Variables	OR	(95% CI)*	*P* value*	OR	(95% CI)*	*P* value*
Study period						
April 2008–March 2009	Ref.					
April 2012–March 2013	0·547	(0·353–0·849)	0·007	0·600	(0·386–0·934)	0·024
Quinolone antibiotics						
No	Ref.					
Yes	0·166	(0·009–2·984)	0·223	0·230	(0·012–4·175)	0·320
Bronchial asthma						
No	Ref.					
Yes	1·536	(0·966–2·441)	0·070	1·429	(0·896–2·280)	0·133

OR, Odds ratio; CI, confidence interval.
* Wald type 95% CIs and *P* values in the logistic regression models were performed based on Firth's penalized likelihood estimation [17].

Antimicrobial susceptibility of *S. pneumoniae* isolates

Antimicrobial susceptibility was determined for 46/62 (in 2008) and 28/31 (in 2012) of the *S. pneumoniae* isolated from sputum samples, and against five (in 2008) and four (in 2012) of the *S. pneumoniae* isolated from blood samples (Table 5). The MIC_{50} of TFLX increased slightly, from $\leqslant 0{\cdot}12$ μg/ml in 2008 to 0·25 μg/ml in 2012. The MIC_{50} for all other tested antibiotics remained unchanged or decreased during this interval, including that for penicillin (from 0·5 μg/ml to 0·25 μg/ml). All isolates in both years were susceptible to meropenem and vancomycin.

DISCUSSION

The introduction of PCV7 in developed countries has resulted in a decreased incidence of CAP [18, 19]. In

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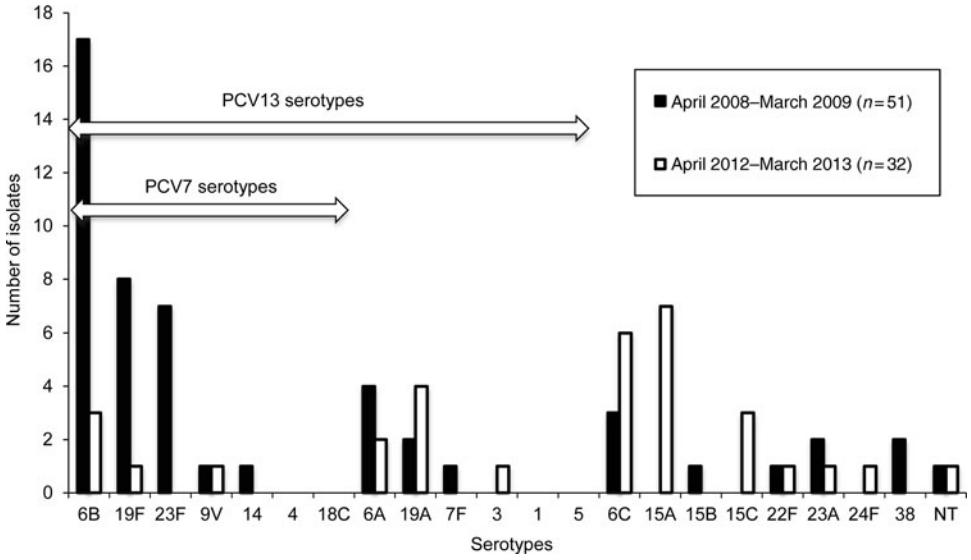

Fig. 1. Serotype distribution and vaccine coverage of *Streptococcus pneumoniae* isolated from blood and sputum samples. Major serotypes in April 2008–March 2009 (2008) which were 6B, 23 F and 19 F markedly declined by April 2012–March 2013 (2012), resulting in a decline in the PCV7 coverage rates of *S. pneumoniae* from 66·6% (34/51) in 2008 to 15·6% (5/32) in 2012 ($P < 0{\cdot}01$). The serotypes covered by PCV13 also declined from 80·4% (41/51) in 2008 to 37·5% (12/32) in 2012 ($P < 0{\cdot}01$).

the present study, the incidence of pneumonia hospitalizations in Japan was 17·6 episodes/1000 child-years in 2008, a value similar to that reported in previous studies [20]. Our findings showed a reduction of 18·9% in the incidence of CAP in children aged <5 years after the introduction of PCV7 in Japan. This reduction in the incidence is in the same range as those reported for children aged <5 years in studies in other countries (e.g. 22% reduction in the UK [21]; 13% reduction in Canada [3]), even though these studies had clearly different methodological parameters.

The reduction of PP in our study, including patients with *S. pneumoniae* isolated from sputum, is similar to the observations seen for invasive pneumococcal disease, which exhibited a reduction after the introduction of PCV7 [21, 22]. However, in contrast to previous studies, the rate of invasive PP in our study did not show a significant reduction [23]. The low prevalence of children with invasive PP in developed countries [9] may have limited the sensitivity of our results for this parameter. Given that *S. pneumoniae* is rarely isolated from blood cultures of childhood pneumonia patients, the washed sputum method used in this study was reliable, and could be used to assess the potential pathogen in children.

In our study, the early effect of PCV7 was a marked decrease in vaccine serotypes (VT). Although non-vaccine serotypes (NVT) such as 6C, 15A, 15C and 19A increased in the post-vaccine period, the decrease in VT was greater than the increase of NVT, resulting in an overall reduction in the prevalence of *S. pneumoniae* isolated from pneumonia patients. The decline in PCV7 serotypes from sputum samples in our study is similar to the results of other studies related to invasive disease and nasal carriage in children [24, 25]. Particularly, serotype 6B markedly decreased not only in numbers, but also in genetic diversity, as STs were reduced from 11 in 2008 to two in 2012. All three patients with VT from sputum had serotype 6B and incomplete PCV7 vaccination. These 'breakthrough cases' are known to occur, especially in relation to serotype 6B in invasive pneumococcal diseases when children have incomplete vaccination [26]. This implies that immunization must be completed for PCV7 to have a reliable effect on PP. Serotypes with increasing prevalence in our study may reflect secular trends in Chiba city, and so may predict future trends, as seen in the increase of serotypes 19A [5–7] and 6C [27] for invasive pneumococcal diseases worldwide after the introduction of PCV7. Serotype 15A increased in Norway for invasive pneumococcal disease [28], and in Canada for nasopharyngeal colonization [29], both of which occurred after the introduction of PCV13. Serotype 15A is also known

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Table 4. *Multilocus sequence typing and allelic profiles of* Streptococcus pneumoniae *isolates from children admitted to five hospitals in Japan before (April 2008–March 2009) and after (April 2012–March 2013) the introduction of PCV7*

Vaccine	Serotype	Sequence type	PMEN clones*	No. of isolates	
				April 2008–March 2009 ($n = 51$)	April 2012–March 2013 ($n = 32$)
PCV7 and PCV13	6B	90	**Spain^{6B}-2**	7	1
		902		1	0
		2224		1	0
		2923		1	0
		2924		1	0
		5232		1	0
		5830		1	0
		5831		1	0
		5834		1	0
		5494		1	0
		5497		1	0
		6410		0	1
		9026		0	1
	19 F	236	**Taiwan^{19F}-14**	6	1
		2993		1	0
		5495		1	0
	23 F	242	**Taiwan^{23F}-15**	4	0
		1437		3	0
	9 V	280		1	1
	14	343		1	0
PCV13	6A	282		1	1
		855		1	0
		5833		2	1
	19A	2331		1	0
		3111		1	3
		5842		0	1
	7 F	191	Netherlands^{7F}-39	1	0
	3	180	Netherlands^{3}-31	0	1
Others	6C	2924		0	1
		5241		0	2
		5832		3	2
		6183		0	1
	15A	63	**Sweden^{15A}-25**	0	7
	15B	199	Netherlands^{15B}-37	1	0
	15C	83		0	1
		199	Netherlands^{15B}-37	0	2
	22 F	433		1	1
	23A	338		1	1
		5246		1	0
	24 F	5496		0	1
	38	393		2	0
	NT	4845		0	1
		5496		1	0

* PMEN clones: clones included in the Pneumococcal Molecular Epidemiology Network. Clones known as multidrug-resistant PMEN clones are shown in bold.

to have multidrug resistance [30]. In our study, seven isolates in 2012 were the Sweden^{15A}-25/ST63 strain, which is known as one of the MDR PMEN clones, and two of the 2012 serotype 15A isolates were from blood samples. This explains the high percentage of MDR PMEN clones in the post-vaccine period

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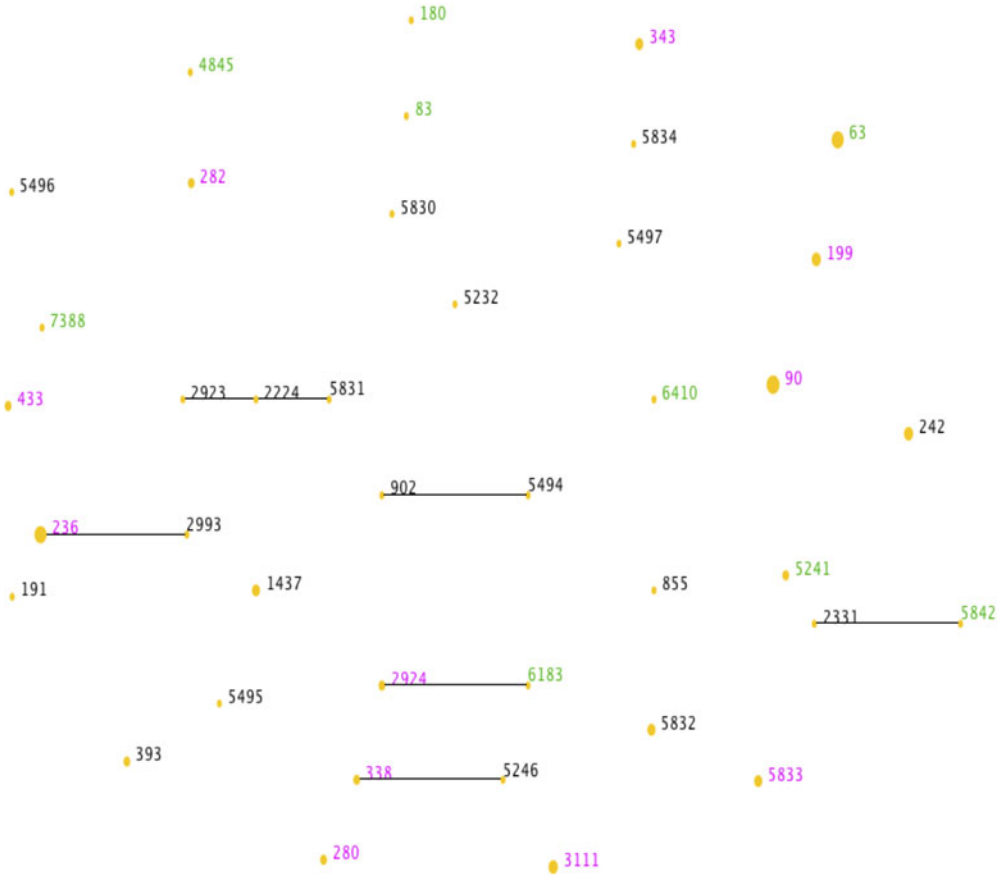

Fig. 2. Population snapshot of *Streptococcus pneumoniae* isolated in April 2008–March 2009 and April 2012–March 2013 using eBURST analysis. Each spot represents a single sequence type (ST) (with ST designation as indicated), such that the size of the spot is proportional to the number of *S. pneumoniae* isolates with each ST. Lines indicate the presence of clonal complex links between/among particular STs. ST designations in black represent STs found only in the April 2008–March 2009 (2008) dataset; ST designations in green represent STs found only in the April 2012–March 2013 (2012) dataset; ST designations in purple represent STs found in both the 2008 and 2012 datasets.

(28·1%), even after the decrease in VT after the introduction of PCV7. Indeed, the high percentage of MDR PMEN clones due to serotype 15A requires careful monitoring in the future.

In our study, which used the latest established breakpoints, no penicillin non-susceptible strains were identified. Furthermore, the MIC_{50} of penicillin decreased with the reduction of VT, as seen in previous studies [8]. Some studies have reported that macrolide susceptibility increased along with the introduction of PCV7 [31, 32]; however, our results show high levels of macrolide resistance in both 2008 and 2012. One reason for this may be that macrolides were widely used for treating childhood respiratory infections during the *Mycoplasma* epidemic in Japan from the second half of 2011 to the end of 2012 [33]. On the other hand, a slight increase of high TFLX MIC isolates was evident in the post-PCV7 era. TFLX is an oral fluoroquinolone developed by Toyama Chemical Co. Ltd in 1990. It was approved for children in Japan in January 2010. TFLX provides a broad spectrum of antibacterial activity against various causative organisms of respiratory tract infections (e.g. *S. pneumoniae, H. influenzae, M. catarrhalis*) [34]. To the best of our knowledge, Japan is the only country with an oral fluoroquinolone licensed for treating children with respiratory tract infections, and TFLX use in Japan markedly increased in 2012. A study of invasive pneumococcal disease patients revealed that those who had received fluoroquinolone treatment prior to developing pneumococcal diseases were 12 times more likely to be infected with fluoroquinolone-resistant isolates [35]. The decrease of TFLX susceptibility also may be due to the increased use of TFLX during the *Mycoplasma* epidemic, resulting from the

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Table 5. *Drug susceptibility of* Streptococcus pneumoniae *isolates from children admitted to five hospitals in Japan*

	April 2008–March 2009 ($N = 51$)		April 2012–March 2013 ($N = 32$)	
Antimicrobials	MIC_{50} (μg/ml)	Range	MIC_{50} (μg/ml)	Range
Penicillin	0·5	⩽0·015–2	0·25	⩽0·015–2
Amoxicillin	1	⩽0·03–4	0·25	⩽0·03–4
Cefditoren	0·25	⩽0·03–2	0·25	⩽0·03–1
Cefotaxime	0·5	⩽0·03–4	0·25	⩽0·03–1
Meropenem	0·12	⩽0·008–0·5	0·06	⩽0·008–0·5
Panipenem	0·03	⩽0·008–0·12	0·015	⩽0·008–0·12
Tebipenem	0·015	⩽0·008–0·12	⩽0·008	⩽0·008–0·12
Erythromycin	⩾8	⩽0·12–⩾8	⩾8	⩽0·12–⩾8
Clindamycin	⩾8	⩽0·12–⩾8	⩾8	⩽0·12–⩾8
Tosfuloxacin	⩽0·12	⩽0·12–0·5	0·25	⩽0·12–0·5
Vancomycin	0·25	0·25–0·5	0·25	⩽0·12–0·25

MIC_{50}, Fifty percent minimum inhibitory concentration.

high macrolide resistance of *Mycoplasma pneumoniae* in Japan [36]. Levofloxacin resistance in *S. pneumoniae* is known to lead to increased unfavourable outcomes for adult CAP patients [37]. Widespread use of TFLX may become a driving force of TFLX resistance of *S. pneumoniae* in Japan. Physicians should be aware of the proper use of broad-spectrum antimicrobial agents for children.

This study has some limitations. First, due to the rapid introduction of PCV13 (after 3 years of PCV7 introduction) in Japan, we were only able to compare two 1-year studies. Second, the diagnosis of pneumonia was not based on a standardized method, for example, the definitions for radiological pneumonia developed by a WHO group [38]. WHO criteria is rarely used in Japan, and thus we followed the same method used in the pre-vaccine study [13].

In November 2013, PCV13 was approved as a vaccine for Japanese children aged from 2 to 71 months. PCV13 contains six new serotypes (1, 3, 5, 6A, 7 F, 19A) in addition to those contained in PCV7. In the United States, PCV13 is showing early benefit on invasive pneumococcal disease, due in part to the high prevalence of serotype 19A [23], which was also identified as one of the increasing serotypes in our study. It is interesting to monitor the frequency of serotype 19A after the introduction of PCV13, and also serotype 6C, because cross-protection is known to occur between serotypes 6A and 6C for nasal carriage [39]. However, the serotypes with the greatest increase in 2012, such as 15A and 15C, are not covered by PCV13. Due to the low coverage rate of PCV13 serotypes in those remaining after the introduction of PCV7 in Chiba city that cause pneumonia, these results suggest that pneumonia admission due to *S. pneumoniae* may not show a great benefit after introducing PCV13 in Japan. To predict future prevalence and effectively prevent pneumococcal disease in Japan, it is important to continue monitoring changes in serotype frequencies during the post-PCV13 period.

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DECLARATION OF INTEREST

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