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Impact of Seven Valent Pneumococcal Conjugate Vaccine on Nasopharyngeal Carriage in Young Children in Okinawa, Japan

Hideki Akeda[1], Bin Chang[2], Yoshihisa Nakamura[3], Hirotsune Hamabata[4], Kenji Ameku[5], Takaya Toma[6], Eiichi Tamanaha[7], Makoto Ohnishi[2]

[1]Okinawa Prefectural Nanbu Medical Center & Children's Medical Center, Okinawa, Japan
[2]Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan
[3]Nakamura Clinic Pediatric, Dermatology and Internal Medicine, Okinawa, Japan
[4]Awase Daiichi Clinic, Okinawa, Japan
[5]Syuri Child Clinic, Okinawa, Japan
[6]Wanpaku Clinic, Okinawa, Japan
[7]The Okinawa Society of Child Health, Okinawa, Japan
Email: akeda_hideki@hosp.pref.okinawa.jp

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Abstract

In Japan, the heptavalent pneumococcal conjugate vaccine (PCV7) became available in February 2010 and was subsidized by the national funding system from May 2011 in Okinawa, after which it was incorporated into the national immunization practice (NIP) in April 2013 using a 3 + 1 schedule for all infants. We conducted an annual survey in 2012 to determine the effect of PCV7 on nasopharyngeal colonization by pneumococcal serotypes and to analyze the risk factors for colonization in infants. Nasopharyngeal swabs for pneumococcal isolation and serotyping were obtained from infant 2 to 22 months of age before and after PCV7 immunization among 4 clinics in Okinawa, Japan. Between January 2012 and December 2012, nasopharyngeal swabs for bacterial cultures were obtained among 782 infants aged 2 to 22 months old and demographic data was obtained among 725 participant infants. Among the 725 evaluable infants, 193 pneumococcal strains were detected in 180 infants for an overall nasopharyngeal carriage of 24.8%. The main capsular serotypes isolated were 6C (16.1%), 19A (12.4%) and 15B (9.8%). Carriage of PCV7 serotypes accounted for 21.8% (42/193). The result of multivariate data analysis showed the pneumococcal carriage rate of non-PCV7 serotypes was significantly ($P < 0.001$) high in infant with siblings and daycare attendance. On the other hand, the result of multivariate data analysis showed that carriage rate of PCV7 serotype had only significantly high risk in infant with siblings and did not have

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a significant risk dependent on age and daycare attendance. Carriage PCV7 serotypes increased in the presence of other siblings, while PCV7 vaccination was shown to eliminate daycare attendance as a risk. The results of this study demonstrates that PCV7 vaccination decrease the overall nasopharyngeal carriage of PCV7 serotypes in vaccinated children including children at risk such as children attending day-care centers.

Keywords

Nasopharynx, Carriage, Pneumococcal Conjugate Vaccine, Japan, Young Children

1. Introduction

The nasopharynx is the primary reservoir of *Streptococcus pneumoniae*, and nasopharyngeal and oropharyngeal carriage usually precedes and, in most of the cases, parallels pneumococcal infection [1] [2]. Although pneumococcal bacteria are widely spread in the community, nasopharyngeal carriage (NPC) rates are particularly high in preschool children attending day-care centers [3]. Additionally, *Streptococcus pneumonia* is a major source of morbidity and mortality worldwide. It is estimated that at least one million children under the age of 5 years old die of pneumococcal disease every year, mostly in developing countries [4]-[7]. Invasive pneumococcal disease (IPD) in children is associated with considerable case-fatality rates and rates of sequelae even in industrialized countries. The heptavalent pneumococcal conjugate vaccine (PCV7) is made by coupling the polysaccharide capsule portion of the organism to a carrier protein. PCV7 includes seven polysaccharide antigen from pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F (Prevenar®). The vaccine contain 2 μg per serotype, except serotype 6B which contains 4 μg. Immunization with the heptavalent pneumococcal conjugate vaccine (PCV7 includes serotype 4, 6B, 9V, 14, 18C, 19F and 23F) has reduced invasive pneumococcal disease (IPD) and other pneumococcal infections among children [4]-[6]. Several studies have shown that receipt PCV7 vaccination is accompanied by a rapid decrease in PCV7 serotypes of IPD and replacement by non-vaccine serotypes [4] [7]. At the same time, increase in nasopharyngeal non-PCV7 serotypes has been associated with an increase in non-PCV7 IPD and pneumococcal infections with antibiotic-resistant pneumococci [8]. Many studies have been conducted overseas on the investigations of nasopharyngeal carriage before and after the introduction of PCV7 [9]-[13]. One report has been published in Japan on the analysis of risk factors after the introduction of PCV7, but pneumococcal serotypes have not been confirmed [14]. With this background, it is considered that there is a strong involvement of nasopharyngeal carriage in pneumococcal infections and the various effects of PCV7 can be promptly confirmed by investigating the nasopharyngeal carriage of pneumococci. PCV7 was released in Japan 10 years after it was released in America, and was subsidized by the national funding system from May 2011 in Okinawa. It is considered that there will be sufficient confirmation of the effects in 2012, second year after the introduction of PCV7. The purpose of our study, conducted in 2012, was to evaluate the impact of PCV7 inclusion to NIP in Okinawa Island of Japan and to evaluate the effect on the nasopharyngeal *S. pneumoniae* colonization.

2. Method

2.1. Study Population

The study target population and inclusion criteria are healthy infants aged 2 - 22 months old who visited 4 pediatric clinics on the main island of Okinawa from January to December 2012 for vaccination. Okinawa Island is located at the south of the Japanese Archipelago and the island can be considered as an appropriate environment for investigating pathogens reservoir due to limited movement of people (especially children) between Okinawa Island and the Japanese mainland. A cotton swab was used to collect nasopharyngeal mucus after obtaining consent from the parents. This study was implemented after registration for clinical trial and with approval from the ethics committee at Okinawa Prefectural Nanbu Medical Center & Children' Medical Center.

2.2. Bacterial Identification and Serotyping

Nasopharyngeal swabs were delivered via a swab transport system (BD culture swab TM) to Department of

Bacteriology I at the National Institute of Infectious Diseases for isolation and identification of *S. pneumoniae*. All of the *S. pneumoniae* isolates were serotyped using Quellung reaction with commercially available antisera (Statens Serum Institute, Copenhagen, Denmark). Homemade antiserum for serotypes 6C and 6D were also used [15].

2.3. Statistical Analysis

Demographic characteristics were also collected including sex, age, PCV7 vaccination history and frequency, daycare attendance, and number of siblings. Assessments and analysis methods included the carriage rate for *S. pneumoniae* based on differences in background factors, and analysis of the impact of risk factors on the *S. pneumoniae* carriage rate and sero-type prevalence using univariate logistic regression analysis and multivariate logistic regression analysis. Age and number of siblings were used as explanation variables for logistic regression analysis by converting to binary data based on the paper of Ueno *et al.* [14]. In the same way, the number of PCV7 vaccinations were divided into two types, the first to the third dose considered as initial immunization and fourth dose considered as booster immunization, and was used by targeting non vaccinated. Odds ratios, 95% confidence intervals (95% CI) and P-values were calculated for each independent variable. In case of duplicate registration, data was only utilized from the initial registration to avoid bias. All statistical analyses were performed using SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA). All P-values were two-sided, and $P < 0.05$ was considered statistically significant.

3. Result

From January 2012 to December 2012, 782 infants were enrolled. The number of evaluated participants dropped to 725 after excluding data of infants registered more than once. Of these, 371 subjects were male and 354 were female. **Table 1** shows the characteristics (subject demographics) for each PCV7 vaccination. The average age was 9 months old ± 5.2 months old, with 7 months old as the median, the youngest age was 2 months old and the oldest 22 months old. By age, 278 cases (38.3%) were 2 months old up to less than 6 months old, 154 cases (21.2%) were 6 months old up to less than 10 months old, 161 cases (22.2%) were 10 months old up to less than 14 months old, 43 cases (5.9%) were 14 months old up to less than 18 months old, 88 cases (12.1%) were 18 months old up to less than 22 months old, and 1 case (0.1%) was 22 months old. For daycare attendance, 158 cases (21.8%) attended daycare, 566 cases (78.2%) did not, and in 1 case it was not clear. No difference was observed in the number of registrations by season (**Table 1**). **Table 2** shows the carriage rate for *S. pneumoniae* for each risk factor and vaccinations. The overall carriage rate for the total number of subjects evaluated was 24.8% (180/725). The carriage rate for infants less than 12 months, which was 16.8% prior to the booster dose cutoff point, while the carriage rate was 37.6% for infants 12 months and older. Thus, the carriage rate was significantly higher for older infants. No significant difference was observed in carriage rate between male and female in terms of PCV7 vaccination. On the other hand, the carriage rate was 58.2% for infants attending daycare and 15.4% for infants not attending daycare, and a significant difference also was seen between infants with siblings (34.2%) and infants without siblings (11.0%). **Table 3** shows the distribution of each serotype of *S. pneumoniae* (193 isolates). For those with a frequency of at least 5%, 6C (31 isolates; 16.1%) was the most prevalent, followed in order by 19A (12.4%), 15B (9.8%), 6B (9.3%), 15C (6.7%), and 23F (5.2%). The isolation rate for 7-valent serotypes was 21.8 % (42/193) and 38.3% (74/193) for 13-valent serotypes. **Table 4** shows the *S. pneumoniae* carriage situation for each PCV7 vaccination. The ratio of carrier was 25.4% in infants not vaccinated with PCV7. In infants that received PCV7 vaccination one to two times, the ratio of carrier was 20.7% and 20.3%, respectively, while the ratio was 29.7% and 37.1% for infants who received three and four vaccinations of PCV7. Thus, the carrier ratio of *S. pneumoniae* for infants who received vaccination three or four times was higher compared to infants who were not vaccinated and received vaccination once or twice (**Table 4**). On the other hand, the ratio for carriers of PCV7 serotypes was 10.7% for non-vaccinated, 5.3% for the first vaccination of PCV7, 5.9% for the second vaccination of PCV7, 3.3% for the third vaccination, and 2.9% for the fourth vaccination (**Table 4**). The number of PCV7 vaccinations received appears to be correlated with the decline of PCV7 serotype carriage. The results of the univariate and multivariate logistic regression analysis of the risk factors of *S. pneumoniae* carriage for PCV7 serotypes and non-PCV7 serotypes are shown in **Table 5** and **Table 6**. These results confirmed that for *S. pneumoniae* carriage for non- PCV7 serotypes (**Table 6**), exposure through daycare attendance and siblings is a significant risk factor, with odds ratios of Crude OR 9.882 (95% CI:

Table 1. Characteristics of the subjects in each PCV7 dose.

		Dose of PCV7					Total
		0	1st dose	2nd dose	3rd dose	4th dose	
Number of subjects		122	169	187	212	35	725
Gender	Male	58 (47.5%)	84 (49.7%)	99 (52.9%)	112 (52.8%)	18 (51.4%)	371 (51.2%)
	Female	64 (52.5%)	85 (50.3%)	88 (47.1%)	100 (47.2%)	17 (48.6%)	354 (48.8%)
Age in months	N	122	169	187	212	35	725
	Mean (SD)	8.0 (5.7)	6.5 (4.4)	7.5 (4.5)	11.5 (4.0)	16.9 (2.8)	9.0 (5.2)
	Median (Q1, Q3)	5.0 (4.0, 12.0)	4.0 (4.0, 7.0)	6.0 (4.0, 12.0)	12.0 (8.0, 13.0)	18.0 (17.0, 18.0)	7.0 (4.0, 12.0)
	(Minimum-Maximum)	(2.0 - 22.0)	(3.0 - 21.0)	(3.0 - 20.0)	(4.0 - 21.0)	(8.0 - 20.0)	(2.0 - 22.0)
	<2 mo	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2 mo - 5 mo	62 (50.8%)	112 (66.3%)	89 (47.6%)	15 (7.1%)	0 (0.0%)	278 (38.3%)
	6 mo - 9 mo	24 (19.7%)	29 (17.2%)	49 (26.2%)	51 (24.1%)	1 (2.9%)	154 (21.2%)
	10 mo - 13 mo	13 (10.7%)	11 (6.5%)	30 (16.0%)	103 (48.6%)	4 (11.4%)	161 (22.2%)
	14 mo - 17 mo	7 (5.7%)	6 (3.6%)	5 (2.7%)	18 (8.5%)	7 (20.0%)	43 (5.9%)
	18 mo - 21 mo	15 (12.3%)	11 (6.5%)	14 (7.5%)	25 (11.8%)	23 (65.7%)	88 (12.1%)
	≥22 mo	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
Group child care attendance	Not attending	105 (86.1%)	147 (87.0%)	161 (86.6%)	135 (63.7%)	18 (51.4%)	566 (78.2%)
	Attending	17 (13.9%)	22 (13.0%)	25 (13.4%)	77 (36.3%)	17 (48.6%)	158 (21.8%)
	Unclear	0	0	1	0	0	1
# of young siblings	0	34 (27.9%)	65 (38.5%)	73 (39.0%)	105 (49.5%)	15 (42.9%)	292 (40.3%)
	1	32 (26.2%)	55 (32.5%)	64 (34.2%)	54 (25.5%)	14 (40.0%)	219 (30.2%)
	2	35 (28.7%)	30 (17.8%)	34 (18.2%)	32 (15.1%)	4 (11.4%)	135 (18.6%)
	3	15 (12.3%)	12 (7.1%)	7 (3.7%)	15 (7.1%)	2 (5.7%)	51 (7.0%)
	≥4	6 (4.9%)	7 (4.1%)	9 (4.8%)	6 (2.8%)	0 (0.0%)	28 (3.9%)
In case with sibling, their daycare attendance	Not attending	15 (17.0%)	17 (16.3%)	25 (21.9%)	9 (8.4%)	5 (25.0%)	71 (16.4%)
	Attending	73 (83.0%)	87 (83.7%)	89 (78.1%)	98 (91.6%)	15 (75.0%)	362 (83.6%)
Season	Mar-May (Spring)	28 (23.0%)	33 (19.5%)	57 (30.5%)	49 (23.1%)	6 (17.1%)	173 (23.9%)
	Jun-Aug (Summer)	41 (33.6%)	62 (36.7%)	43 (23.0%)	59 (27.8%)	10 (28.6%)	215 (29.7%)
	Sep-Nov (Fall)	24 (19.7%)	33 (19.5%)	42 (22.5%)	59 (27.8%)	14 (40.0%)	172 (23.7%)
	Dec-Feb (Winter)	29 (23.8%)	41 (24.3%)	45 (24.1%)	45 (21.2%)	5 (14.3%)	165 (22.8%)

Table 2. The carriage rate in various factors (Dose number of PCV7).

		Dose of PCV7															All subject		
		0			1st dose			2nd dose			3rd dose			4th dose					
		Carrier of *S. pneumonia*		Carriage rate	Carrier of *S. pneumonia*		Carriage rate	Carrier of *S. pneumonia*		Carriage rate	Carrier of *S. pneumonia*		Carriage rate	Carrier of *S. pneumonia*		Carriage rate	Carrier of *S. pneumonia*		Carriage rate
		No	Yes	(%)	No	Yes	(%)	No	Yes	(%)	No	Yes	(%)	No	Yes	(%)	No	Yes	(%)
Age	<12 mo	70	17	19.5	121	21	14.8	116	23	16.5	63	14	18.2	1	0	0.0	371	75	16.8*
	≥12 mo	21	14	40.0	13	14	51.9	33	15	31.3	86	49	36.3	21	13	38.2	174	105	37.6*
Gender	Female	49	15	23.4	67	18	21.2	76	12	13.6	70	30	30.0	8	9	52.9	270	84	23.7
	Male	42	16	27.6	67	17	20.2	73	26	26.3	79	33	29.5	14	4	22.2	275	96	25.9
Daycare	Not attending	86	19	18.1	126	21	14.3	138	23	14.3	113	22	16.3	16	2	11.1	479	87	15.4*
	Attending	5	12	70.6	8	14	63.6	11	14	56.0	36	41	53.2	6	11	64.7	66	92	58.2*
Sibling	No	30	4	11.8	62	3	4.6	71	2	2.7	87	18	17.1	10	5	33.3	260	32	11.0*
	Has	61	27	30.7	72	32	30.8	78	36	31.6	62	45	42.1	12	8	40.0	285	148	34.2*
Season	Mar-May	21	7	25.0	26	7	21.2	49	8	14.0	37	12	24.5	4	2	33.3	137	36	20.8
	Jun-Aug	29	12	29.3	52	10	16.1	32	11	25.6	43	16	27.1	7	3	30.0	163	52	24.2
	Sep-Nov	20	4	16.7	26	7	21.2	33	9	21.4	34	25	42.4	10	4	28.6	123	49	28.5
	Dec-Feb	21	8	27.6	30	11	26.8	35	10	22.2	35	10	22.2	1	4	80.0	122	43	26.1
																Total	545	180	24.8

*:Significant difference is determined when $P < 0.001$.

Table 3. The distribution of serotypes in healthy children.

		N	(%)
Carriage situation of *S. pneumoniae*	non carrier	545	(75.2%)
	carrier	180	(24.8%)
Total number of serotypes		193	
The number of each serotype (The denominator is total number of isolated serptypes which includes carriage of multiple serotypes)	6B	18	(9.3%)
	23F	10	(5.2%)
	19F	8	(4.1%)
	14	4	(2.1%)
	18C	2	(1.0%)
	19A	24	(12.4%)
	6A	8	(4.1%)
	6C	31	(16.1%)
	15B	19	(9.8%)
	Untypeable	19	(9.8%)
	15C	13	(6.7%)
	15A	9	(4.7%)
	23A	9	(4.7%)
	11A	6	(3.1%)
	11E	5	(2.6%)
	10A	3	(1.6%)
	22F	3	(1.6%)
	38	1	(0.5%)
	35B	1	(0.5%)
The number of PCV7 serotypes isolated	non-PCV7	151	(78.2%)
	PCV7	42	(21.8%)

Table 4. The number of *S. pneumonia* carriers among subjects (ratio of carrier) in various PCV7 dose status.

		PCV7 dose status									
		Non	(n = 122)	1shot	(n = 169)	2shots	(n = 187)	3shots	(n = 212)	4shots	(n = 35)
The number of *S.pneumonia* carriers among all subjects	non-carrier	91	(74.6%)	134	(79.3%)	149	(79.7%)	149	(70.3%)	22	(62.9%)
	carrier	31	(25.4%)	35	(20.7%)	38	(20.3%)	63	(29.7%)	13	(37.1%)
The number of PCV7 serotypes carriers among all subjects	non-carrier	109	(89.3%)	160	(94.7%)	176	(94.1%)	205	(96.7%)	34	(97.1%)
	PCV7 types carriers	13	(10.7%)	9	(5.3%)	11	(5.9%)	7	(3.3%)	1	(2.9%)

Table 5. Univaluate and multivaluate logistic regression analysis.

Outcome variable: carriage of PCV7 serotypes (N = 724)							
		Crude OR	95% CI	P	Adjusted OR	95% CI	P
Age in months	≥12 mo vs < 12 mo	1.268	0.672 - 2.395	0.464	1.580	0.740 - 3.376	0.238
Gender	Male vs Female	0.904	0.481 - 1.697	0.753	0.849	0.444 - 1.625	0.622
Daycare attendance	Attending vs Not attending	1.166	0.559 - 2.433	0.682	1.073	0.459 - 2.505	0.871
Sibiling	Has vs Non	9.261*	2.832 - 30.285	< 0.001	8.184*	2.484 - 26.963	< 0.001
PCV7 status	1 - 3 shots vs non-vaccination	0.418*	0.209 - 0.837	0.014	0.491	0.240 - 1.003	0.051
	4 shots vs non-vaccination	0.247	0.031 - 1.954	0.185	0.240	0.028 - 2.036	0.191

*Significant difference is determined when P < 0.05.

Table 6. Univaluate and multivaluate logistic regression analysis.

Outcome variable: carriage of non-PCV7 serotypes (N = 724)							
		Crude OR	95% CI	P	Adjusted OR	95% CI	P
Age in months	≥12 mo vs < 12 mo	3.416*	2.331 - 5.006	<0.001	1.325	0.794 - 2.212	0.281
Gender	Male vs Female	1.187	0.820 - 1.717	0.363	0.980	0.640 - 1.500	0.926
Daycare attendance	Attending vs Not attending	9.882*	6.523 - 14.968	<0.001	8.010*	4.910 - 13.069	<0.001
Sibiling	Has vs Non	3.164*	2.038 - 4.911	<0.001	3.404*	2.079 - 5.574	<0.001
PCV7 status	1 - 3 shots vs non-vaccination	1.302	0.765 - 2.216	0.331	1.164	0.643 - 2.108	0.616
	4 shots vs non-vaccination	2.828	1.206 - 6.633	0.017	1.420	0.525 - 3.839	0.490

* Significant difference is determined when P < 0.05.

6.523-14.968), Adjusted OR 8.010 (95%CI: 4.910 - 13.069) and Crude OR 3.164 (95% CI: 2.038 - 4.911), Adjusted OR 3.404 (95%CI: 2.079 - 5.574), respectively. Moreover, carrier risk of non-PCV7 streptococcus *pneumoniae* serotype for ages 12 months or more and 4 vaccinations was significantly higher with only univariate analysis. On the other hand, it was confirmed that the exposure to siblings was a significant risk factor with odds at Crude OR 9.261 (95% CI: 2.832 - 30.285), Adjusted OR 8.184 (95% CI: 2.484 - 26.963), while it was indicated that PCV7 vaccination eliminates daycare attendance as a risk factor in carriage of *S. pneumoniae* for PCV7 serotypes (**Table 5**). In addition, the risk of PCV7 carrier serotype for the group with one to three PCV7 vaccinations reduced significantly compared to non vaccinated group with univariate analysis, Crude OR 0.418 (95% CI: 0.21 - 0.84).

4. Discussion

We studied nasopharyngeal carriage in healthy children for one year in an environment where PCV7 vaccination

rates were increasing. The *S. pneumoniae* carriage rate in registered infants aged 2 - 22 months old was 24.8%, while the carriage rate in infants attending daycare was higher at 58.2%. Overseas reports have also stated the carriage rate to be 20% - 40% in healthy children, and 60% or more in children attending daycare [3] [9]-[13]. Those reports are in line with the results of our study. It is believed that *S. pneumoniae* is carried at nearly the same rate among developed countries, even though there may be regional differences. Also, just as stated in existing reports, this study confirmed that siblings were a risk factor in the carriage of *S. pneumoniae*. Serotype 6C is the most prevalent for the diffusion of carried *S. pneumoniae* serotypes, followed by 19A and 15B. As expected, it is confirmed that the carriage rate for non-PCV7 serotypes is high in an environment with PCV7 vaccination. This study was conducted in the second year after PCV7 vaccinations increased through a public subsidy program. Serotype replacement was confirmed in a research implemented during the same period on causative agents for IPD in Japan [16]. It was surmised to be antecedent to IPD with serotype replacement appearing in the nasopharynx. In regard to the correlation between number of PCV7 vaccinations and the *S. pneumoniae* carriage rate, looking at *S. pneumoniae* as a whole, the carriage rate increased as the number of PCV7 vaccinations increased, but this can be attributed to interference from external factors. With the increase in number of vaccinations, the child also grows older, opportunities for contact with external environments grow, and the carriage rate increases [17]. To support this, while the overall carriage rate for *S. pneumoniae* increased, the carriage rate declined for heptavalent serotypes correlated with number of PCV7 vaccinations. A multivariate analysis conducted on various risk factors for *S. pneumoniae* carriage rate showed that the higher the number of PCV7 vaccinations received, the more significant the decrease in carriage of *S. pneumonia*e heptavalent serotypes becomes, however, no significant changes were recognized with respect to the carriage of *S. pneumoniae* non-heptavalent serotypes. Also, in the case of non-heptavalent serotypes, the risk for carriage increased due to exposure through daycare attendance (nursery school, kindergarten) since there was no PCV7 intervention. However, in the case of heptavalent serotypes, exposure due to daycare attendance was not a risk because of PCV7 intervention. Daycare attendance and differences in race/ethnic groups were risk factors for IPD infection based on epidemiological research conducted on IPD in the United States, but after introduction of vaccine these factors were no longer reported to be risks [18]. Having a sibling was a significant risk factor for both heptavalent serotypes and non- heptavalent serotypes. The difference with daycare attendance can be attributed to disparities in the activity of carrier infants, degree of contact with carrier infants, and amount of exposure. That is, contact with older children is limited in daycare and the majority of time is spent with children of the same age, but if an older sibling is present at home, there is a wider scope of activities and the opportunity to come in contact with the sibling increases. Additionally, it can also be considered that contact between young children (degree of contact) is not very high in daycares and a hygienic environment is maintained with wash rooms, etc., compared to home, while longer time is spent with siblings in enclosed spaces. The limit for the present research was up to 1 year after the introduction of PCV7, therefore collective regional effect could not be confirmed since the transition of pneumococcal serotype was not checked over many years, and also only infants who had visited the clinic for vaccination were considered. However, we considered that effects on pneumococcal serotype and risk factors depending on PCV7 vaccination could be confirmed.

To summarize the above results, when rate of immunization following the introduction of PCV7 is high, nasopharyngeal carriage rate for *S. pneumoniae* heptavalent serotypes clearly decreases, while the ratio of *S. pneumonia* non-heptavalent serotypes increases. Daycare attendance ceases to be a risk factor for the carriage of heptavalent serotype *S. pneumoniae* due to PCV7 vaccination. Therefore, it can be surmised that vaccination for *S. pneumoniae* at an early stage prior to entering daycare will be extremely beneficial, in other words, from the age of 2 months old. In conclusion, PCV7 have impacted nasopharyngeal carriage for PCV7 serotypes *S. pneumoniae* among vaccinated children. Same research has to be conducted in the future after the introduction of 13-valent pneumococcal conjugate vaccine in Japan.

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