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Original Article

Changes in *Streptococcus pneumoniae* Serotypes in the Nasopharynx of Japanese Children after Inoculation with a Heptavalent Pneumococcal Conjugate Vaccine

Masahiro Ueno[1,2]*, Yoshikazu Ishii[3], Kazuhiro Tateda[3], Yoshiko Anahara[4], Akiko Ebata[4], Masaei Iida[4], Fumie Mizuno[5], Seiko Inamura[6], Kaori Takahata[7], Yoko Suzuki[7], Bin Chang[8], Akihito Wada[8,9], Minoru Sugita[2], Taichiro Tanaka[2], and Yuji Nishiwaki[2]

[1]*Department of Pediatrics and*
[5]*Department of Otorhinolaryngology, Hasuda Issinkai Hospital, Saitama 349-0123;*
[2]*Department of Environmental and Occupational Health and*
[3]*Department of Microbiology and Infectious Diseases, School of Medicine, Toho University, Tokyo 143-8540;*
[4]*Department of Bacteriology, Ageo Medical Laboratories, Inc., Saitama 340-0808;*
[6]*Yashio Ekimae Internal Medicine and Pediatric Clinic, Saitama 340-0822;*
[7]*Department of Pediatrics, Tokyo Women's Medical University Medical Center East, Tokyo 116-8567;*
[8]*Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo 162-8640; and*
[9]*Yonaguni Medical Clinic, Okinawa 907-1801, Japan*

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SUMMARY: In this study, we prospectively investigated changes in *Streptococcus pneumoniae* serotypes among Japanese children after heptavalent pneumococcal conjugate vaccine (PCV7) inoculation. We acquired nasopharyngeal swabs from the children at each routine PCV7 inoculation and again at least 2 months after the last PCV7 inoculation. We defined 2 periods with regard to each culture: the inoculation period as ''the period of pre- or incomplete vaccination'' and post-inoculation as ''the period of post- or completed vaccination.'' The prevalence of vaccine-type (VT) pneumococci was significantly reduced from 9.5% in the inoculation-period cultures to 2.9% in the post-inoculation cultures ($P < 0.01$). There was no statistical difference in the prevalence of non-vaccine-type pneumococci between the inoculation-period and post-inoculation cultures (24.1% versus 23.4%). The protection of PCV7 against nasopharyngeal colonization was inferred from the decrease in VT carriage post-inoculation. The decrease in VT carriage may be conducive to reducing VT transmission within the study area.

INTRODUCTION

Streptococcus pneumoniae is part of the commensal flora of the nasopharynx, but it is also a clinically important pathogen that causes invasive pneumococcal diseases, such as sepsis, meningitis, and pneumonia, in children (1). In the United States, the introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) resulted in a decreased incidence of invasive pneumococcal diseases through a reduction in the prevalence of vaccine-type (VT) (serotypes included in PCV7) pneumococci among vaccinated children (2,3). It also had indirect effects on pneumococcal transmission through herd immunity (4).

PCV7 was introduced in Japan in 2010 and has been administered under the National Health Insurance System since 2011. The standard PCV7 schedules in Japan are as follows: 3 doses between the ages of 2 months and 7 months, followed by 1 booster dose after the age of 12 months (3 + 1-dose); 2 doses between the ages of 7 months and 11 months, followed by 1 booster dose after the age of 12 months (2 + 1-dose); 2 doses between the ages of 12 months and 23 months (2-dose); and a single dose after the age of 24 months (1-dose).

The present study is the first to prospectively investigate changes in *S. pneumoniae* serotypes in the nasopharynx of Japanese children inoculated with PCV7.

MATERIALS AND METHODS

Study population: During the study period (July 2010 through November 2012), 137 healthy children were enrolled from 3 institutions in Saitama Prefecture and Tokyo: Hasuda Issinkai Hospital, Yashio Ekimae Internal Medicine, and Pediatric Clinic and Tokyo Women's Medical University Medical Center East. Parents were informed about the study when they brought their children for their first routine PCV7 inoculation, and

*Corresponding author: Mailing address: Department of Environmental and Occupational Health, School of Medicine, Toho University, 5-21-16 Omorinishi, Ota-ku, Tokyo 143-8540, Japan. Tel: +81 337624151, ext. 2405, Fax: +81 354935416, E-mail: m10d026u@oc.toho-u.ac.jp

written informed consent was obtained from those who agreed to participate. All subjects were aged between 2 months and 6 years, and none had, at registration, a prior history of immunization with a pneumococcal vaccine or an obvious infection. This study (22026) was approved by the Ethical Review Board of Toho University.

Specimen collection and testing: Pediatricians acquired nasopharyngeal swabs (Amies charcoal transport medium) from the children at each routine PCV7 inoculation and again at least 2 months after the last PCV7 inoculation. The samples were plated on agar medium containing 5% sheep blood and incubated overnight at 35°C in 5% CO_2. Pneumococci were isolated and identified on the basis of colony morphology and optochin susceptibility. Colonies that were difficult to identify with the optochin susceptibility test were confirmed by means of polymerase chain reaction analysis of *lytA* gene (5).

Characteristics of the study population: The following information was collected from the medical records of each participating child and/or through a questionnaire completed by their parents or guardians: age (<2 or 2–6 years), sex, day-care attendance (yes/no), presence of older/younger siblings (yes/no), and antibiotic treatment within the preceding 3 months (yes/no).

Serogrouping and serotyping: Serogrouping and serotyping of *S. pneumoniae* isolates from the children were performed using the quellung reaction at the Department of Bacteriology I, National Institute of Infectious Diseases. Isolates that had negative reactions to all pooled sera and omni serum were considered to be nontypeable. Isolates were classified as either VT pneumococci (4, 6B, 9V, 14, 18C, 19F, and 23F) or non-vaccine-type (NVT) pneumococci (all others).

Definition of ''inoculation-period'' and ''post-inoculation'' cultures: Pediatricians used flexible, sterile, dry nasopharyngeal cotton-wool swabs to transnasally acquire nasopharyngeal samples from the subjects during each PCV7 inoculation, which was performed according to the standard PCV7 schedules described above. A total of 233 culture samples created from these swab samples were defined as ''inoculation-period'' cultures (i.e., the period of pre- or incomplete vaccination) (Fig. 1). In addition, 2 months or more after the last PCV7 inoculation, nasopharyngeal swab samples were acquired again and cultured (defined as ''post-inoculation'' cultures, i.e., the period of completed vaccination) (Fig. 1).

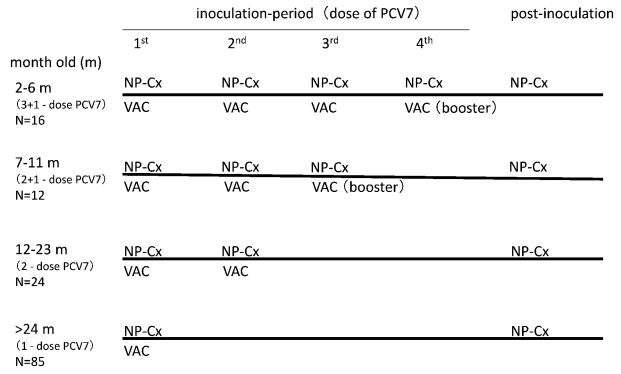

Fig. 1. Study procedures in 137 children receiving 3 + 1-dose of PCV7, 2 + 1-dose of PCV7, 2-dose of PCV7 or 1-dose of PCV7. NP-Cx, nasopharyngeal sample obtained for culture; VAC, vaccination given.

Statistical analysis: McNemar's test was used to analyze the differences between the prevalence of VT and NVT pneumococci in the inoculation-period and post-inoculation cultures. A 2-sided P of <0.05 was considered statistically significant. STATA ver. 12 (STATA Corporation, College Station, Tex., USA) was used for all data analyses.

RESULTS

The demographic characteristics of the study participants at their first routine PCV7 inoculation are shown in Table 1.

S. pneumoniae was isolated from 32 of the 137 (23%) children during the first PCV7 vaccination in the inoculation period. The pneumococci obtained at the second, third, and fourth vaccinations during the inoculation period were isolated from 11 of 52 (21%), 7 of 28 (25%), and 5 of 16 (31%) samples, respectively, and the pneumococci obtained at post-inoculation were isolated from 35 of 137 (26%) samples. The serotype-specific prevalence of pneumococcal isolates is shown in Table 2. The percentages of VT and NVT pneumococci at the first PCV7 vaccination in the inoculation-period cultures were 30% and 70%, respectively. The corresponding figures for the post-inoculation cultures were 11% and 89%, respectively. The most prevalent serotype was 6C in the first PCV7 vaccination in both the inoculation-period (15%) and post-inoculation (22%) cultures. Among the VT pneumococci isolates, serotype 6B was isolated from both the inoculation-period and post-inoculation cultures, but serotypes 14, 19F, and 23F were not isolated from the post-inoculation cultures. Among the NVT pneumococci isolates, serotypes

Table 1. Characteristics of the 137 subjects at 1st visit

Characteristic	No. (%) of children
Age	
<2 years old	52 (38)
2–6 years old	85 (62)
Sex	
Male	77 (56)
Female	60 (44)
Day care attendance	
Yes	45 (33)
No	92 (67)
Presence of older siblings	
Yes	60 (44)
No	77 (56)
Presence of younger siblings	
Yes	32 (23)
No	102 (74)
Not known	3 (3)
Antibiotic treatment within the preceding 3 months	
Yes	55 (40)
No	81 (59)
Not known	1 (1)

Table 2. Pneumococcus serotypes in inoculation-period and post-inoculation cultures

Serotype	Inoculation period								Post-inoculation	
	1st		2nd		3rd		4th			
	No.	Column %	No.	Column %	No.	Column %	No.	Column %	No.	Column %
VT										
6B	4	12.12	2	16.67	2	25.00	0	—	4	11.11
14	3	9.09	0	—	0	—	0	—	0	—
19F	1	3.03	0	—	0	—	0	—	0	—
23F	2	6.06	0	—	0	—	0	—	0	—
total	10	30.30	2	16.67	2	25.00	0	—	4	11.11
NVT										
3	0	—	0	—	0	—	0	—	1	2.78
6A	1	3.03	1	8.33	0	—	0	—	1	2.78
6C	5	15.15	4	33.33	3	37.50	1	20.00	8	22.22
10A	0	—	0	—	0	—	0	—	1	2.78
15A	1	3.03	1	8.33	0	—	2	40.00	4	11.11
15B	2	6.06	0	—	0	—	1	20.00	3	8.33
15C	3	9.09	1	8.33	1	12.50	0	—	3	8.33
19A	3	9.09	0	—	0	—	0	—	2	5.56
22F	2	6.06	1	8.33	0	—	0	—	3	8.33
23A	0	—	0	—	0	—	0	—	2	5.56
33F	3	9.09	0	—	0	—	0	—	0	—
34	0	—	0	—	0	—	0	—	2	5.56
35B	2	6.06	1	8.33	0	—	0	—	1	2.78
38	1	3.03	0	—	0	—	0	—	0	—
UT	0	—	1	8.33	2	25.00	1	20.00	1	2.78
total	23	69.70	10	83.33	6	75.00	5	100.00	32	88.89
Total	33*	100.00	12*	100.00	8*	100.00	5	100.00	36*	100.00

*There are overlaps in the total numbers because some different serotypes were detected in several subjects.
PCV7, heptavalent pneumococcal conjugated vaccine; VT, vaccine-type; NVT, non-vaccine-type.

Table 3. Number of pneumococcal carriages (VT/NVT pneumococci) detected in inoculation-period and post-inoculation cultures

S. pneumoniae (VT or NVT)		No. of children	
Inoculation period (at 1st-4th dose)	Post-inoculation (>2 mo after last inoculation)	VT	NVT
negative	negative	123	86
positive	negative	10	19
negative	positive	1	18
positive	positive	3	14
	total	137	137

VT, vaccine-type; NVT, non-vaccine-type.

33F and 38 were absent from the post-inoculation cultures, but serotypes 3, 10A, 23A, and 34 were newly isolated from the same cultures.

The mean duration (±SD) from the last PCV7 inoculation to the last sampling was 139.4 (±79.3) days.

The numbers of pneumococcal carriages, categorized as VT or NVT, detected during each period are shown in Table 3. The prevalence of VT pneumococci was significantly reduced from 9.5% in the inoculation-period cultures to 2.9% in the post-inoculation cultures ($P < 0.01$). There was no statistical difference in the prevalence of NVT pneumococci between the inoculation-period and post-inoculation cultures (24.1% versus 23.4%).

DISCUSSION

To the best of our knowledge, this is the first study in Japan to prospectively investigate changes in *S. pneumoniae* serotypes in nasopharynx samples of children following PCV7 inoculation. Reports from other countries have indicated that the prevalence of VT pneumococci is individually reduced after PCV7 administration (6,7). Millar et al. reported that VT pneumococcal carriage was lower among adults and unvaccinated children living with others who received the PCV7 vaccine (8). In the United States, the decreased nasopharyngeal carriage of VT strains among PCV7-immunized children led to decreased transmission to nonimmunized

children and adults (i.e., herd immunity) (4). In our study, we found that the prevalence of VT was significantly reduced in the post-inoculation cultures compared with that in the inoculation-period cultures (9.5% to 2.9%).

Serotype 6B was isolated from both the inoculation-period and post-inoculation cultures. Dagan et al. reported that in subjects carrying VT pneumococci before the first dose of PCV7, the serum IgG response to the carried serotype after 2 or 3 doses was significantly lower than that in noncarriers (9). Furthermore, the prevalence of pneumococcal phenotypes is associated with the structure of the capsular polysaccharide (10,11). Serotypes such as 6B, which have capsules with larger zones of dextran exclusion, were more resistant to neutrophil-mediated killing and more prevalent in carriage (10).

Serotype 6C was highly prevalent in both the inoculation-period and post-inoculation cultures. Increases in the prevalence of serotype 6C have received attention in the United States and Europe as the cause of invasive pneumococcal disease (12–14). According to a report recently released by Nariai (15), an increase in the prevalence of serotype 6C was reported among children with lower respiratory infection in Japan. In our multilocus sequence typing (MLST) analysis, 5 of 11 serotypes, which were identified from these serotype 6C isolates, were confirmed as new serotypes (unpublished data) from the database at http://www.mlst.net. One of the hypotheses was that asymptomatic 6C, which is always prevalent in this area, may have been detected by chance in this study.

The primary limitation of our study was the lack of a non-vaccinated control group, which made it difficult to definitively interpret the effects of PCV7.

In summary, we prospectively investigated changes in *S. pneumoniae* serotypes among Japanese children after PCV7 inoculation. The protection of PCV7 against nasopharyngeal colonization was inferred from the decrease in VT carriage after the completed vaccination. The decrease in VT carriage may be conducive to reducing VT transmission within the study area. Concomitantly with the decrease in colonization with VT, it is necessary to monitor emerging new pneumococcal serotypes not included in PCV7. Thus, it is critical to conduct constant surveillance of serotype replacement by NVT within the study area.

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Conflict of interest None to declare.

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