

Impact of the Pneumococcal Heptavalent Conjugated Vaccine on *Streptococcus pneumoniae* Nasopharyngeal Carriage and Antimicrobial Susceptibility in Children 2-5-Year-Old in Beijing, China

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How to cite this paper: Lv, M., Bai, S., Sun, Y.N., Zhang, T.G., Li, A.H. and Wu, J. (2017) Impact of the Pneumococcal Heptavalent Conjugated Vaccine on *Streptococcus pneumoniae* Nasopharyngeal Carriage and Antimicrobial Susceptibility in Children 2-5-Year-Old in Beijing, China. *World Journal of Vaccines*, 7, 27-41.

<https://doi.org/10.4236/wjv.2017.73003>

Received: August 18, 2017

Accepted: August 27, 2017

Published: August 30, 2017

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Abstract

Streptococcus pneumoniae is a primary cause of illness and death among children younger than 5 years in China. The heptavalent pneumococcal conjugate vaccine (PCV7) was the only conjugated vaccine (PCV) available in China from 2008 to 2013. This randomized, controlled, open-label study conducted at 46 Beijing clinics involved 3281 healthy 2-5-year-old Chinese children, randomized 1:1 to receive one dose of the *S. pneumoniae* heptavalent conjugated vaccine (PCV7) (n = 1643) or *Haemophilus influenzae* type b conjugate vaccine (Hib) (n = 1638). The main objective of this study was to investigate the impact of PCV7 against that of Hib vaccination in the nasopharyngeal *S. pneumoniae* colonization in healthy Chinese children. Nasopharyngeal (NP) samples for culture, serotyping and antimicrobial susceptibility testing were collected before vaccination and at Day 60 and 180 post-vaccination. A total 3281 children were enrolled in the study. Demographic characteristics were similar among both study groups: 1641 children received PCV7. Before immunization, *S. pneumoniae* was isolated in 338 and 360 children in the PCV7 (144 PCV7 isolates) and Hib groups (145 PCV7 isolates), respectively. At Day 180, PCV7 vaccination was more effective than Hib vaccination in reduction NP carriage (20.2% [$P = 0.052$]) and new acquisition (19.0% [$P = 0.066$]). When reductions in NP carriage and new acquisition of PCV7 VT plus 6A was analyzed, reduction in the PCV7 vaccinated group achieved statistical significance ($P = 0.034$ and $P = 0.042$ versus Hib, respectively). NP carriage of NVT increased in both groups ($P = 0.305$ between study groups at Day 180). PCV7 decreased NP carriage of non-susceptible VT to amoxicillin ($P = 0.000$),

ceftriaxone ($P = 0.047$) and MDR ($P = 0.024$) versus Hib. PCV7 vaccination in Chinese children 2 to 5 years of age was more effective than vaccination with Hib in the reduction of *S. pneumoniae* nasopharyngeal carriage, new acquisition and non-susceptible isolates.

Keywords

Heptavalent Pneumococcal Vaccine, *Streptococcus Pneumoniae*, Serotypes, Nasopharyngeal Carriage, Antimicrobial Non-Susceptibility

1. Introduction

Globally, *Streptococcus pneumoniae* is the most common cause of vaccine-preventable deaths in young children [1]. The World Health Organization (WHO) estimated that in 2008, 0.5 million children died of *S. pneumoniae* infections [2]. In China, *S. pneumoniae* is a primary cause of morbidity and mortality among children younger than 5 years [1]: 136,551 cases of severe community-acquired pneumonia and 14,202 deaths were caused by *S. pneumoniae* in 2010 and 2011, respectively [3].

Nasopharyngeal (NP) colonization by *S. pneumoniae* is the prerequisite for mucosal and invasive pneumococcal infections [4]. The prevalence of NP colonization varies by season, age, and other genetic and sociological factors. Rates are higher in children younger than 24 months, those whose families have lower socioeconomic status, during winter, and among children who attend daycare centers or who live with multiple siblings [5] [6] [7]. Pneumococcal colonization is usually asymptomatic, but spread from the nasopharynx can lead to sinusitis, otitis media, pneumonia, and invasive diseases such as bacteremia, sepsis and meningitis [2] [8].

Pneumococcal conjugate vaccines (PCV) reduce pneumococcal disease by preventing: 1) NP acquisition and the duration or density of carriage of *S. pneumoniae*, and 2) the progression of pneumococcal colonization to disease [4] [9]. The impact of PCVs on pneumococcal NP colonization and carriage is an indicator of efficacy against disease, and has become an important factor for consideration as a supplementary or alternative endpoint in vaccine licensure [9] [10].

The licensure of the heptavalent pneumococcal conjugate vaccine (PCV7) which includes serotypes 4, 6B, 9V, 14, 18C, 19F and 23F in many countries in 2000, reduced the incidence of vaccine-serotype (VT)-associated invasive pneumococcal disease (IPD) by 79% - 100% in countries where the vaccine was included in National Immunization Programs [11] [12]. Reducing NP carriage decreases the transmission of VT *S. pneumoniae*, thereby driving the herd immunity effect observed in unvaccinated individuals where PCV immunization has been implemented [2] [4] [13]. Since most antibiotic non-susceptible *S. pneumoniae* serotypes are covered by PCV7 [14], the vaccine also decreases an-

tibiotic—non-susceptible carriage and disease caused by these VT, leading to a reduction of antimicrobial usage [15]. Due to its effectiveness, WHO recommended that PCV be included in childhood vaccination programs worldwide [13]. However, the use of PCV has led to replacement NP colonization by non-vaccine serotypes (NVT) [16] [17], and a relative increase in disease caused by antibiotic non-susceptible NVT [14] [18].

Higher-valent PCVs (ten valent vaccine, PCV10 and the 13-valent vaccine, PCV13) are available on the market in various countries although PCV13 is the only one available in the US; however, PCV7 was the only pneumococcal vaccine available in China during the study period [13]. Although estimations and models of the health and economic impact of PCV7 in China have been published [1] [13], the direct effects of PCV7 immunization on NP carriage among children in China have not yet been described.

The main objectives of this study were to investigate the effectiveness of PCV7 in reducing NP carriage and new acquisition of VT *S. pneumoniae* in healthy 2-5-year old children in China, and its impact on non-vaccine-type *S. pneumoniae* (NVT) carriage and pneumococcal antimicrobial susceptibility 6 months after vaccination.

2. Methods

2.1. Study Design and Definitions

This study was a parallel-group, randomized, controlled, open-label, interventional trial conducted at 46 clinics in Beijing, China, between June 2012 and March 2014 (ClinicalTrials.gov Identifier: NCT02133469). Enrolled children who met the eligibility criteria were randomized 1:1 to receive a single dose of PCV7 or *Haemophilus influenzae* type b conjugate vaccine (Hib) (Day 0). A baseline NP sample was obtained before vaccination (Day 0) and subsequent samples were collected from each participant on days 60 and 180 after vaccination. All samples were analyzed by *S. pneumoniae* culture and serotyping. After all study visits were completed, participants in the PCV7 group were vaccinated with Hib and those in the Hib group were vaccinated with PCV7.

The study protocol and corresponding informed consent were approved by the Ethics Committee of the Beijing Center for Disease Prevention and Control, and the study was conducted in accordance with the Declaration of Helsinki.

The study main objectives were to investigate efficacy of a single PCV7 dose, compared to Hib vaccination, in the prevention of NP carriage of VT *S. pneumoniae* and vaccine-related serotype 6A [19] [20] in 2-5-year-old Chinese children. The impact of PCV7 on carriage rates of NVT and antibiotic-nonsusceptible and resistant *S. pneumoniae* was also evaluated.

S. pneumoniae isolates were classified as either VT pneumococci (4, 6B, 9V, 14, 18C, 19F, and 23F) or non-PCV7 pneumococci (NVT) for all other serotypes. New acquisition rates were defined as the proportion of participants who on the initial NP sample had a negative culture or positive culture for a specific

serotype but at the follow-up NP culture (Day 180) carried a different serotype than the one detected on Day 0.

2.2. Study Population

Study participants were healthy children aged 2 - 5 years who lived in Beijing (Xicheng, Huairou, Daxing, Chaoyang, Fengtai and Dongcheng Districts). The parent or legal guardian of each participant was required to provide informed consent for their child to participate in the study.

Exclusion criteria included febrile illness at the time of vaccination (axillary temperature $> 37.0^{\circ}\text{C}$); previous immunization with or contraindications to any pneumococcal or Hib vaccine; previous treatment with any biological product containing *S. pneumoniae* or Hib components; conditions associated with a prolonged bleeding coagulation time that would contraindicate intramuscular injection; confirmed or suspected immune deficiency; treatment with immunosuppressive agents; congenital malformation or injury of the nasopharynx that would prevent the taking of an NP swab; significant neurological disorders; and receipt of blood products or gamma-globulin (including hepatitis B immunoglobulin and monoclonal antibodies) within 12 weeks before enrollment.

2.3. Vaccination

Participants were vaccinated either with the heptavalent pneumococcal conjugate vaccine [PCV7; Prevenar, imported by Pfizer (Shanghai) International Trade Co., Ltd.] containing the aforementioned seven serotypes covalently linked to a cross-reacting diphtheria variant (CRM197) protein carrier; or with *Haemophilus influenzae* type b conjugate vaccine (Act-Hib; sub-packaged by Shenzhen Sanofi Pasteur Biological Products Co., Ltd.).

Each 0.5 mL dose of PCV7 contained 2 μg each of serotypes 4, 9V, 14, 18C, 19F and 23F; 4 μg of serotype 6B; and 20 μg of CRM197 carrier protein, adsorbed on adjuvant aluminum phosphate (0.5 mg), along with sodium chloride and water. Each Hib vaccine dose contained 10 μg *Haemophilus influenzae* type b polysaccharide conjugated with tetanus protein, hydroxymethyl aminomethane, and sucrose, diluted to 0.5 mL with diluent containing sodium chloride and water.

Both vaccines were administered as a single dose by intramuscular injection in the lateral deltoid of the upper arm. After vaccination, subjects were observed by trained study staff over 30 minutes for potential reactions.

2.4. Nasopharyngeal Sampling

NP samples were collected at the clinics by otolaryngologists before vaccination (Day 0) and on Days 60 + 15 and 180 + 15 after vaccination. A single flexible aluminum shaft swab with a rayon tip (Copan Diagnostics, Inc., Italy) was passed through the anterior nares as far as the posterior pharynx and rotated 180 degrees before removal. Samples were transported to the serotyping laboratory

(the Beijing Center for Disease Prevention and Control) in Amies medium within 6 hours of being taken.

2.5. Identification, Serotyping and Antimicrobial Susceptibility Testing of *S. pneumoniae*

NP samples were plated on agar medium containing 5% sheep blood (Columbia Blood Agar, Oxoid, England), and incubated for 18 - 24 hours at 35°C in 5% CO₂. Pneumococci were isolated and identified on the basis of optochin susceptibility (where the diameter of the bacterial inhibition zone was 7 - 14 mm in diameter) and confirmed by the bile solubility test.

S. pneumoniae isolates were serotyped using the Quellung reaction carried out by trained, certified laboratory personnel. Ten per cent of the NP samples were sent to a third-party laboratory for serotyping validation.

Susceptibility testing for the following representative antimicrobials penicillin, erythromycin, ceftriaxone and levofloxacin was done by minimal inhibitory concentration (MIC) by means of the E-test (PDM Epsilometer, AB Biodisk, Solma, Sweden). The *S. pneumoniae* isolates were classified as susceptible, intermediate and resistant to these antibiotics based on cutoff concentrations in the manufacturer's instructions for the E-test reagent. In the statistical analyses, intermediate and resistant isolates were collectively considered to be antibiotic-nonsusceptible. *S. pneumoniae* strain ATCC49619 was used for quality control for all tests and Interpretation of the results was according to the National Committee for Clinical Laboratory Standards recommendations.

2.6. Statistical Analyses

Sample size calculation was based on efficacy of carriage prevalence rate on Day 180. A sample size of 3270 was required to show efficacy of PCV7 in reducing VT NP carriage with 90% power, assuming a 10% loss to follow-up, 50% VT coverage, NP carriage rate of 25% and VT carriage reduction rate of 30%. Demographic and baseline characteristics of the study participants were compared in each study group to evaluate inter-group comparability. Comparisons were performed on the culture/serotyping results of pre-vaccination (Day 0) NP swabs versus NP swabs obtained on Days 60 and 180 between PCV and Hib study groups, and for Day 0 versus Day 180 data. Antimicrobial non-susceptibility and resistance data from samples isolated on Days 0 and 180 were similarly analyzed.

A four-fold table χ^2 test was used for inter-group comparisons of categorical variables such as NP carriage and/or resistance rates. $P < 0.05$ was considered statistically significant.

All statistical analyses were carried out using SPSS 19.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Population

In total 3281 children were enrolled in the study: 1643 children received PCV7

Table 1. Study group characteristics and number of nasopharyngeal samples obtained in Chinese children.

Variables	Participants		
	PCV7 Group	Hib Group	Total
1. Number of children enrolled	1643	1638	3281
2. Number of children with Nasopharyngeal sample collected at Day 0	1643	1638	3281
3. Number of children with Nasopharyngeal sample collected at Day 60	1535	1518	3053
4. Number of children with Nasopharyngeal sample collected at Day 180	1475	1475	2950
5. Gender, male (%)	820 (49.9%)	850 (51.8%)	1670 (50.9%)
6. Age, mean \pm SD (years)	3.2 \pm 0.8	3.0 \pm 0.8	3.1 \pm 0.8
7. Height, mean \pm SD (cm)	98.8 \pm 8.0	98.6 \pm 8.1	98.7 \pm 8.0
8. Weight, mean \pm SD (kg)	16.2 \pm 3.2	16.2 \pm 3.2	16.2 \pm 3.2

and 1638 received Hib vaccine on Day 0. Among enrolled children, follow-up visits at Day 60 and 180 were completed in 1535 children and 1475 children, respectively in the PCV7 group and in 1518 children and 1475 children, respectively in the Hib group. Demographic characteristics among both study groups were similar (**Table 1**).

3.2. Culture

Upon enrollment (Day 0) a nasopharyngeal swab for culture was obtained in 1643 children in the PCV7 group and in 1638 children in the Hib group. On Day 60 a sample was obtained in 1535 children in the PCV7 group and in 1518 children in the Hib group and on Day 80 in 1475 children and 1475 children, respectively.

A total of 9284 nasopharyngeal swabs were obtained at all study visits and 2047 *S. pneumoniae* strains were collected among both study groups for an overall pneumo-coccal detection rate of 22%. Among the 2047 strains, 66 isolates were not included in the analysis as these strains serotype could not be confirmed by reference laboratory.

At baseline (before immunization) (Day 0), *S. pneumoniae* was isolated in 338 children (21%) and 360 children (22%) in the PCV7 and Hib groups, respectively. Among the *S. pneumoniae* strains isolated at baseline, 144 isolates (9%) and 145 isolates (9%) in the PCV7 and Hib group, respectively belonged to the serotypes included in the heptavalent pneumococcal conjugate vaccine.

Non-vaccine serotypes were detected at baseline in 11% (180/1643) and 13% (210/1638) isolates in the PCV7 and Hib group, respectively. Among the non-PCV 7 serotypes (390 isolates), the most common serotypes detected at baseline were 6A (19%), 6C (12.5%), 19A and 15B (7.1% each).

At baseline, susceptibility testing was performed in 687 *S. pneumoniae* isolates, including 334 strains in the PCV7 group and 353 strains in the Hib group. There were no significant differences in terms of antimicrobial resistance among the two study groups. Among PCV7 and Hib groups most of the baseline isolates were susceptible to penicillin (99.7% each), amoxicillin (99% each) and levofloxacin (98% each) but less commonly to ceftriaxone (59% and 61%, respectively) and erythromycin (1% and 0%, respectively). Among all the erythromycin and ceftriaxone—non-susceptible—*S. pneumoniae* strains isolated at baseline, 41% (285/687) strains and 26% (173/687) strains, were serotypes included in the heptavalent conjugated vaccine and among non-VT-strains the most common erythromycin and ceftriaxone—non-susceptible serotypes were: 6A (18% and 7%, respectively), 6C (11% and 4%, respectively) and 19A (7% and 5%, respectively).

Resistance to >3 antimicrobials (MDR) was observed in 27% (90/343) of isolates obtained in the PCV 7 group and in 23% (83/353) of isolates collected from children randomized to Hib vaccine. MDR serotypes were more common among serotypes included in the heptavalent conjugated vaccine (25% [173/687]) than among non-vaccine serotypes (13% [95/687]) and were equally distributed among the PCV7 group (39% [132/334]) and the Hib group (38% [136/353]).

3.3. Effect of Vaccination on Carriage of Pneumococci

The overall *S. pneumoniae* nasopharyngeal carriage among both study groups remained similar at Day 60 and Day 180. Nasopharyngeal carriage rates at Day 60 and at Day 180 for the PCV 7 group was 20% and 24% respectively, and 21% and 25% respectively for children randomized to receive Hib vaccine.

Nasopharyngeal carriage of vaccine-type isolates at Day 60 and Day 180 for the PCV7 group was 8% (117/1535) and 8% (116/1475) respectively, and 8% (117/1518) and 10% (146/1475) respectively in the Hib vaccinated group. At Day 180, there was a non-statistical significant reduction in the nasopharyngeal carriage of *S. pneumoniae* in the PCV7 group versus the group of children vaccinated with Hib (20% [95% CI: -0.3, 37.1]). A significant vaccine effect on pneumococcal carriage was observed at Day 180 among children vaccinated with PCV7 against those vaccinated with Hib if PCV7 related serotype 6A was added to the seven vaccine serotypes (20% [95% CI: 1.6, 35.0]) (Table 2).

The carriage of non-PCV7 serotypes from Day 0 to Day 180 increased from 11% (178/1535) to 16% (234/1475) in the PCV7 vaccinated group and from 13% to 14% in the Hib group. Overall, the increase of non-vaccine serotypes in the PCV7 group (45% [95% CI: 20.8, 73.6]) was significantly higher than that observed in the group of children vaccinated with Hib (13% [95% CI: -5.2, 35.1]).

The most common NVT in the PCV7 group at Day 180 were 6A (2%), 15A (2%), and 19A, 8 and 6C (1% each). The NVT that showed the greatest NP carriage rate increases in the PCV7 group at Day 180 were serotype 15C (from 0.1% to 0.6%; an increase of 401%; $P = 0.022$ versus Day 0), 15A (from 0.5% to 2%; an

increase of 234%; $P = 0.002$), serotype 3 (from 0.4% to 1%; an increase of 197%; $P = 0.017$) and serotypes 42 and 11A (these changes were not significant). In the Hib group, none of the changes in NP carriage of individual NVT at Day 180 differed significantly from Day 0.

At Day 180, the acquisition rate of new VT *S. pneumoniae* was 8% in the PCV7 group and 10% in the Hib group (Table 3). The 20% lower rate in new

Table 2. Comparison of nasopharyngeal carriage rate of heptavalent vaccine type *S. pneumoniae* serotypes and of serotype 6A before (Day 0) and post-vaccination (Day 180) comparison of nasopharyngeal carriage rates between study groups at Day 180 in Chinese children.

Serotype	<i>S. p</i> isolates in PCV7 group (% of participants) ^a		<i>S. p</i> isolates in Hib group (% of participants) ^a		Comparison of NP carriage rate at Day 180 between PCV7 and Hib groups		
	Day 0 n = 1643	Day 180 n = 1475	Day 0 n = 1638	Day 180 n = 1475	Percentage decrease of Nasopharyngeal <i>S. p</i> carriage rate from Day 0 to Day 180 in PCV7 group vs. Hib group (95% CI)	χ^2 value	Pvalue
4	3 (0.2)	1 (0.1)	2 (0.1)	1 (0.1)	0.0 (−1,497.2, 93.7)	0.00	1.000
6B	32 (2.0)	29 (2.0)	31 (1.8)	36 (2.4)	16.7 (−30.7, 50.3)	0.54	0.380
9V	1 (0.1)	1 (0.1)	0 (0.0)	3 (0.2)	50.0 (−220.1, 96.5)	0.25	0.617
14	28 (1.7)	16 (1.1)	21 (1.2)	29 (2.0)	44.8 (−1.1, 69.9)	3.81	0.051
18C	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0	/	/
19F	56 (3.4)	45 (3.1)	54 (3.4)	57 (3.9)	21.1 (−15.9, 46.2)	1.33	0.249
23F	24 (1.5)	24 (1.6)	36 (2.2)	20 (1.4)	−14.3 (−116.3, 33.4)	0.37	0.543
Total VT	144 (8.8)	116 (7.9)	145 (8.9)	146 (9.9)	20.2 (−0.3, 37.1)	3.77	0.052
6A	30 (1.8)	28 (1.9)	45 (2.7)	34 (2.3)	17.7 (−35.1, 49.8)	0.59	0.441
Total VT plus 6A	174 (10.6)	144 (9.8)	190 (11.6)	180 (12.2)	20.0 (1.6, 35.0)	4.49	0.034

a. Data are number of isolates (percentage of total isolates in study group at each time point).

Table 3. Vaccine type *Streptococcus pneumoniae* acquisition rate^a at Day 180 post-vaccination among Chinese children vaccinated with heptavalent conjugated *S. pneumonia* vaccine or *Haemophilus influenzae* type b vaccine.

Serotype	<i>S. p</i> isolates in PCV7 group (% of participants) n = 1475	<i>S. p</i> isolates in Hib group (% of participants) n = 1475	Percentage reduction between PCV7 and Hib groups(95% CI)	χ^2	P
4	1 (0.1)	1 (0.1)	0 (−1,497.2, 93.7)	0.00	1.000
6B	27 (1.8)	35 (2.4)	22.8 (−26.8, 53.1)	0.25	0.617
9V	1 (0.1)	3 (0.2)	65.0 (−220.1, 96.5)	0.25	0.617
14	16 (1.1)	29 (2.0)	45.2 (−1.1, 69.9)	3.81	0.051
18C	0 (0.0)	0 (0.0)	0	/	/
19F	44 (3.0)	55 (3.8)	20.0 (−18.1, 45.8)	1.27	0.261
23F	24 (1.7)	18 (1.2)	−33.3 (−144.6, 27.3)	0.87	0.351
Total VT	113 (7.7)	141 (9.6)	19.9 (−1.5, 36.8)	3.38	0.066
6A	26 (1.76)	32 (2.17)	18.9 (−35.6, 51.3)	0.63	0.426
Total VT plus 6A	139 (9.4)	173 (11.7)	19.7 (0.8, 35.0)	4.14	0.042

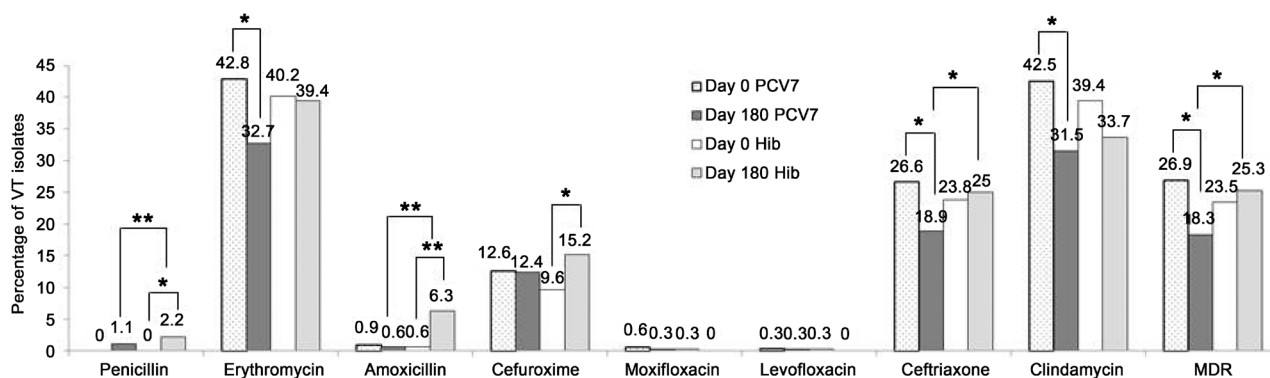
a. Data are number of isolates (percentage of participants in study group).

acquisitions in the PCV7 versus the Hib group was not statistically significant ($P = 0.066$). However, when acquisition rates for serotype 6A were taken into account, the overall new acquisition rate of PCV7 serotypes plus 6A was 9.4% in the PCV7 group and 11.7% in the Hib group representing a 20% reduction in the PCV7 group versus the Hib group ($P = 0.042$).

On Day 180, 723 *S. pneumoniae* isolates were culture and tested for antimicrobial susceptibility; 355 isolates in the PCV7 group and 368 isolates in the Hib group. Nasopharyngeal carriage rates of *S. pneumoniae* that were non-susceptible to these antibiotics were also similar between study groups at baseline. The non-susceptibility and resistance of VT isolates to tested antimicrobials and MDR were compared before and 180 days after PCV7 or Hib vaccination (Figure 1). The proportions of VT *S. pneumoniae* that were non-susceptible to each antibiotic were similar in the PCV7 and Hib groups at Day 0. At Day 180, the proportion of VT isolates that were non-susceptible decreased in the PCV7 group for all antibiotics except for penicillin (which increased from 0 to 1%; not significant). These decreases after PCV7 vaccination were statistically significant for erythromycin, cefuroxime, ceftriaxone, clindamycin, and MDR *S. pneumoniae* ($P < 0.05$) versus Day 0.

By contrast, in the Hib-vaccinated group, the proportion of non-susceptible VT isolates increased by Day 180 for all antibiotics except erythromycin, and le-

(a) Non-susceptible



(b) Resistant

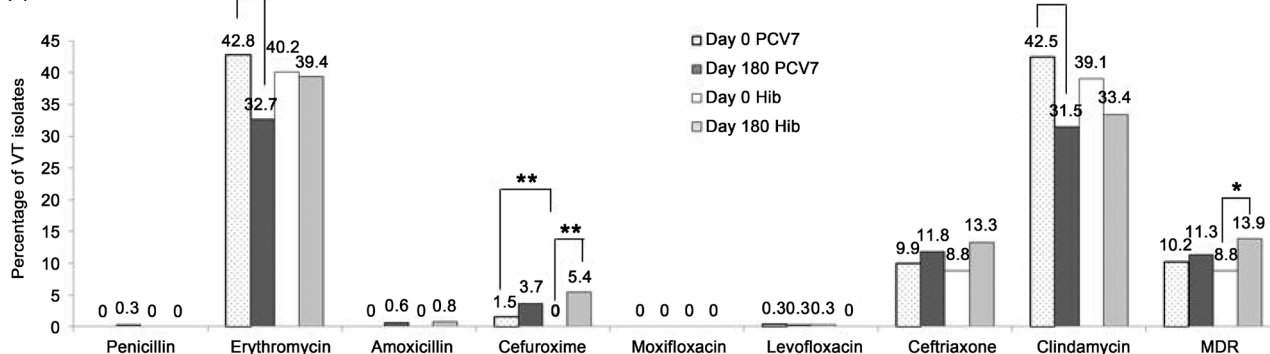


Figure 1. Distribution of antimicrobial non-susceptibility (a) and resistance (b) of PCV7 serotypes to selected antibiotics before and 180 days after PCV7 or Hib vaccination. * $P < 0.05$; ** $P < 0.001$.

vofloxacin. The increases in non-susceptible VT 180 days after Hib vaccination were statistically significant for amoxicillin ($P < 0.001$), penicillin and cefuroxime ($P < 0.05$).

When the non-susceptibility rates among VT isolates at Day 180 were compared between study groups, significantly lower rates were observed in the PCV7 group for penicillin, amoxicillin ($P < 0.001$), ceftriaxone ($P < 0.05$) and MDR *S. pneumoniae* ($P < 0.05$) versus Hib.

Different impacts on resistance rates to the different antibiotics were observed among VT isolates after PCV7 and Hib vaccination. Before vaccination, the antimicrobial-resistant rates of VT were similar in both study groups. A statistically significant difference in resistance rates between Hib and PCV7 groups was only seen for cefuroxime at this time point ($P < 0.001$).

At Day 180 after vaccination, the proportion of erythromycin-resistant and clindamycin-resistant VT decreased significantly in the PCV7 group ($P < 0.05$), and non-significantly in the Hib group. However, proportions of VT that was resistant to amoxicillin, cefuroxime, ceftriaxone and MDR increased in both study groups, although the increases only reached statistical significance for cefuroxime and MDR ($P < 0.05$ versus Day 0) in the Hib group. A comparison of Day 180 VT resistance rates between study groups showed no significant differences.

4. Discussion

To our knowledge, this is the first report on the impact of a single PCV7 dose on the nasopharyngeal carriage, new acquisition, and antimicrobial susceptibility of *S. pneumoniae* in a large cohort of 2-5-year-old children in China. This study was designed and completed while PCV7 was the sole PCV registered in China. However, due to the registration of PCV13 in October 2016, indirect expectation on the value of PCV13 in China could be inferred. PCV7 serotypes comprised 41.4% of the *S. pneumoniae* isolated from all participants at Day 0 while PCV13 serotypes 3, 6A, and 19A comprised an additional 18.1% of the isolates. PCV7 reduced nasopharyngeal carriage by 20.2% and new acquisition of PCV7 VT by 19.0% compared to Hib. When the analyses included serotype 6A, the reductions in nasopharyngeal carriage and new VT acquisition with PCV7 achieved statistical significance ($P = 0.034$ for decreased nasopharyngeal carriage of VT and $P = 0.042$ for reduction in new acquisition versus Hib immunization). The reductions in PCV7 VT carriage were within the <20% - 80% range for reduction in carriage rates observed with multiple dosing schedules (2 + 0, 2 + 1, 3 + 0 and 3 + 1) in a meta-analysis of studies in which the primary PCV7 dose was given in the first 6 months of life [8]. By contrast, PCV13 serotypes 3 and 19A increased in both groups over this period.

In agreement with previous reports [11], the children vaccinated with PCV7 had a greater increase in NVT carriage at Day 180 than those vaccinated with Hib, although the difference in overall NVT carriage rates between the study

groups was not significant. Statistically significant increases in NVT carriage rates were seen for serotypes 3, 15A, and 15C in the PCV7 group. The vast majority of NVT observed at Day 180 in the PCV7 group were nasopharyngeal colonizers that rarely lead to mucosal or invasive disease, except for PCV13 serotypes 3 and 19A, which have been strongly associated with acute otitis media and conjunctivitis [16], and 19A with IPD [21]. IPD caused by serotype 19A accounted for 53.6% of IPD cases among children aged ≤ 5 years in Taiwan in 2011-2012 [21]. Although the increase in nasopharyngeal carriage of serotype 19A was not statistically significant in this study, serotypes 19A and 19F were the most common *S. pneumoniae* serotypes isolated from patients younger than 14 years during 2013-2014 at Beijing Children's Hospital [22]. In many other countries, nasopharyngeal carriage rates of additional serotypes included in PCV13 increased with wider use of PCV7 [21] [23] [24] [25]. Hence, to offer enhanced protection against pneumococcal carriage and disease, PCV13 was registered in China in 2016.

Macrolides and cephalosporins are commonly prescribed by Chinese pediatricians, particularly for respiratory tract infections, and cephalosporin use has increased at tertiary hospitals [26]. PCV7 vaccination led to decreases in nasopharyngeal carriage of VT that were non-susceptible to erythromycin, ceftriaxone, clindamycin, and MDR *S. pneumoniae* ($P < 0.05$; **Figure 1(a)**) versus Day 0. Nasopharyngeal carriage of NVT that were non-susceptible to cefuroxime were lower in the PCV7 group than the Hib group at Day 180. This is an important finding, given the high non-susceptibility rates among *S. pneumoniae* isolated from Beijing hospitalized children younger than 14 years, of 8.1% for amoxicillin-clavulanic acid, 89.9% for cefuroxime, 99.5% to erythromycin and 20.8% to ceftriaxone during 2013-2014 [22].

The impact of PCV7 vaccination on antibiotic resistance among VT was more variable. The proportion of VT that was resistant to erythromycin and clindamycin decreased after both PCV7 and Hib vaccination (but significantly after PCV7). However, rates of resistance to amoxicillin, cefuroxime, ceftriaxone and MDR-VT increased in both study groups, with the increases for cefuroxime and MDR-VT significant after Hib vaccination. PCV13 VT isolates 3, 6A and 19A contributed almost an additional one-fifth of isolates that were non-susceptible and/or resistant to erythromycin before vaccination. This proportion had increased further by Day 180 in the PCV7 group.

Colonization endpoints are important when analyzing data from studies of PCV, because pneumococcal colonization is a precondition for pneumococcal disease [10]. Nasopharyngeal carriage was recently recognized as an important proxy for PCV impact assessments [4]. The one-fifth reduction in *S. pneumoniae* nasopharyngeal carriage observed in the current study with PCV7 is likely to reduce substantially the burden of VT pneumococcal disease among Chinese children, given that a modeling study predicted that 31 million cases of IPD and hospitalized pneumonia would have occurred during 2008-2018 in Chinese

children younger than 5 years in the absence of PCV7 [1]. In terms of absolute number of disease cases prevented, the indirect effect of PCV7 could have been even greater than its direct effect because the unvaccinated population outnumbered the vaccinated population [1] [9]. Additional benefits would be conferred in terms of preventing nasopharyngeal carriage of VT that are non-susceptible to numerous antibiotics commonly used in China. The impact of PCV13 on nasopharyngeal carriage and, hence, pneumococcal disease, will likely be even greater, as shown in other studies [14] [27] [28].

A study limitation is that it was conducted in just one city, so the findings cannot necessarily be extrapolated to the rest of the country, particularly for rural areas where PCV7 immunization uptake is even lower [29]. However, the study population was large, and was drawn from all parts of Beijing. Another limitation is that the impact was measured in elder children who are not the routinely recommended population. And the effectiveness may be influenced by previous exposure to the same serotypes compared with younger children who are less possibly colonized. Therefore, we explore that the PCV will induce similar or bigger impact in younger children.

In conclusion, a single dose of PCV7 given to 2-5-year-old children was effective in preventing new acquisition of PCV7 VT and serotype 6A, and nasopharyngeal carriage of VT that were non-susceptible to amoxicillin, erythromycin and ceftriaxone. PCV7 may thus have reduced *S. pneumoniae* transmission in the Chinese population since its registration in 2008. Additional societal and cost benefits may have resulted from the herd effect, and from reduced pneumococcal disease caused by VT, particularly serotypes non-susceptible to antibiotics. These benefits would likely be dramatically augmented if PCV7 (and even more so, if PCV13) were to be included in China's national immunization program.

Acknowledgements

We thank Dr. Liu Liying, Dr. Li Chao, Dr. Hou Wenjun, Dr. Chen Liyan, Dr. Chu Yanhui, Dr. Sun Qiang, Dr. Shi Nianmin, Dr. Lili, Dr. Wang Qinghai, Dr. Zhai Lijun, Dr. Di Mingzhi, Dr. Guo Fangru, Dr. Wu Jin, Dr. Wang Jing, Dr. Sun Hao, Dr. Wang Zhongzhan for their contributions to project coordination and data acquisition, Dr. Wen Feng, Dr. Duan Xing, Dr. Wang Zhan, Dr. Bai Jing, Dr. Yan Zhanfeng, Dr. Xue Junfang for their contributions to nasopharyngeal sampling, and Dr. Yao Kaihu for his contributions to strain identification.

The study was sponsored by the Beijing CDC. Funding was provided by Pfizer.

Partial editorial support during preparation of the manuscript was provided by Samantha Santangelo at MIMS (Hong Kong).

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