의학석사 학위논문

Changes in Serotype Distribution and Antibiotic Resistance of Nasopharyngeal Isolates of *Streptococcus pneumoniae* from Children in Korea, after the Introduction of the Extended-Valency Pneumococcal Conjugate Vaccine

광범위 단백결합 백신 사용 후 국내 소아청소년 비인두에서 분리된 폐구균의 혈청형 분포와 항생제 감수성의 변화

2017 년 2 월

서울대학교 대학원

의학과 소아과학 과정

이준기

A thesis of the Master of Science in Medicine

광범위 단백결합 백신 사용 후 국내 소아청소년 비인두에서 분리된 폐구균의 혈청형 분포와 항생제 감수성 변화

Changes in Serotype Distribution and Antibiotic Resistance of Nasopharyngeal Isolates of *Streptococcus pneumoniae* from Children in Korea, after the Introduction of the Extended-Valency Pneumococcal Conjugate Vaccine

February 2017

The Department of Medicine

Seoul National University Graduate School

Joon Kee Lee

Changes in Serotype Distribution and Antibiotic Resistance of Nasopharyngeal Isolates of *Streptococcus pneumoniae* from Children in Korea, after the Introduction of the Extended-Valency Pneumococcal Conjugate Vaccine

by

Joon Kee Lee

A thesis submitted to the Department of Pediatrics

in partial fulfillment of the requirements for the

Degree of Master of Science in Medicine (Pediatrics)

at Seoul National University

October 2016

Professor _____ Chairman

Professor _____ Vice chairman

Professor _____

광범위 단백결합 백신 사용 후
국내 소아청소년 비인두에서 분리된
폐구균의 혈청형 분포와 항생제 감수성 변화

지도교수 최 은 화

이 논문을 의학석사 학위논문으로 제출함

2016 년 10 월

서울대학교 대학원

의학과 소아과학 전공

이 준 기

이준기의 의학석사 학위논문을 인준함

2016 년 12 월

위 원 장 (인)

부위원장 (인)

위 원 (인)

ABSTRACT

Changes in Serotype Distribution and Antibiotic Resistance of Nasopharyngeal Isolates of *Streptococcus pneumoniae* from Children in Korea, after the Introduction of the Extended-Valency Pneumococcal Conjugate Vaccine

Joon Kee Lee

Pediatrics, Medicine

The Graduate School

Seoul National University

Introduction: The aim of this study is to investigate the serotype distribution and antimicrobial resistance of nasopharyngeal isolates of *Streptococcus pneumoniae* from children after the introduction of the extended-valency pneumococcal conjugate vaccines (PCVs) in Korea.

Methods: From July 2010 to June 2015, 3,820 nasopharyngeal aspirates obtained from infants and children who presented with respiratory symptoms at the Seoul National University Children's Hospital were plated on trypticase soy agar containing 5% sheep blood for isolation of pneumococci. Serotype was determined by Quellung reaction and antimicrobial susceptibility was tested by E-test. Trend analysis was

performed for serotype distribution and antimicrobial non-susceptibility rates.

Results: *S. pneumoniae* was isolated from a total of 397 (10.4%) specimens. The most common serotypes were 19A (14.0%), 23A (12.8%), 11A (10.1%), 6C (7.8%), 6A (6.3%), and 19F (6.0%) among typeable pneumococci (n=335). Overall, the proportions of PCV serotypes were 33.4%, non-PCV serotypes were 66.6%, PCV7 serotypes were 12.5%, and PCV13 specific serotypes were 20.9%. There was a significant decrease in the proportions of PCV serotypes (59.1% in 2010/11 to 17.0% in 2014/15, $P<0.001$). The proportion of non-PCV serotypes was 40.9% in 2010/11, and increased to 83.0% in 2014/15 ($P<0.001$). Non-susceptibility rates of penicillin (oral), penicillin (parenteral, non-meningitis), cefotaxime, and erythromycin were 97.8%, 22.8%, 27.7%, and 95.5%, respectively. PCV serotypes showed significantly higher non-susceptibility to penicillin (parenteral, 41.4% vs 13.9%, $P<0.001$) and cefotaxime (40.2% vs 21.7%, $P=0.001$) than non-PCV serotypes. The proportions of PCV serotypes that are responsible for non-susceptibility to penicillin (parenteral, non-meningitis) and multidrug resistance showed a significant decrease (80.8% to 21.1%, $P<0.001$, 64.3% to 12.3%, $P<0.001$, respectively) between 2010/11 and 2014/15.

Conclusions: This study found that the proportion of PCV serotypes decreased among nasopharyngeal carriage pneumococci after the introduction of the extended-valency PCVs in Korea, while non-PCV

serotypes increased. Although antimicrobial non-susceptibility rates for penicillin and erythromycin remain high, the proportion of PCV serotypes responsible for antimicrobial resistance has decreased over time.

Keywords: ***Streptococcus pneumoniae*****; Serotype; Antimicrobial Resistance; Pneumococcal Conjugate Vaccines**

Student number: 2012-21708

CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

IPD; Invasive Pneumococcal Disease

NP; Nasopharyngeal

PCV; Pneumococcal Conjugate Vaccine

PCV7; 7-valent Pneumococcal Conjugate Vaccine

PCV10; 10-valent Pneumococcal Conjugate Vaccine

PCV13; 13-valent Pneumococcal Conjugate Vaccine

PCR; Polymerase Chain Reaction

MIC; Minimum Inhibitory Concentration

MDR; Multidrug Resistance

INTRODUCTION

Streptococcus pneumoniae is a leading cause of serious illness among children worldwide (1). The natural route of infection with *S. pneumoniae* starts with colonization, which may progress to invasive disease if natural immunological barriers are compromised. Preventing nasopharyngeal (NP) colonization of *S. pneumoniae* might also decrease horizontal spread of pneumococcal strains, thus providing herd immunity (2). The efficacy and effectiveness of the seven valent pneumococcal conjugate vaccine (PCV7) were demonstrated in large randomized trials before licensure and in surveillance for invasive pneumococcal disease (IPD) after licensure in the United States (US) and other countries; however, an increase of IPD caused by non-vaccine serotypes has been observed (3, 4). Drug-resistant strains of *S. pneumoniae* were relatively uncommon in the US through the 1980s, and penicillin was widely accepted as the drug of choice for empiric treatment (5). During the 1990s, however, drug-resistant strains, including those with reduced susceptibility to multiple antibiotics, became increasingly prevalent (6). Therefore, development and use of effective pneumococcal vaccines was required to treat and prevent disease caused by these strains. Data from Active Bacterial Core surveillance in the US indicate declines in antibiotic-nonsusceptible IPD after the introduction to PCV7 in 2000 (7). PCV13 (Prevnar 13, Pfizer Inc.) reduced IPD across all age groups when used routinely in children and colonization by 6 additional serotypes among children in the US (8-10).

The serotype distribution among nasopharyngeal carriage isolates varies significantly by country, age group, and type of cohort. For example, a study conducted in the Netherlands showed 19F (19%), 6B (16%), 6A (13%), 9V (7%), and 23F (7%) as the most frequently found serotype among children under 3 years of age (11). Likewise, in the USA, serotypes 6B, 14,

19F, and 23F were common (12). Influence of PCV introduction was not limited to IPD but including pneumococcal carriage. Most studies evaluating the effects of universal immunization with the PCV7 on NP carriage have shown that vaccination does not change the overall rate of pneumococcal carriage (13, 14). But the carriage of vaccine serotypes almost disappeared and replacement with non-vaccine serotypes were clearly observed (15, 16). A study conducted in Anchorage, Alaska clinics observed declines in carriage of PCV7 serotypes and trimethoprim-sulfamethoxazole nonsusceptible strains, but not in penicillin non-susceptible serotypes (15). These results implied that with persistent pressure of some antimicrobials, reductions in carriage of antimicrobial nonsusceptible PCV7 serotypes may be offset by increases in carriage of non-susceptible non-PCV7 serotypes. Similar results were also observed in a study that gathered information from primary care practices in 8 Massachusetts communities (16). This study picked out serotypes 19A and 35B as the serotypes with rapid replacement with penicillin-nonsusceptibility after the introduction of PCV7.

As was with the PCV7, PCV13 also affected changes in NP carriage. Introduction of PCV13 for universal infant use was associated with significant reductions in nasopharyngeal carriage of PCV13 serotypes and resistant strains (17). A study from eight children's hospitals in the US found a decline of the proportion of isolates non-susceptible to penicillin and ceftriaxone after the introduction of PCV13 (18). Another recent study conducted in the US showed that PCV13 has a significant added benefit over PCV7 in reducing carriage of antibiotic-nonsusceptible *S. pneumoniae*, expecting that PCV13 will provide protection against antibiotic-nonsusceptible *S. pneumoniae* disease that exceeds protection provided by PCV7 (19). Universal vaccination with PCV in infants appears to influence the antibiotic resistance of strains carried in the nasopharynx as well as on those causing invasive disease.

Despite the added protection of PCV13 among IPD, residual diseases caused by non-PCV13 serotypes were shown in a US study. A surveillance following introduction of PCV13 indicates that the most common non-PCV13 serotypes isolated from children with IPD were 22F, 33F, 38, 35B, 15B, 15C, 23B and 12F (8). A Norwegian study which invesigated IPDs among children <5 yr of age after the switch to PCV13, no trend for serotype replacement could be observed in the targeted age group, likely due to small numbers (20). As regards of NP carriage, emergence of serotypes 35B, 23B, 21 and 15A/B/C was observed after the introduction of PCV13 in the US (17, 21). In the European studies, various serotypes including serotypes 11A, 15A, 23B, 24F, and 35F were included in the replacement of NP carriage (22-24).

In Korea, PCV7 was introduced in November 2003 as an optional vaccine, and the vaccine uptake rate increased gradually, reaching 40% for 3 primary series in 2005 and 60% in 2007 (25). PCV10 and PCV13 were introduced in July 2010 also as an optional vaccine. Nationwide survey of immunization in 2013 which included the PCV coverage rate of infants younger than two years revealed that 83.4% had 1 or more doses of PCV and 70.4% had 4 doses of PCV (26). Since May 2014, PCV10 and PCV13 were included in the National Immunization Program (NIP). To date, the data regarding serotype distribution and antimicrobial susceptibilities of pneumococci that are carried in the NP from children after the introduction of PCV10 and PCV13 is limited.

The aim of this study is to investigate the distribution of serotypes and antimicrobial susceptibilities of pneumococcal carriage isolates from children after the introduction of extended-valency PCVs in Korea, thereby analyzing their influence on the NP carriage.

MATERIALS AND METHODS

Isolation of pneumococcal strains

NP aspirates were obtained from infants and children aged 18 years or less who presented with respiratory symptoms at the Seoul National University Children's Hospital from July 2010 to June 2015 (defined as post-PCV10/13 period in this study).

NP aspirate was inoculated onto a 5% defibrinated sheep blood agar plate within 72 hours from the collection and incubated overnight at 37℃ in a 5% CO_2 chamber. Identification of *S. pneumoniae* was based on the presence of alpha-hemolysis and inhibition by optochin. Subsequent pneumococci with the same serotype isolated from the same child were excluded from the analysis and only initial isolates were included in the study. Different serotypes from the same child were included in the analysis.

Serotype determination

Serotype was determined by Quellung reaction using antiserum (Statens Serum Institute, Copenhagen, Denmark). To assign serotypes 6A, 6B, 6C, and 6D, all serogroup 6 strains were screened for $wciN_{\beta}$ and $wciP_{6B}$ genes by using 2 simplex PCRs and subsequent sequencing analyses were performed as previously described (27). The *wciN* gene was amplified with the forward primer (5106) 5′-TAC CAT GCA GGG TGG AAT GT-3′ and the reverse primer (3101) 5′-CCA TCC TTC GAG TAT TGC-3′, resulting in product sizes of 1.8 kb for serotypes 6C and 6D for the $wciN_{\beta}$ gene. Following this reaction, presence of an G or A at position 584, a characteristic of 6A and 6B *wciP* ($wciP_{6A}$ and $wciP_{6B}$) respectively, was confirmed by sequencing analysis of the *wciP* gene using the forward primer 5′-AAT TTG TAT TTT ATT

CAT GCC TAT ATC TGG-3′ and the reverse primer 5′-TTA GCG GAG ATA ATT TAA AAT GAT GAC TA-3′. Strains that carry *wciP*$_{6A}$ genes are assigned as type 6A or 6C and those that carry *wciP*$_{6B}$ are assigned as type 6B or 6D. Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were classified as PCV7 serotypes. Serotypes 1, 5, and 7F were classified as PCV10/13 additional serotypes. Serotype 3, 6A, and 19A were classified as PCV13 specific serotypes. PCV serotypes were defined as any serotypes that are included in PCV13. Non-PCV serotypes include all others.

Antimicrobial susceptibility

All pneumococcal isolates obtained from children aged <5 years except non-typeable strains and two strains which failed to regrow were tested for minimal inhibitory concentrations (MICs) of 8 antimicrobials (penicillin, cefotaxime, chloramphenicol, tetracycline, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, and levofloxacin) by E-test (BioMérieux, Marcy-l'Étoile, France). Breakpoints of Clinical and Laboratory Standards Institute 2014 Guideline were used for antimicrobial susceptibility (28). For penicillin, susceptibility was analyzed by two different breakpoints, the oral penicillin breakpoint (0.06 μg/mL) and the non-meningitis parenteral breakpoint (2.0 μg/mL). Mainly, non-meningitis parenteral breakpoint was used in this study, unless specified. For cefotaxime, non-meningitis breakpoint (1.0 μg/mL) was applied. Multidrug resistance (MDR) was defined as non-susceptibility to 3 or more antimicrobial drug classes applying the non-meningitis parenteral breakpoint for penicillin (2.0 μg/mL).

Statistical analysis

Statistical analysis was performed by using the SPSS software version 23.0 (SPSS, Chicago, USA). Rates and proportions were compared using the chi-square or Fisher exact test, as appropriate. For trend analysis, linear-by-linear association model was used by the Pearson's chi-squared test. A P value <0.05 was considered statistically significant.

Ethics statements

The study protocol was approved by the institutional review board of the Seoul National University Hospital (IRB registration number-H-1105-051-361). Informed consent was exempted, since NP aspirates were obtained as a standard of patient care to identify etiologic agents of respiratory tract infections including viruses.

RESULTS

Pneumococcal carriage and demographic findings

A total of 3,820 NP aspirates were collected from July 2010 through June 2015. Fifty-eight percent of the subjects (2,230/3,820) were male and the median age of the children was 2.08 yr (range: 0.01-18.83 yr). Three hundred and ninety-seven pneumococcal isolates were recovered, with a detection rate of 10.4% (397/3,820). The male to female ratio was 1:0.64 (242:155) and the median age of the children was 2.00 yr (range: 0.08-18.25 yr) among those with pneumococcal colonization. The proportions of the carriers by age group were 49.9% (< 2 yr), 30.0% (2-4 yr), and 20.2% (≥ 5 yr), respectively. The pneumococcal detection rate of each age group was 10.7% (198/1,857) among children aged < 2 yr, 13.4% (119/888) among children aged 2-4 yr, and 7.4% (80/1,075) among children aged ≥ 5 yr. The detection rate was significantly higher among children aged between 2-4 yr (by chi-squared test, P=0.01). Table 1 shows the clinical characteristics and detection rate of pneumococcal carriage enrolled in the study.

Table 1. Clinical characteristics and detection rate of pneumococcal carriage

Variables		No. of NP aspirates (%)	No. of Pneumococcal carriage (%)	Detection rate (%)	*P* value
Gender	Male	2,230 (58.3%)	242 (61.0%)	10.9% (242/2,230)	0.60
	Female	1,590 (41.7%)	155 (39.0%)	9.7% (155/1,590)	0.51
Age	< 2 yr	1,857 (48.6%)	198 (49.9%)	10.7% (198/1,857)	0.79
	2-4 yr	888 (23.2%)	119 (30.0%)	13.4% (119/888)	0.01*
	≥ 5 yr	1,075 (28.1%)	80 (20.2%)	7.4% (80/1,075)	<0.01*
Total		3,820 (100.0%)	397 (100.0%)	10.4% (397/3,820)	

*Statistically significant by Pearson's chi-squared test ($P<0.05$)

Serotype distributions in post-PCV10/13 period

Among the 397 pneumococcal isolates, 62 (15.6%) isolates were non-typeable. Figure 1 shows the serotype distribution in post-PCV10/13 period from 2010/11 to 2014/15. The serotype distribution of pneumococci demonstrated that the most common serotypes were 19A (n = 47, 14.0%), 23A (n = 43, 12.8%), 11A (n = 34, 10.1%), 6C (n = 26, 7.8%), 6A (n = 21, 6.3%), and 19F (n = 20, 6.0%) in decreasing order of frequency. PCV serotypes accounted for 33.4% (112 isolates) while non-PCV serotypes accounted for 66.6% (223 isolates). The proportion of PCV7 serotypes accounted for 12.5% (42 isolates) and PCV13 specific serotypes accounted for 20.9% (70 isolates). Among non-PCV serotypes the most common serotypes were 23A (n = 43, 12.8%), 11A (n = 34, 10.1%), and 6C (n = 26, 7.8%) in decreasing order of frequency.

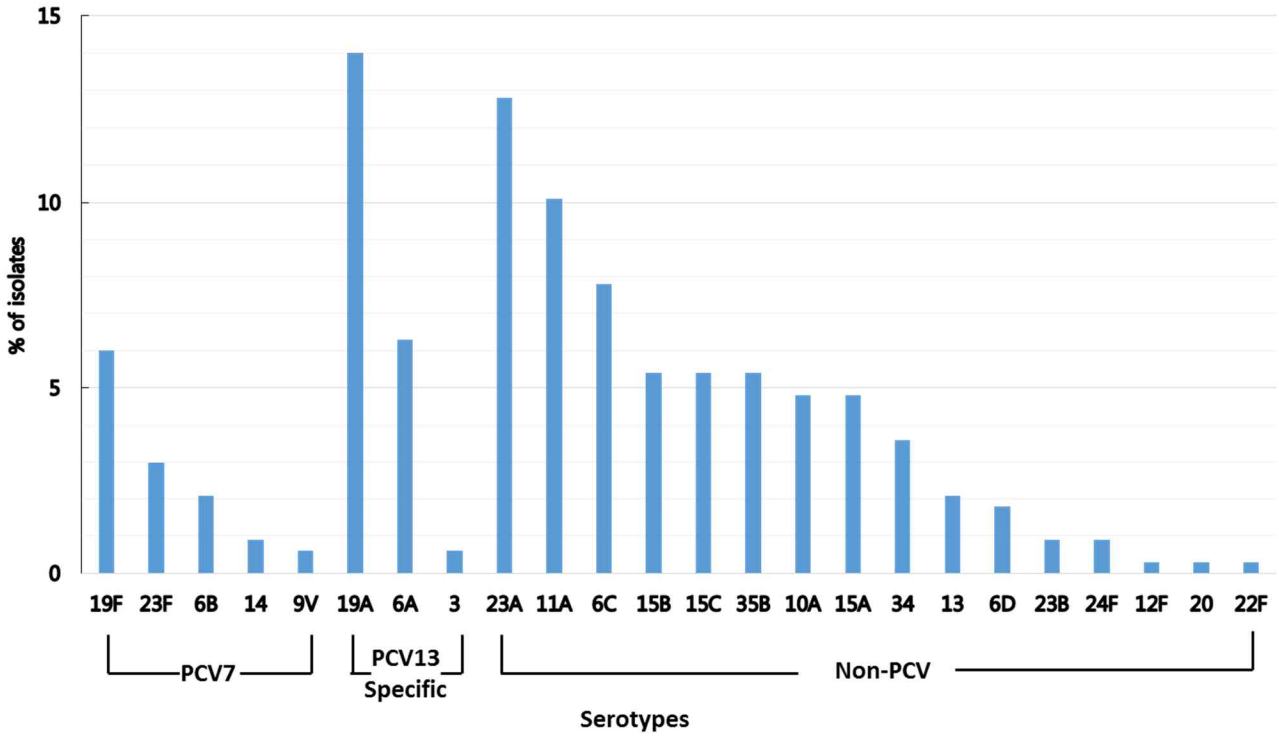

Figure 1. Serotype distributions with regards to pneumococcal conjugate vaccines (PCVs) among pneumococcal carriage isolates from children in post-PCV10/13 period, 2010-2015. PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F); PCV13 specific serotypes (3, 6A, and 19A). There was no PCV10/13 additional serotype (1, 5, and 7F).

Figure 2 shows serotype distribution by age group (< 2 yr, 2-4 yr, and ≥ 5 yr). The proportions of PCV serotypes, non-PCV serotypes, PCV7 serotypes, and PCV13 specific serotypes did not show statistical significance among different age groups. When looked at specific serotype in detail, none of the individual serotypes showed statistical significance among different age groups (data not shown).

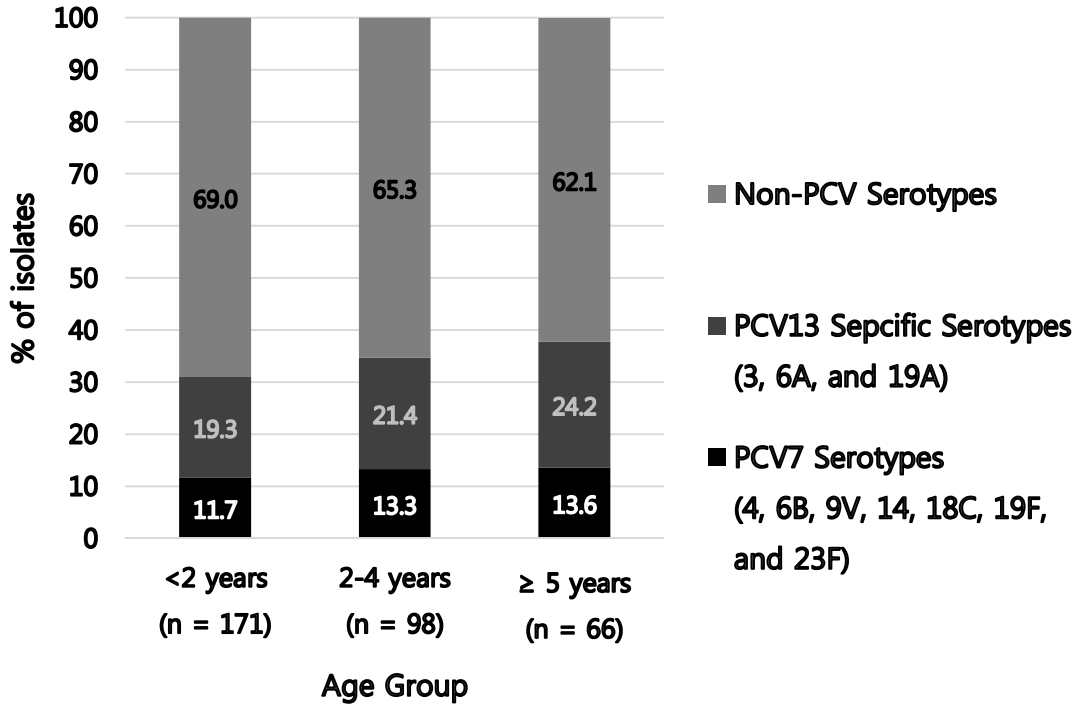

Figure 2. Serotype distributions among pneumococcal carriage isolates by age group in post-PCV10/13 period, 2010-2015. There was no PCV10/13 additional serotype (1, 5, and 7F).

Changes in serotype distributions in post-PCV10/13 period

Table 2 and Figure 3 show serotype distribution during 2010/11-2014/15, by year. The trend analysis of proportions of PCV serotypes (from 59.1% to 17.0%, $P<0.001$), PCV7 serotypes (from 25.8% to 8.5%, $P<0.001$), and PCV13 specific serotypes (from 33.3% to 8.5%, $P<0.001$) showed significant decrease from 2010/11 to 2014/15. As for individual serotypes, 6B (from 6.5% to 0%, $P=0.002$), 23F (from 6.5% to 1.1%, $P=0.033$), 6A (from 22.6% to 1.1%, $P=0.010$), and 19A (from 22.6% to 7.4%, $P=0.002$) showed statistically significant decrease. On the contrary, the trend analysis of proportions of non-PCV serotypes showed significant increase (from 40.9% to 83.0%, $P<0.001$). Among non-PCV serotypes, 6C (from 4.3% to 16.0%, $P=0.003$), 10A (from 1.1% to 9.6%, $P=0.001$), 23A (from 4.3% to 16.0%, $P=0.002$), and 23B (from 0.0% to 3.2%, $P=0.031$) showed statistically significant increase, while serotype 6D (from 4.3% to 0.0%, $P=0.004$) demonstrated significant decrease.

Table 2. Changes in serotype distributions in post-PCV10/13 period, 2010-2015

Serotype	No. (%) isolates						*P* for trend
	Total	2010/11	2011/12	2012/13	2013/14	2014/15	
PCV	**112 (33.4)**	**55 (59.1)**	**23 (37.1)**	**9 (23.1)**	**9 (19.1)**	**16 (17.0)**	**<0.001**
PCV7	*42 (12.5)*	*24 (25.8)*	*6 (9.7)*	*2 (5.1)*	*2 (4.3)*	*8 (8.5)*	*<0.001*
6B	7 (2.1)	6 (6.5)	1 (1.6)				0.002
9V	2 (0.6)	1 (1.1)	1 (1.6)				
14	3 (0.9)	1 (1.1)				2 (2.1)	
19F	20 (6.0)	10 (10.8)	2 (3.2)	2 (4.4)	1 (2.1)	5 (5.3)	
23F	10 (3.0)	6 (6.5)	2 (3.2)		1 (2.1)	1 (1.1)	0.033
PCV13 Specific	*70 (20.9)*	*31 (33.3)*	*17 (27.4)*	*7 (17.9)*	*7 (14.9)*	*8 (8.5)*	*<0.001*
3	2 (0.6)	1 (1.1)	1 (1.6)				
6A	21 (6.3)	9 (9.7)	6 (9.7)	2 (5.1)	3 (6.4)	1 (1.1)	0.010
19A	47 (14.0)	21 (22.6)	10 (16.1)	5 (12.8)	4 (8.5)	7 (7.4)	0.002
Non-PCV	**223 (66.6)**	**38 (40.9)**	**39 (62.9)**	**30 (76.9)**	**38 (80.9)**	**78 (83.0)**	**<0.001**
6C	26 (7.8)	4 (4.3)	3 (4.8)	1 (2.6)	3 (6.4)	15 (16.0)	0.003
6D	7 (2.1)	4 (4.3)	3 (4.8)				0.010
10A	16 (4.8)	1 (1.1)	1 (1.6)		5 (10.6)	9 (9.6)	0.001
11A	34 (10.1)	10 (10.8)	8 (12.9)	3 (7.7)	4 (8.5)	9 (9.6)	
12F	1 (0.3)					1 (1.1)	
13	6 (1.8)	3 (3.2)		3 (7.7)			
15A	16 (4.8)	1 (1.1)	4 (6.5)	5 (12.8)	2 (4.3)	4 (4.3)	
15B	18 (5.4)	1 (1.1)	5 (8.1)	2 (5.1)	3 (6.4)	7 (7.4)	
15C	18 (5.4)		6 (9.7)	5 (12.8)	1 (2.1)	6 (6.4)	
20	1 (0.3)					1 (1.1)	
22F	1 (0.3)					1 (1.1)	
23A	43 (12.8)	4 (4.3)	6 (9.7)	6 (15.4)	12 (25.5)	15 (16.0)	0.002
23B	3 (0.9)					3 (3.2)	0.027
24F	3 (0.9)	1 (1.1)		2 (5.1)			
34	12 (3.6)	2 (2.2)	2 (3.2)	1 (2.6)	3 (6.4)	4 (4.3)	
35B	18 (5.4)	7 (7.5)	1 (1.6)	2 (5.1)	5 (10.6)	3 (3.2)	
Total	**335 (100.0)**	**93 (100.0)**	**62 (100.0)**	**39 (100.0)**	**47 (100.0)**	**94 (100.0)**	

PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F); PCV13 specific serotypes (3, 6A, and 19A).

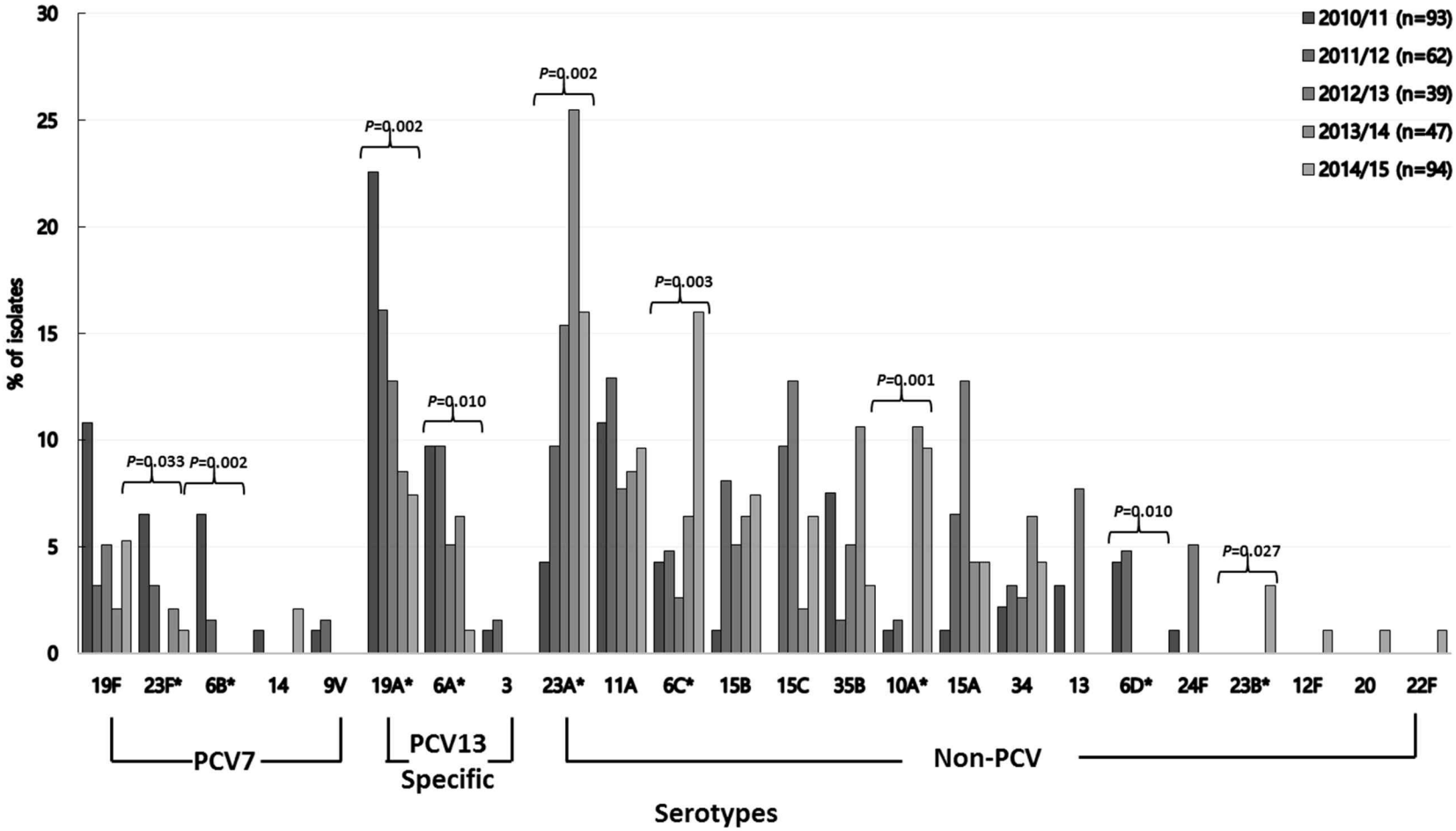

Figure 3. Changes in serotype distributions with regards to pneumococcal conjugate vaccines (PCVs) among pneumococcal carriage isolates from children in post-PCV10/13 period, 2010-2015. PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F); PCV13 specific serotypes (3, 6A, and 19A). There was no PCV10/13 additional serotype (1, 5, and 7F). *Statistically significant *P* value <0.05 by linear-by-linear analysis are shown.

Antimicrobial susceptibility in post-PCV10/13 period

Antimicrobial susceptibility data are shown in Table 3. A total number of 267 isolates were included for antibiotic susceptibility test. Susceptibility rates were 2.2% for penicillin (oral), 77.2% for penicillin (parenteral, non-meningitis), 72.3% for cefotaxime, and 4.5% for erythromycin. Non-susceptibility rates for penicillin (oral), penicillin (parenteral, non-meningitis), cefotaxime, erythromycin and multidrug (MDR) per each serotype are shown in Table 4. Overall non-susceptibility rates for penicillin (oral) and penicillin (parenteral, non-meningitis) were 97.8% and 22.8%, respectively. For cefotaxime and erythromycin, non-susceptibility rates were 27.7% and 95.5%, repsectively. The most common non-susceptible serotype to non-meningitis penicillin breakpoint was 19A (34.4%, n = 21), followed by 19F (14.8%, n = 9) and 23A (14.8%, n = 9). For cefotaxime, 19A (33.8%, n = 25) was the most common serotype followed by 23A (20.3%, n = 15) and 11A (16.2%, n = 12). There were significant differences in antibiotic non-susceptibility between PCV and non-PCV serotypes for penicillin (non-meningitis, 41.4% versus 13.9%, $P<0.001$) and cefotaxime (40.2% versus 21.7%, $P=0.001$).

Multidrug resistance rate was 91.0%. Multidrug resistance rate was significantly higher in PCV serotypes than non-PCV serotypes (97.7% versus 87.8%, $P=0.006$).

Table 3. Antimicrobial susceptibility testing in post-PCV10/13 period*

Antimicrobial agents	No. (%) isolates		
	Susceptible	Intermediate	Resistant
Penicillin (oral)†	6 (2.2)	75 (28.1)	186 (69.7)
Penicillin (non-meningitis)‡	206 (77.2)	43 (16.1)	18 (6.7)
Cefotaxime (non-meningitis)	193 (72.3)	62 (23.2)	12 (4.5)
Erythromycin	12 (4.5)	1 (0.4)	254 (95.1)
Clindamycin	62 (23.2)	2 (0.7)	203 (76)
Tetracycline	25 (9.4)		242 (90.6)
Chloramphenicol	195 (73.0)		72 (27.0)
TMP/SMX§	92 (34.5)	44 (16.5)	131 (49.1)
Levofloxacin	266 (99.6)		1 (0.4)

*Susceptibility criteria of 2014 CLSI guideline were employed.

†Breakpoints of MIC≤0.06 were employed for the classification of susceptibility.

‡Breakpoints of MIC≤2 were employed for the classification of susceptibility.

§ TMP/SMX, trimethoprim/sulfamethoxazole.

Table 4. Non-susceptibility rates for penicillin, cefotaxime, erythromycin and multidrug (MDR)*

Serotype (No.)	No. (%) of non-susceptible isolates				
	Penicillin† (oral)	Penicillin‡, ‖ (non-meningitis)	Cefotaxime ‖ (non-meningitis	Erythromycin	Multidrug§
PCV (n=87)	**85 (97.7)**	**36 (41.4)**	**35 (40.2)**	**85 (97.7)**	**85 (97.7)**
PCV7 (n=33)	*33 (100.0)*	*14 (42.4)*	*8 (24.2)*	*32 (97.0)*	*32 (97.0)*
6B (n=5)	5 (100.0)	4 (80.0)		5 (100.0)	5 (100.0)
9V (n=2)	2 (100.0)			2 (100.0)	2 (100.0)
14 (n=2)	2 (100.0)			1 (50.0)	1 (50.0)
19F (n=16)	16 (100.0)	9 (56.3)	7 (43.8)	16 (100.0)	16 (100.0)
23F (n=8)	8 (100.0)	1 (12.5)	1 (12.5)	8 (100.0)	8 (100.0)
PCV13 Specific (n=54)	*52 (96.3)*	*22 (40.7)*	*27 (50.0)*	*53 (98.1)*	*53 (98.1)*
3 (n=2)				1 (50.0)	1 (50.0)
6A (n=17)	17 (100.0)	1 (5.9)	2 (11.8)	17 (100.0)	17 (100.0)
19A (n=35)	35 (100.0)	21 (60)	25 (71.4)	35 (100.0)	35 (100.0)
Non-PCV (n=180)	**176 (97.8)**	**25 (13.9)**	**39 (21.7)**	**170 (94.4)**	**158 (87.8)**
6C (n=18)	18 (100.0)	1 (5.6)	1 (5.6)	17 (94.4)	16 (88.9)
6D (n=3)	3 (100.0)	1 (33.3)		3 (100.0)	3 (100.0)
10A (n=12)	12 (100.0)		2 (16.7)	9 (75.0)	9 (75.0)
11A (n=31)	30 (96.8)	7 (22.6)	12 (38.7)	31 (100.0)	30 (96.8)
13 (n=5)	5 (100.0)	1 (20.0)	1 (20.0)	5 (100.0)	5 (100.0)
15A (n=13)	13 (100.0)		2 (15.4)	13 (100.0)	13 (100.0)
15B (n=13)	13 (100.0)	2 (15.4)	2 (15.4)	13 (100.0)	13 (100.0)
15C (n=16)	16 (100.0)	3 (18.8)	4 (25.0)	16 (100.0)	14 (87.5)
22F (n=1)				1 (100.0)	
23A (n=36)	36 (100.0)	9 (25.0)	15 (41.7)	34 (94.4)	32 (88.9)
23B (n=2)				2 (100.0)	
24F (n=3)	3 (100.0)			3 (100.0)	3 (100.0)
34 (n=12)	12 (100.0)	1 (8.3)		8 (66.7)	8 (66.7)
35B (n=15)	15 (100.0)			15 (100.0)	12 (80.0)
Total (n=267)	**261 (97.8)**	**61 (22.8)**	**74 (27.7)**	**255 (95.5)**	**243 (91.0)**

*Susceptibility criteria of 2014 CLSI guideline were employed.

†Breakpoints of MIC≤0.06 were employed for the classification of susceptibility.

‡Breakpoints of MIC≤2 were employed for the classification of susceptibility.

§Multidrug resistance was defined as non-susceptibility to ≥ 3 antimicrobial drug classes.

[||] Statistically significant *P* value <0.05 by chi-square between PCV and non-PCV serotypes.

Changes of penicillin non-susceptible serotypes in post-PCV10/13 period

The changes of penicillin non-susceptible serotypes in post-PCV10/13 period are shown in Table 5. The trend analysis of penicillin (parenteral, non-meningitis) susceptibility testing from 2010/11 to 2014/15 did not show statistical significance (non-susceptibility from 33.3% to 26.4%, P=0.381). The proportion of PCV serotypes among isolates with non-susceptibility to penicillin decreased from 80.8% in 2010/2011 to 21.1% in 2014/2015 (P<0.001) while that of non-PCV serotypes increased from 19.2% in 2010/2011 to 78.9% in 2014/2015 (P<0.001). The PCV7 serotypes also showed statistically significant decrease in the proportion among isolates with non-susceptibility (from 42.3% to 5.3%, P<0.001). Nevertheless, the PCV13 specific serotypes did not show significant change (from 38.5% to 15.8%, P=0.086).

Table 5. Changes of penicillin non-suscptible serotypes in post-PCV10/13 period*

Serotype (No.)	No. (%) of non-susceptible isolates†					*P* for trend
	2010/11 (n=78)	2011/12 (n=47)	2012/13 (n=33)	2013/14 (n=37)	2014/15 (n=72)	
PCV (n=36)	**21 (80.8)**	**5 (83.3)**	**5 (83.3)**	**1 (25.0)**	**4 (21.1)**	**<0.001**
PCV7 (n=14)	*11 (42.3)*	*1 (16.7)*	*1 (16.7)*		*1 (5.3)*	*0.003*
6B (n=4)	4 (15.4)					0.041
19F (n=9)	6 (23.1)	1 (16.7)	1 (16.7)		1 (5.3)	
23F (n=1)	1 (3.8)					
PCV13 specific (n=22)	**10 (38.5)**	**4 (66.7)**	**4 (66.7)**	**1 (25.0)**	**3 (15.8)**	
6A (n=1)		1 (16.7)				
19A (n=21)	10 (38.5)	3 (50.0)	4 (66.7)	1 (25.0)	3 (15.8)	
Non-PCV (n=25)	**5 (19.2)**	**1 (16.7)**	**1 (16.7)**	**3 (75.0)**	**15 (78.9)**	**<0.001**
6C (n=1)					1 (5.3)	
6D (n=1)	1 (3.8)					
11A (n=7)	3 (11.5)			1 (25.0)	3 (15.8)	
13 (n=1)	1 (3.8)					
15B (n=2)		1 (16.7)			1 (5.3)	
15C (n=3)					3 (15.8)	0.022
23A (n=9)			1 (16.7)	2 (50.0)	6 (31.6)	0.001
34 (n=1)					1 (5.3)	
Overall (n=61)	**26 (33.3)‡**	**6 (12.8)‡**	**6 (18.2)‡**	**4 (10.8)‡**	**19 (26.4)‡**	**0.381**

*Susceptibility criteria of 2014 CLSI guideline were employed. Breakpoints of MIC>2 (parenteral, non-meningitis) were employed for the classification of non-susceptibility.

†Percentage represents proportion of each serotype within the penicillin non-susceptible serotypes of each separate study period.

‡Percentage represents proportion of penicillin non-susceptible serotypes within the total serotypes detected in each study period.

Changes of MDR serotypes in post-PCV10/13 period

The changes of MDR serotypes in post-PCV10/13 period are shown in Table 6. The trend analysis of MDR from 2010/11 to 2014/15 did not show statistical significance (from 89.7% to 90.3%, P=0.998). The proportion of PCV serotypes consisting MDR decreased significantly from 64.3% in 2010/11 to 12.3% in 2014/15 (P<0.001) while non-PCV serotypes increased from 35.7% in 2010/11 to 87.7% in 2014/15 (P<0.001). PCV13 specific serotypes also showed significant decrease in proportion (from 32.9% to 7.7%, P<0.001). Among the PCV7 serotypes 6B (from 7.1% to 0.0%, P=0.007) and 23F (from 8.6% to 0.0%, P=0.010), and among the PCV13 specific serotypes 19A (from 22.9% to 6.2%, P=0.002) and 6A (from 8.6% to 1.5%, P=0.047) showed significant decrease in proportion. Serotype 23A (from 2.9% to 20.0%, P<0.001), 10A (from 0.0% to 10.8%, P<0.001), 6C (from 2.9% to 13.8%, P=0.013), and 15B (from 0.0% to 9.2%, P=0.048) showed proportional increase among non-PCV10/13 serotypes.

Table 6. Changes of multidrug resistant serotypes in post-PCV10/13 period*

Serotype (No.)	No. (%) of multidrug resistant (≥ 3) isolates†					*P* for trend
	2010/11 (n=78)	2011/12 (n=47)	2012/13 (n=33)	2013/14 (n=37)	2014/15 (n=72)	
PCV (n=85)	**45 (64.3)**	**18 (41.9)**	**8 (25)**	**6 (18.2)**	**8 (12.3)**	**<0.001**
PCV7 (n=32)	*22 (31.4)*	*4 (9.3)*	*1 (3.1)*	*2 (6.1)*	*3 (4.6)*	*<0.001*
6B (n=5)	5 (7.1)					0.007
9V (n=2)	1 (1.4)	1 (2.3)				
14 (n=1)	1 (1.4)					
19F (n=16)	9 (12.9)	2 (4.7)	1 (3.1)	1 (3.0)	3 (4.6)	
23F (n=8)	6 (8.6)	1 (2.3)		1 (3.0)		0.010
PCV13 specific (n=53)	*23 (32.9)*	*14 (32.6)*	*7 (21.9)*	*4 (12.1)*	*5 (7.7)*	*<0.001*
3 (n=1)	1 (1.4)					
6A (n=17)	6 (8.6)	6 (14)	2 (6.3)	2 (6.1)	1 (1.5)	0.047
19A (n=35)	16 (22.9)	8 (18.6)	5 (15.6)	2 (6.1)	4 (6.2)	0.002
Non-PCV (n=158)	**25 (35.7)**	**25 (58.1)**	**24 (75)**	**27 (81.8)**	**57 (87.7)**	**<0.001**
6C (n=16)	2 (2.9)	2 (4.7)	1 (3.1)	2 (6.1)	9 (13.8)	0.013
6D (n=3)	2 (2.9)	1 (2.3)				
10A (n=9)				2 (6.1)	7 (10.8)	<0.001
11A (n=30)	9 (12.9)	7 (16.3)	2 (6.3)	3 (9.1)	9 (13.8)	
13 (n=5)	3 (4.3)		2 (6.3)			
15A (n=13)		4 (9.3)	4 (12.5)	2 (6.1)	3 (4.6)	
15B (n=13)		4 (9.3)	1 (3.1)	2 (6.1)	6 (9.2)	0.048
15C (n=14)		4 (9.3)	4 (12.5)	1 (3.0)	5 (7.7)	
23A (n=32)	2 (2.9)	2 (4.7)	6 (18.8)	9 (27.3)	13 (20.0)	<0.001
24F (n=3)	1 (1.4)		2 (6.3)			
34 (n=8)		1 (2.3)	1 (3.1)	3 (9.1)	3 (4.6)	
35B (n=12)	6 (8.6)		1 (3.1)	3 (9.1)	2 (3.1)	
Overall (n=243)	**70 (89.7)‡**	**43 (91.5)‡**	**32 (97.0)‡**	**33 (89.2)‡**	**65 (90.3)‡**	**0.998**

*Susceptibility criteria of 2014 CLSI guideline were employed. Breakpoints of MIC>2 (parenteral, non-meningitis) to penicillin were employed for the classification of non-susceptibility.

†Percentage represents proportion of each serotype within the multidrug resistant serotypes of each separate study period.

‡Percentage represents proportion of multidrug resistant serotypes within the total serotypes detected in each study period.

DISCUSSION

This study highlights the changes in serotype distribution of NP carriage in children after the introduction of PCV10 and PCV13 in a country where PCVs have already been used for 12 years since 2003. This study investigates the latter 5 years which is the period of extended-valency PCVs. During the 5-year study period, serotype distribution showed further decrease of PCV7 serotypes and decrease of PCV13 specific serotypes. But the proportion of non-PCV serotypes significantly increased. Serotype 19A which showed marked increase after the introduction of PCV7 (29) decreased by the introduction of extended-valency PCVs. Decrease in serotype 19A and 6A was most significant. While, the serotype 19F which remained in considerable proportion even after the introduction of PCV7 did not show significant change.

In the studies that investigated the serotype distribution of pneumococcal carriage after routine PCV7 immunization, PCV7 reduced the carriage of PCV7 serotype pneumococci, which was replaced with non-PCV7 serotypes, making only a slight decrease or even no change in the overall pneumococcal carriage rate (15, 30, 31). This phenomenon was also observed after the introduction of PCV13 in major studies conducted in the US (10, 17). These results seem to be consistent with the results in our study, where the proportion of PCV10/13 serotype isolates decreased and non-PCV10/13 serotypes increased.

Serotype 19A has been a major issue since the introduction of PCV in both the IPD and the pneumococcal carriage. A large study conducted in the US after the introduction of PCV7 in October 2000 showed that even though IPD caused by PCV7 serotypes declined through 2005, overall IPD rates leveled off beginning in 2002, primarily because of increases in the incidence of IPD caused by non-PCV7 serotype 19A (3). In regarding of the pneumococcal carriage, disappearance of vaccine serotypes occurred in young children in the US after introduction of

PCV7, with rapid replacement with penicillin-nonsusceptible non-vaccine serotypes, particularly 19A (15, 16). The current study focuses on the change in serotype distribution after the introduction of PCV10/13 and demonstrates the decrease of serotype 19A, which has been shown in many studies. We have also found that non-PCV10/13 serotypes have increased and serotypes 23A, 6C, and 10A take considerable part in the increase in Korea. A previous report which was performed four years after the introduction of PCV10/13 based on children who were attending daycare centers in Korea also showed the most common serotype as 23A, which is consistent with our study (32).

Change in the serotype distribution may differ by region, time, the PCV being implemented, and immunization policies. A well designed study performed in Atlanta, Georgia, in the US showed serotype 35B as the most increasing non-vaccine serotype in pneumococcal carriage after the introduction of PCV13 (17). The study also demonstrated steeper decrease (from 25.8% to 3%, $P<0.0001$) and increase (from 68.4% to 97%, $P<0.0001$) of PCV10/13 serotypes and non-PCV10/13 serotypes compared to our observations. Another study from the US showed emergence of serotypes 35B, 23B, 21 and 15A/B/C (21). In a different study conducted in France which collected the NP carriages of young children with acute otitis media after the introduction of PCV13, though increase of non-vaccine serotypes was not clearly observed, serotype 15A and 11A was detected from 5% of NP carriages (22). In the United Kingdom, a study also showed continued reduction in carriage of PCV7 serotypes and additional protection against carriage of the PCV13 specific serotypes across all age groups within two years of PCV13 replacing PCV7, with serotypes of 11A, 23B, 24F, and 35F being the most frequent in 2012/2013 (24). A study conducted in Italy which assessed NP carriage at 6 months and 12 months following PCV13 vaccination in healthy Italian children aged 3–59 months observed a

decrease in NP colonization caused by PCV13 serotypes, while substantial increase of 15A was noticed (23).

A previous study that was performed in the same institute investigated the NP carriage after the introduction of PCV7. In this study, 19F was the most common serotype among PCV7 serotypes and showed modest reduction during the study period (29). The explanation for this was that serotype 19F showed the lowest geometric mean titer (GMT) in the opsonophagocytic assay (OPA) among PCV7 serotypes and this might mean a weaker vaccine-induced mucosal immunity (33). A recent study demonstrated the decreased acquisition of 19F and significantly higher IgG responses elicited by PCV13 for this specific serotype (34). PCV13 may have enhanced activity against serotype 19F due to cross-reactive antibodies induced by 19A (35). Although there is a trend of decrease in serotype 19F, our findings did not demonstrate a significant decrease of serotype 19F. Rather, our study demonstrates marked reduction of serotype 6A. A study conducted in Massachusetts, US, showed reduction of serotype 6A in pneumococcal carriage after introduction of PCV7 which was not observed in the previous study conducted in Korea (29, 36). It was assumed that as OPA GMT of serotype 6A reached similar levels with that of serotype 19F, there could be a delayed reduction where PCV7 coverage is modest. It is reasonable to concede that effect of delayed reduction by PCV7 was added to the direct effect of PCV13.

In the post-PCV7 and pre-PCV10/13 era, despite the decrease of penicillin non-susceptible PCV7 serotype pneumococci, non-susceptibility to penicillin remained steady in many of the previous studies, mostly due to an increased proportion of penicillin non-susceptible non-PCV7 serotype pneumococci, serotype 19A in particular (15, 16, 30). In the post-PCV10/13 era, several studies show decrease in non-susceptibility to penicillin or MDR. A recent study from

42 medical centers in the US investigated antimicrobial susceptibilities for pneumococci from invasive and noninvasive disease among all age groups from 2008/09 to 2012/13. The study revealed significant decrease in the overall non-susceptibility to penicillin and MDR among pneumococcal isolates after the introduction of PCV13 (37). This study also demonstrates that the increasing proportion of non-PCV13 serotypes exhibited non-susceptibility to penicillin or MDR. Of the penicillin non-susceptible or multidrug resistant serotypes, 35B, 23A, 23B, and 15B, were the serotypes that replaced the decreasing serotype 19A. This current study reveals unique changes in the serotype distribution among penicillin non-susceptible and multidrug resistant isolates. In terms of proportion of isolates non-susceptible to penicillin, the major serotypes were PCV10/13 serotypes in 2010/11 period but non-PCV10/13 serotypes in 2014/15. This study showed significant decrease in proportions of serotypes 6B, 6A, 19A, and 23F, of which are known for the high level of antibiotics resistance. On the other hand, serotypes with increasing proportions for penicillin non-susceptibility were 23A and 15C, while those for multidrug resistant were 23A, 10A, 6C, and 15B. This result is partly consistent with previous study conducted in Korea which demonstrated 23A, 15B, and 15C as the antibiotic non-susceptible serotypes with high rates of carriage following the introduction of extended-valency PCVs in Korea (32). While a study conducted in the US which assessed the potential additional impact of PCV13 over PCV7 revealed that of the most commonly isolated nonvaccine serotypes, those having the highest proportion of nonsusceptible isolates were 10B, 11A, 15A, 15B/C, 16F, 17F, 21, 23B, 34, 35B, and 38 (19). We have not yet found one dominant non-PCV10/13 serotype that was highly associated with antibiotic non-susceptibility among NP carriage isolates, for example, serotype 19A in the post-PCV7 and pre-PCV10/13 period. Continued surveillance is needed to monitor trends of non-vaccine serotypes that might emerge to be highly associated with antibiotic non-susceptibility.

This study has several limitations. First, because pneumococcal carriage isolates were collected at a single center in a large city, this sample may not represent the whole national data. Multicenter-based data collection is needed to investigate the change of pneumococcal carriage to evaluate the impact of newly introduced pneumococcal conjugate vaccines on serotype distribution. Second, this study did not include the individual's vaccination status and previous antibiotic history. Our study brings up results from further invesigations of extended cases, although the results are partly consistent with a study that investigated nasopharyngeal swabs collected from children who were attending daycare centers in Korea which showed high rates of carriage caused by non-PCV13 and sustaining antibiotics non-susceptibility after the introduction of extended-valency PCVs (32).

This study may have several implications for the future pneumococcal immunization programs. The replacement phenomenon which is also being demonstrated in PCV10/13 is a challenging situation for the current vaccine strategy in Korea. It might also implicate that polysaccharide-based pneumococcal conjugate vaccine may not be the fundamental solution against IPD and carriage. Considering the unique situation of consistent antibiotics non-susceptibility and MDR rate despite the decrease of PCV serotypes, there is a need for judicious antibiotics use in Korea.

REFERENCES

1. Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children - use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine - recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports*. 2010;59(Rr-11):1-18.

2. Bogaert D, De Groot R, Hermans PW. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *The Lancet Infectious diseases*. 2004;4(3):144-54.

3. Centers for Disease Control and Prevention. Invasive pneumococcal disease in children 5 years after conjugate vaccine introduction--eight states, 1998-2005. *MMWR Morbidity and mortality weekly report*. 2008;57(6):144-8.

4. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. *The Lancet Infectious diseases*. 2011;11(10):760-8.

5. Spika JS, Facklam RR, Plikaytis BD, Oxtoby MJ. Antimicrobial resistance of Streptococcus pneumoniae in the United States, 1979-1987. The Pneumococcal Surveillance Working Group. *The Journal of infectious diseases*. 1991;163(6):1273-8.

6. Hofmann J, Cetron MS, Farley MM, Baughman WS, Facklam RR, Elliott JA, et al. The prevalence of drug-resistant Streptococcus pneumoniae in Atlanta. *The New England journal of medicine*. 1995;333(8):481-6.

7. Hampton LM, Farley MM, Schaffner W, Thomas A, Reingold A, Harrison LH, et al. Prevention of antibiotic-nonsusceptible Streptococcus pneumoniae with conjugate vaccines. *The Journal of infectious diseases*. 2012;205(3):401-11.

8. Moore MR, Link-Gelles R, Schaffner W, Lynfield R, Lexau C, Bennett NM, et al. Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children and adults in the USA: analysis of multisite, population-based surveillance. *The Lancet Infectious diseases*. 2015;15(3):301-9.

9. Gounder PP, Bruce MG, Bruden DJ, Singleton RJ, Rudolph K, Hurlburt DA, et al. Effect of the 13-valent pneumococcal conjugate vaccine on nasopharyngeal colonization by Streptococcus pneumoniae--Alaska, 2008-2012. *The Journal of infectious diseases*. 2014;209(8):1251-8.

10. Lee GM, Kleinman K, Pelton SI, Hanage W, Huang SS, Lakoma M, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccination on Streptococcus pneumoniae Carriage in Young Children in Massachusetts. *Journal of the Pediatric Infectious Diseases Society*. 2014;3(1):23-32.

11. Bogaert D, Engelen MN, Timmers-Reker AJ, Elzenaar KP, Peerbooms PG, Coutinho RA, et al. Pneumococcal carriage in children in The Netherlands: a molecular epidemiological study. *Journal of clinical microbiology*. 2001;39(9):3316-20.

12. Yeh SH, Zangwill KM, Lee H, Chang SJ, Wong VI, Greenberg DP, et al. Heptavalent pneumococcal vaccine conjugated to outer membrane protein of Neisseria meningitidis serogroup b and nasopharyngeal carriage of Streptococcus pneumoniae in infants. *Vaccine*. 2003;21(19-20):2627-31.

13. Huang SS, Platt R, Rifas-Shiman SL, Pelton SI, Goldmann D, Finkelstein JA. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. *Pediatrics*. 2005;116(3):e408-13.

14. Millar EV, O'Brien KL, Watt JP, Bronsdon MA, Dallas J, Whitney CG, et al. Effect of community-wide conjugate pneumococcal vaccine use in infancy on nasopharyngeal carriage

through 3 years of age: a cross-sectional study in a high-risk population. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2006;43(1):8-15.

15. Park SY, Moore MR, Bruden DL, Hyde TB, Reasonover AL, Harker-Jones M, et al. Impact of conjugate vaccine on transmission of antimicrobial-resistant Streptococcus pneumoniae among Alaskan children. *The Pediatric infectious disease journal*. 2008;27(4):335-40.

16. Huang SS, Hinrichsen VL, Stevenson AE, Rifas-Shiman SL, Kleinman K, Pelton SI, et al. Continued impact of pneumococcal conjugate vaccine on carriage in young children. *Pediatrics*. 2009;124(1):e1-11.

17. Desai AP, Sharma D, Crispell EK, Baughman W, Thomas S, Tunali A, et al. Decline in Pneumococcal Nasopharyngeal Carriage of Vaccine Serotypes After the Introduction of the 13-Valent Pneumococcal Conjugate Vaccine in Children in Atlanta, Georgia. *The Pediatric infectious disease journal*. 2015;34(11):1168-74.

18. Kaplan SL, Barson WJ, Lin PL, Romero JR, Bradley JS, Tan TQ, et al. Early trends for invasive pneumococcal infections in children after the introduction of the 13-valent pneumococcal conjugate vaccine. *The Pediatric infectious disease journal*. 2013;32(3):203-7.

19. Dagan R, Juergens C, Trammel J, Patterson S, Greenberg D, Givon-Lavi N, et al. Efficacy of 13-valent pneumococcal conjugate vaccine (PCV13) versus that of 7-valent PCV (PCV7) against nasopharyngeal colonization of antibiotic-nonsusceptible Streptococcus pneumoniae. *The Journal of infectious diseases*. 2015;211(7):1144-53.

20. Steens A, Bergsaker MA, Aaberge IS, Ronning K, Vestrheim DF. Prompt effect of replacing the 7-valent pneumococcal conjugate vaccine with the 13-valent vaccine on the epidemiology of invasive pneumococcal disease in Norway. *Vaccine*. 2013;31(52):6232-8.

21. Kaur R, Casey JR, Pichichero ME. Emerging Streptococcus pneumoniae Strains Colonizing the Nasopharynx in Children After 13-valent Pneumococcal Conjugate Vaccination in Comparison to the 7-valent Era, 2006-2015. *The Pediatric infectious disease journal*. 2016;35(8):901-6.

22. Cohen R, Levy C, Bingen E, Koskas M, Nave I, Varon E. Impact of 13-valent pneumococcal conjugate vaccine on pneumococcal nasopharyngeal carriage in children with acute otitis media. *The Pediatric infectious disease journal*. 2012;31(3):297-301.

23. Mameli C, Fabiano V, Daprai L, Bedogni G, Faccini M, Garlaschi ML, et al. A longitudinal study of streptococcus pneumoniae carriage in healthy children in the 13-valent pneumococcal conjugate vaccine era. *Human vaccines & immunotherapeutics*. 2015;11(4):811-7.

24. van Hoek AJ, Sheppard CL, Andrews NJ, Waight PA, Slack MP, Harrison TG, et al. Pneumococcal carriage in children and adults two years after introduction of the thirteen valent pneumococcal conjugate vaccine in England. *Vaccine*. 2014;32(34):4349-55.

25. Choe YJ, Yang JJ, Park SK, Choi EH, Lee HJ. Comparative estimation of coverage between national immunization program vaccines and non-NIP vaccines in Korea. *Journal of Korean medical science*. 2013;28(9):1283-8.

26. Korea Centers for Disease Control and Prevention. National Immunization Survey in South Korea, 2013. *Public Health Weekly Report*. 2013;7(21):449-54.

27. Choi EH, Lee HJ, Cho EY, Oh CE, Eun BW, Lee J, et al. Prevalence and genetic structures of Streptococcus pneumoniae serotype 6D, South Korea. *Emerging infectious diseases*. 2010;16(11):1751-3.

28. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 18th information supplement, CLSI document M100-S24. *Wayne PA:*

National Committee for Clinical and Laboratory Standards. 2014.

29. Cho EY, Kang HM, Lee J, Kang JH, Choi EH, Lee HJ. Changes in serotype distribution and antibiotic resistance of nasopharyngeal isolates of Streptococcus pneumoniae from children in Korea, after optional use of the 7-valent conjugate vaccine. *Journal of Korean medical science*. 2012;27(7):716-22.

30. Moore MR, Hyde TB, Hennessy TW, Parks DJ, Reasonover AL, Harker-Jones M, et al. Impact of a conjugate vaccine on community-wide carriage of nonsusceptible Streptococcus pneumoniae in Alaska. *The Journal of infectious diseases*. 2004;190(11):2031-8.

31. Dunais B, Bruno-Bazureault P, Carsenti-Dellamonica H, Touboul P, Pradier C. A decade-long surveillance of nasopharyngeal colonisation with Streptococcus pneumoniae among children attending day-care centres in south-eastern France: 1999-2008. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*. 2011;30(7):837-43.

32. Choe YJ, Lee HJ, Lee H, Oh CE, Cho EY, Choi JH, et al. Emergence of antibiotic-resistant non-vaccine serotype pneumococci in nasopharyngeal carriage in children after the use of extended-valency pneumococcal conjugate vaccines in Korea. *Vaccine*. 2016;34(40):4771-6.

33. Bryant KA, Block SL, Baker SA, Gruber WC, Scott DA, Group PCVIS. Safety and immunogenicity of a 13-valent pneumococcal conjugate vaccine. *Pediatrics*. 2010;125(5):866-75.

34. Dagan R, Patterson S, Juergens C, Greenberg D, Givon-Lavi N, Porat N, et al. Comparative immunogenicity and efficacy of 13-valent and 7-valent pneumococcal conjugate vaccines in reducing nasopharyngeal colonization: a randomized double-blind trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*.

2013;57(7):952-62.

35. Grant LR, O'Brien SE, Burbidge P, Haston M, Zancolli M, Cowell L, et al. Comparative immunogenicity of 7 and 13-valent pneumococcal conjugate vaccines and the development of functional antibodies to cross-reactive serotypes. *PloS one*. 2013;8(9):e74906.

36. Nahm MH, Lin J, Finkelstein JA, Pelton SI. Increase in the prevalence of the newly discovered pneumococcal serotype 6C in the nasopharynx after introduction of pneumococcal conjugate vaccine. *The Journal of infectious diseases*. 2009;199(3):320-5.

37. Richter SS, Diekema DJ, Heilmann KP, Dohrn CL, Riahi F, Doern GV. Changes in pneumococcal serotypes and antimicrobial resistance after introduction of the 13-valent conjugate vaccine in the United States. *Antimicrobial agents and chemotherapy*. 2014;58(11):6484-9.

국문 초록

서론: 폐구균(*Streptococcus pneumoniae*)은 소아에서 중이염, 부비동염, 폐렴, 패혈증, 수막염 등의 질환을 일으키는 주요 원인균이며 상기도에 무증상으로 집락하며, 상기도염 등의 선행 요인이 있을 때 혈행성 감염 또는 주변의 점막 감염을 일으킨다. 우리나라에서는 7가 폐구균 단백결합 백신(PCV7)이 2003년부터 사용되었으며, 2010 년에는 PCV10/13이 PCV7을 대체하여 사용되었다. 본 연구는 우리나라에서 PCV10/13이 사용된 이후 소아청소년의 비인두로부터 분리된 폐구균의 혈청형 분포와 항생제 감수성을 조사하고자 하였다.

방법: 2011 년 7 월부터 2015 년 6 월까지 서울대학교병원 어린이병원에 호흡기 증상을 주소로 방문한 18 세 이하의 소아청소년의 비인두 검체 3,820 개로부터 폐구균을 분리하였다. Quellung reaction 및 Multiplex PCR 법을 통하여 폐구균의 혈청형을 결정하였으며 항생제 감수성은 E-test 법으로 검사하였다 (penicillin, cefotaxime, chloramphenicol, tetracycline, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, and levofloxacin). 연도별로 혈청형 분포의 변화와 항생제 감수성의 변화를 분석하였다.

결과: 비인두 검체 3,820개로부터 폐구균 총 397 균주를 분리하여 보균율은 10.4%이었다. 비피막형 62 균주를 제외한 335 균주의 혈청형 분포는 19A (14.0%), 23A (12.8%), 11A (10.1%), 6C (7.8%), 6A (6.3%), 19F (6.0%) 순이었다. 전체적으로, PCV 에 포함된 혈청형은 33.4%, PCV 에 포함되지 않은 혈청형은 66.6%를 차지하였다. PCV7 에 포함된 혈청형은 12.5% 였으며 PCV13 에만 포함된 혈청형은 20.9% 를 차지하였다. 폐구균의 혈청형 분포의 변화를 분석한 결과, PCV 혈청형은 2010/2011년에 59.1%, 2014/15년에 17.0%로 의미 있게 감소하였다 ($P<0.001$). 반면, PCV에 포함되지 않은 혈청형은 2010/2011년에 40.9%, 2014/15년에 83.0%로 증가하는 추세였다 ($P<0.001$). Penicillin (경구), penicillin (정맥, 비수막염), cefotaxime, erythromycin 의 비감수성률은 각각 97.8%, 22.8%, 27.7%, 95.5% 이었다. PCV 혈청형이 PCV에 포함되지 않은 혈청형에 비하여 penicillin (정맥, 비수막염, 41.4% vs 13.9%, $P<0.001$) 과 cefotaxime (40.2% vs 21.7%, $P=0.001$)에 대한 비감수성률이 더 높았다. PCV 혈청형은 2010/11 과 2014/15 의 기간 동안 penicillin (정맥, 비수막염) 비감수성 (80.8% vs 21.1%, $P<0.001$) 과 다제내성 (64.3% vs 12.3%, $P<0.001$) 에서 차지하는 비율이 유의하게 감소하였다.

결론: 우리나라에서 PCV10/13이 사용된 이후 소아청소년의 비

인두에서 분리된 폐구균의 PCV 혈청형의 분포는 감소하였고 PCV에 포함되지 않은 혈청형은 증가하였다. Penicillin (경구) 과 erythromycin 에 대한 내성률은 여전히 높은 상태이나 페니실린 비감수성과 다제내성을 차지하는 PCV 혈청형의 비율은 감소하였다.

주요어: 폐구균, 혈청형, 항생제 내성, 폐구균 단백결합 백신

학 번: 2012-21708