의학석사 학위논문

Serotype and Antibiotic Resistance of *Streptococcus pneumoniae* Isolates from Invasive Disease in Children after Optional Use of the 7-valent Conjugate Vaccine; A Multicenter Study, 2006-2010

7가 단백결합 폐구균 백신 사용 후 국내 소아청소년 침습성 감염에서 분리된 폐구균의 혈청형과 항생제 내성; 다기관 연구, 2006-2010

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by
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ABSTRACT

Introduction: *Streptococcus pneumoniae* is a major cause of invasive bacterial infections in children. Serotype distribution of pneumococcus is different according to the study period and region, and it is also influenced by type of vaccine used. The purpose of this study is to investigate serotype distribution and antimicrobial susceptibility of pneumococcal isolates from invasive infections in children by a multicenter study, between 2006 and 2010, when 7-valent pneumococcal conjugate vaccine (PCV7) had been widely used and 10 (PCV10) or 13-valent (PCV13) vaccine has just been introduced in Korea.

Methods: From January 2006 to December 2010, 140 pneumococcal isolates from invasive infections in children younger than 18 years collected in 8 centers in Korea were included in this study. Serotype was determined by Quellung reaction or multiplex polymerase chain reaction. Antimicrobial susceptibility was tested by E-test and interpreted according to the 2003 CLSI guideline for epidemiological comparison. Medical records of the patients were reviewed retrospectively.

Results: The most common serotypes among 140 isolates were

19A (n=32, 22.9%), 19F (n=17, 12.1%), 6B (n=12, 8.6%), and 23F (n=11, 7.9%). Overall, the proportions of PCV7 serotypes were 45.0%, additional 3 serotypes in PCV10 were 2.9%, and 3 PCV13-specific serotypes were 29.3%. In a trend analysis of yearly serotype proportion between 2006 and 2010, PCV7 serotype had decreased from 62.5% to 21.4% (P=0.002), whereas additional 6 serotypes in PCV13 had increased from 21.9% to 46.4% (P=0.031). Antimicrobial susceptibility test was done for 123 isolates, nonsusceptibility rates of penicillin, cefotaxime, and erythromycin were 88.6%, 23.6%, and 87.0%, respectively. Penicillin nonsusceptibility rates of PCV7 serotypes, additional 3 serotypes in PCV10, 3 PCV13-specific serotypes, and nonvaccine serotypes were 91.2%, 0.0%, 94.4%, and 85.2%; cefotaxime nonsusceptibility rates were 17.5%, 0.0%, 47.2%, and 7.4%; and erythromycin nonsusceptibility rates were 94.7%, 33.3%, 91.7%, and 70.4%, respectively.

Conclusions: In this study of pneumococcal isolates from pediatric invasive infection between 2006 and 2010, the proportion of PCV7 serotypes decreased, while additional serotypes in PCV13 increased over time. Antimicrobial resistance rates were higher in PCV7 serotypes and additional

serotypes in PCV13. These findings can be the basis of pneumococcal vaccine policy and treatment strategy of pneumococcal infections in children in Korea.

Keywords: *Streptococcus pneumoniae*; Serotype; Drug Resistance; Heptavalent pneumococcal conjugate vaccine
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CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

IPD; Invasive Pneumococcal Disease

PCV7; 7-valent Pneumococcal Conjugate Vaccine

PCV10; 10-valent Pneumococcal Conjugate Vaccine

PCV13; 13-valent Pneumococcal Conjugate Vaccine

PCR; Polymerase Chain Reaction

NVT; Nonvaccine Type (Nonvaccine serotype)

MIC; Minimum Inhibitory Concentration

MDR; Multidrug resistance

INTRODUCTION

Streptococcus pneumoniae is a leading cause of serious illness and death, manifested by bacteremia, meningitis, and pneumonia in children and adults worldwide. It is also a major cause of respiratory tract infections including sinusitis and acute otitis media (1). In a multicenter study of etiology of invasive bacterial infections in immunocompetent children in the Republic of Korea during the period of 1996–2005, *S. pneumoniae* (45.3%) was the most common cause in children aged 3 months to 5 years. Moreover, when classified according to the site of infection, *S. pneumoniae* was a common cause of meningitis (41.6%), bacteremia without localization (40.0%), and bacteremic pneumonia (74.1%) in this age group (2).

The capsule of the *Streptococcus pneumoniae* consists of polysaccharides, which constitutes a major virulence factor for the bacterium. Currently, more than 90 serotypes of *S. pneumoniae* have been identified on the basis of antigenicity of their capsular polysaccharides, yet a relatively limited number of serotypes cause the majority of invasive pneumococcal disease (IPD) (1).

In 2000 in the United States, a 7-valent pneumococcal conjugate vaccine (PCV7; Prevenar®, Wyeth) was licensed and Advisory Committee on Immunization Practices (ACIP) recommended its routine use in children aged 2-23 months. In the United States, the seven serotypes in PCV7 (4, 6B, 9V, 14, 18C, 19F, and 23F) were the most common serotypes isolated from the blood or CSF in children aged < 5 years, and accounted for 80% of invasive pneumococcal infections. In addition, antimicrobial resistance was detected most frequently among the serotypes included in PCV7 (1).

In 2009, the Committee for Medicinal Products for Human Use (CHMP) of European Medicines Agency (EMEA) recommended the 10-valent pneumococcal conjugate vaccine (PCV10; Synflorix®, GlaxoSmithKline) for children aged from 6 weeks up to 2 years in Europe. PCV10 includes 10 serotypes; 7 serotypes included in PCV7 and additional 3 serotypes (1, 5, and 7F) (3). And in 2010, the 13-valent pneumococcal conjugate vaccine (PCV13; Prevenar 13®, Pfizer) was licensed in U.S., and ACIP recommended routine use of PCV13 in all children aged 2-59 months. PCV13 includes 13 serotypes: 7

serotypes included in PCV7 and additional 6 serotypes (1, 3, 5, 6A, 7F, and 19A) (4).

After the introduction of PCV7 in 2000, data from Active Bacterial Core Surveillance (ABCs) in U.S. showed that the overall incidence of IPD among children aged < 5 years decreased by 79%. The reductions in overall IPD resulted from mainly by the seven serotypes included in PCV7. But the decreases have been offset partially by increases in IPD caused by serotypes not included in the PCV7, in particular serotype 19A (4). In 2008, overall serotype 19A IPD rate remained relatively constant, but the proportion of penicillin-resistant serotype 19A increased to 43.7%, becoming a major concern in antimicrobial selection and treatment in clinical settings (5).

In Korea, PCV7 was introduced in November 2003 for optional use. In a single center study that included 138 IPD isolates spanning 15 years in Korea, during 2001–2003 (just before the introduction of PCV7), the overall proportion of PCV7 serotypes and PCV7-related serotypes were 54% and 10%, respectively (6). Interestingly, the serotype 19A had been noted as the most common serotype before introduction of PCV7. In the study, the percentage of serotype 19A increased

from 0% during 1991-1994 to 18% during 2001-2003, just before the use of PCV7. With increase of 19A, serotype coverage by PCV7 dropped from 65% during 1991-1994 to 54% during 2001-2003.

The multilocus sequence typing (MLST) analysis of 19A isolates showed homogenous pattern of ST320, related to the multidrug-resistant clone (6). Korea is one of the country with the highest prevalence of antibiotic resistance among clinical isolates of *S. pneumoniae* from children (7), and antimicrobial drug use may have resulted selective pressure that would give this highly resistant 19A strain an advantage over other strains (6).

It is expected that PCV7 had a great impact on the incidence and serotypes of IPD in Korean children since its introduction. However, limited data on the serotypes of pneumococci isolated from children after introduction of PCV7 is available. In the previous single center study of IPD isolates, during 2004-2006, just after introduction of PCV7 when the vaccine coverage rate was low, the proportion of PCV7 serotypes was 43% and 19A was 14% among the 14 isolates (6).

Since July 2010, PCV10 and PCV13 have been introduced, and had replaced the PCV7 use in Korea; the change in epidemiology and serotype distribution following the use of PCV7 is of importance at this point. To date, the data regarding serotype distribution after introduction of PCV7 and before PCV10 or PCV13 is limited; the data available are those from only a single institute, include noninvasive isolates, or include isolates from adults as well as children (6, 8–9). There has been no multicenter study data on serotype distribution of IPD isolates from Korean children after introduction of PCV7.

This is a retrospective multicenter study performed to determine the distribution of serotypes and antibiotic susceptibility of *S. pneumoniae* responsible for IPD in children during the period from January 2006 to December 2010 in Korea.

MATERIALS AND METHODS

1. Patients and pneumococcal isolates

Eight university hospitals participated in this study; Seoul National University Children's Hospital (Seoul), Severance Hospital (Seoul), Samsung Seoul Hospital (Seoul), Asan Medical Center (Seoul), Gachon University Gil Medical Center (Incheon), Korea University Ansan Hospital (Ansan, Gyeonggi-do), Seoul National University Bundang Hospital (Seongnam, Gyeonggi-do), and Chonbuk National University Hospital (Jeonju, Jeollabuk-do). All eight hospitals have independently continued hospital-wide surveillance to monitor pneumococcal diseases as a part of routine clinical care. If a pneumococcal isolate was identified based on the presence of α-hemolysis and inhibition by optochin, the isolate was stored in -80℃ deep freezer in each center.

A total of 140 invasive pneumococcal isolates from infants and children aged younger than 18 years isolated from 8 participating hospitals from January 2006 to December 2010 were included in this study. Invasive isolates were defined as the isolates from a normally sterile body fluid; such as blood,

cerebrospinal fluid, pleural fluid, pericardial fluid, joint fluid, bone aspirate, or deep tissue abscess. For subsequent isolates collected from the same child, only initial isolates were included in the study.

Medical records of demographic data, clinical diagnosis, and outcome of each IPD patient were reviewed retrospectively.

2. Serotype determination

Out of 140 isolates, 123 isolates were successfully recovered and subjected to serotype determination by Neufeld's Quellung reaction using pool, type and factor-specific antisera (Statens Serum Institut, Copenhagen, Denmark) (8).

However, other 17 isolates failed to grow and were serotyped by 8 sequential multiplex polymerase chain reactions (PCR) (9, 10). The DNA from pneumococcal stocks were extracted using QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's protocol.

Thirty primer pairs were designed to target 53 serotypes; 1, 3, 4, 5, 6A/6B/6C, 7C/7B/40, 7F/7A, 9N/9L, 9V/9A, 10A, 11A/11D, 12F/12A/44/46, 13, 14, 15A/15F, 15B/15C, 18A/18B/18C/18D, 19A, 19F, 20, 21, 22F/22A, 23A, 23F,

24A/24B/24F, 33F/33A/37, 34, 35B, 35F/47F, and 38/25F (10). A universal capsular primer pair targeting the *cpsA* locus was included in each PCR for internal control (9). Eight multiplex reactions were sequentially performed in 25 μL volumes, with each reaction mixture containing the following: 2.5 μL of × 10 Tris-HCl buffer (100 mM, pH 8.3, Mg^{2+} free), 2.0 μL of 2.5 mM dNTPs, 2.0 μL of $MgCl_2$, 0.25 μL of 5.0 U/μL *Taq* DNA polymerase (Takara Bio Inc., Otsu, Japan), primers with pre-determined concentrations, and distilled water to a final volume of 20 μL. Finally, 5 μL of the DNA extract from the clinical specimens was added to each reaction mixture. Thermal cycling was performed in a PTC-200 Peltier Thermal Cycler DNA engine (MJ Research, Watertown, USA) under the following conditions: 95°C for 15 min followed by 35 amplification cycles of 94°C for 30 sec, 63°C for 90 sec, and 72°C for 60 sec (10). If PCR for the *cpsA* gene and 8 multiplex PCR reactions yielded all negative results, the isolate was considered as "noncapsular". The isolate which has positive PCR result for the *cpsA* gene but with negative results of all 8 multiplex PCR reactions was considered as "untypeable".

To assign serotypes 6C and 6D, all serogroup 6 strains were screened for *wciN*$_{\beta}$ and *wciP*$_{6B}$ genes by using 2 simplex PCRs and subsequent sequencing analysis. First, 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 or 6D for the *wciN*$_{\beta}$ gene. Following this reaction, presence of a 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 were assigned as type 6A or 6C and those that carry *wciP*$_{6B}$ were assigned as type 6B or 6D (10, 11).

Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were classified as PCV7 serotypes. PCV10 includes 3 more serotypes than PCV7; serotypes 1, 5, and 7F. PCV13 includes 3 more serotypes than PCV10; 3, 6A, and 19A. Nonvaccine serotypes (NVT) included all the other serotypes.

3. Antimicrobial drug susceptibility testing

All 123 pneumococcal isolates except 17 strains that failed to recover were tested for minimal inhibitory concentrations (MICs) of 8 antimicrobial drugs (penicillin, cefotaxime, chloramphenicol, tetracycline, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, and levofloxacin) by E-test (AB Biodisk, Solna, Sweden).

Clinical and Laboratory Standards Institute (CLSI) changed penicillin breakpoints in 2008 and cefotaxime breakpoints in 2003 (12-15). For penicillin and cefotaxime, susceptibility criteria of 2003 CLSI guideline were employed for epidemiological comparison (16). Susceptible, intermediate, and resistant MIC breakpoints for penicillin were ≤0.06, 0.12-1, and ≥2 μg/mL, respectively; and each MIC breakpoints for cefotaxime were ≤0.5, 1, and ≥2 μg/mL, respectively. For reference, penicillin susceptibility data interpreted by 2008 CLSI guideline (susceptible, intermediate, and resistant MIC breakpoints were ≤2, 4, and ≥8 μg/mL) was also analysed.

Multidrug resistance (MDR) was defined as nonsusceptibility to ≥ 3 antimicrobial drug classes, according to 2003 CLSI guideline.

4. Statistical analysis

Statistical analysis was performed by using the SPSS software version 19.0 (SPSS, Chicago, IL, USA). Rates and proportions were compared using chi-square test or Fisher exact test, as appropriate. For trend analysis, linear-by-linear association model was used by the Pearson chi-square test. A P value < 0.05 was considered statistically significant.

5. Ethics statements

The study protocol was approved by the institutional review board of Seoul National University Hospital (IRB registration number-H-1206-036-412), and other each participating institute. Informed consent was exempted, since the pneumococcal isolates were obtained as a standard of routine patient care.

RESULTS

1. Patients and pneumococcal isolates

Among 140 patients of IPD, 80 (57.1%) were male and the median age of children was 2.58 years (range: 0.00–16.85 years). The percentages of the patients by age group were 39.3% (< 2 yr), 39.3% (2–4 yr), and 21.4% (≥ 5 yr), respectively.

The numbers of collected pneumococcal isolates by year were as follows; 32 isolates (22.9%) in 2006, 28 isolates (20.0%) in 2007, 29 isolates (20.7%) in 2008, 23 isolates (16.4%) in 2009, and 28 isolates (20.0%) in 2010. Fifty-five percent (77 isolates) of 140 isolates were collected in hospitals located in Seoul, 18.6% (26 isolates) in Gyeonggi-do, 14.3% (20 isolates) in Incheon, and 12.1% (17 isolates) in Jeollabuk-do.

Ninety-five patients (67.9%) were previously healthy immunocompetent children. Remaining 45 patients (32.1%) had medical conditions at high-risk for pneumococcal infections; 27 patients were in immune-compromised conditions (20 on immunosuppressive drugs, 5 chronic renal failure or nephrotic

syndrome, and 2 congenital immunodeficiency), 16 patients had underlying medical conditions (8 chronic heart disease, 7 cerebrospinal fluid leaks, and 1 cochlear implant), and 2 patients had asplenia.

Clinical diagnoses of 140 IPD patients were reviewed. There were 55 cases (39.3%) of bacteremia without focus, 50 cases (35.7%) of bacteremic pneumonia or empyema, 28 cases (20.0%) of meningitis, and 7 cases of other IPD associated with bacteremia (4 cases of osteomyelitis, 2 cases of peritonitis, and 1 case of endocarditis).

2. Serotype distribution

The most common serotypes of 140 isolates were 19A (n = 32, 22.9%), 19F (n = 17, 12.1%), 6B (n = 12, 8.6%), 23F (n = 11, 7.9%), 14 (n = 10, 7.1%), and 9V (n = 8, 5.7%) in decreasing order of frequency. The proportion of PCV7 serotypes accounted for 45.0% (63 isolates), additional 3 serotypes in PCV10 (1, 5, and 7F) accounted for 2.9% (4 isolates), and 3 PCV13-specific serotypes (3, 6A, and 19A) accounted for 29.3% (41 isolates).

Table 1 and Figure 1 show serotype distribution during 2006-2010, by year. The proportion of PCV7, additional 3 serotypes in PCV10, and 3 PCV13-specific serotypes were 62.5%, 3.1%, and 18.8% in 2006, and they changed to 21.4%, 3.6%, and 42.9% in 2010, respectively. The trend analysis of proportions of each vaccine serotypes from 2006 to 2010 showed, PCV7 serotypes significantly decreased (from 62.5% to 21.4%, P = 0.002), additional 3 serotypes in PCV10 didn't change significantly, and 3 PCV13-specific serotypes increased over time (from 18.8% to 42.9%, P = 0.016). For additional 6 serotypes in PCV13 (1, 3, 5, 6A, 7F, and 19A), the proportions increased from 21.9% to 46.4% (P=0.031).

Table 1. Distribution of serotypes among 140 invasive pneumococcal isolates in children in Korea by year, 2006–2010*

Serotype	No. (%) isolates by year						*P* for trend‡
	2006	2007	2008	2009	2010	Total	
PCV7	20 (62.5)	13 (46.4)	15 (51.7)	9 (39.1)	6 (21.4)	63 (45.0)	0.002
4			1 (3.4)	1 (4.3)		2 (1.4)	
6B	2 (6.3)	5 (17.9)	2 (6.9)	2 (8.7)	1 (3.6)	12 (8.6)	
9V	2 (6.3)	2 (7.1)	2 (6.9)	1 (4.3)	1 (3.6)	8 (5.7)	
14	6 (18.8)	2 (7.1)	1 (3.4)		1 (3.6)	10 (7.1)	0.012
18C	1 (3.1)	1 (3.6)	1 (3.4)			3 (2.1)	
19F	5 (15.6)	1 (3.6)	5 (17.2)	3 (13.0)	3 (10.7)	17 (12.1)	
23F	4 (12.5)	2 (7.1)	3 (10.3)	2 (8.7)		11 (7.9)	
additional types in PCV10	1 (3.1)	2 (7.1)			1 (3.6)	4 (2.9)	
1	1 (3.1)	1 (3.6)			1 (3.6)	3 (2.1)	
7F		1 (3.6)				1 (0.7)	
PCV13–specific serotypes	6 (18.8)	5 (17.9)	10 (34.5)	8 (34.8)	12 (42.9)	41 (29.3)	0.016
3		2 (7.1)		1 (4.3)		3 (2.1)	
6A	1 (3.1)		3 (10.3)		2 (7.1)	6 (4.3)	
19A	5 (15.6)	3 (10.7)	7 (24.1)	7 (30.4)	10 (35.7)	32 (22.9)	0.018
NVT	5 (15.6)	8 (28.6)	4 (13.8)	6 (26.1)	9 (32.1)	32 (22.9)	
6C			1 (3.4)			1 (0.7)	

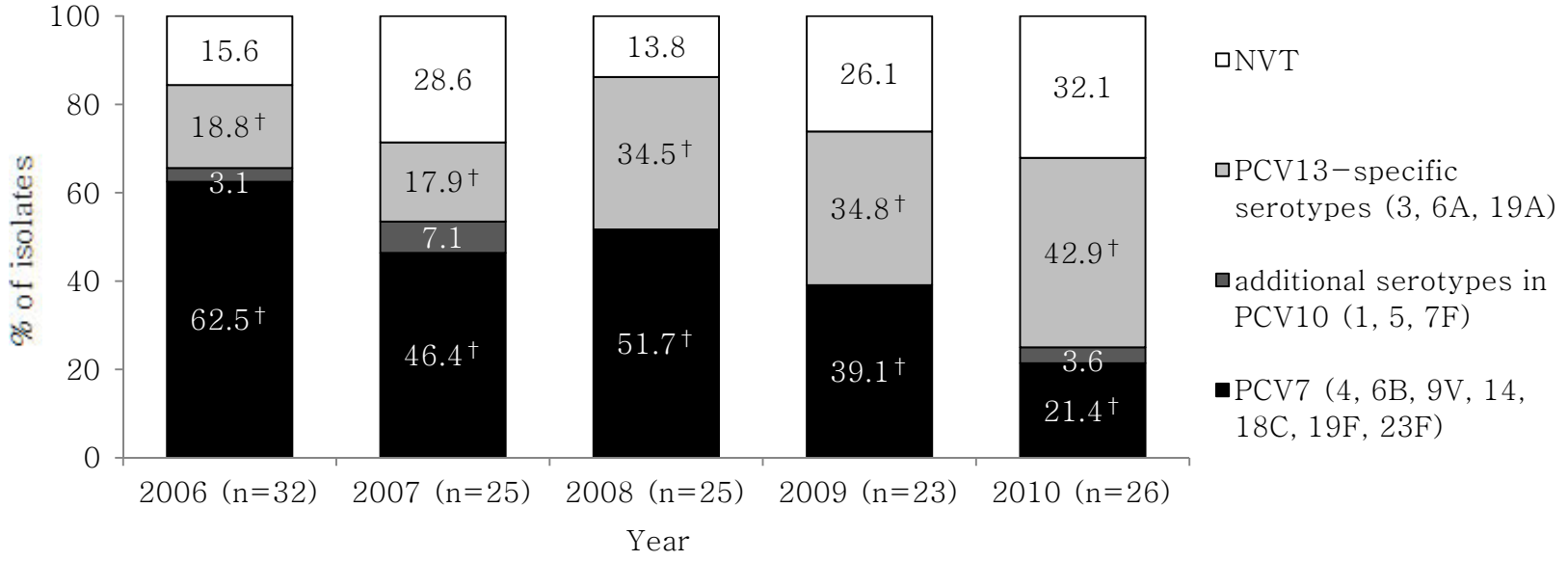
6D			1 (3.4)			1 (0.7)	
10A		1 (3.6)		2 (8.7)	2 (7.1)	5 (3.6)	
11A				2 (8.7)	2 (7.1)	4 (2.9)	
12F		1 (3.6)				1 (0.7)	
15A/B/C	2 (6.3)	2 (7.1)	1 (3.4)		1 (3.6)	6 (4.3)	
23A	2 (6.3)		1 (3.4)		1 (3.6)	4 (2.9)	
24F	1 (3.1)					1 (0.7)	
33F				1 (4.3)		1 (0.7)	
34				1 (4.3)	2 (7.1)	3 (2.1)	0.033
35B		1 (3.6)			1 (3.6)	2 (1.4)	
Untypeable†		1 (3.6)				1 (0.7)	
Noncapsular		2 (7.1)				2 (1.4)	
Total	32 (100.0)	28 (100.0)	29 (100.0)	23 (100.0)	28 (100.0)	140 (100.0)	

*PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; NVT, nonvaccine type

†Untypeable; The isolate with positive *cpsA* gene but serotype could not be determined by 8 multiplex PCR reactions.

‡Statistically significant *P* value <0.05 by linear-by-linear analysis are shown.

Figure 1. Distribution of serotypes, according to vaccine serotypes, among 140 invasive pneumococcal isolates in children in Korea, 2006–2010*



* PCV7, 7–valent pneumococcal conjugate vaccine; PCV10, 10–valent pneumococcal conjugate vaccine; PCV13, 13–valent pneumococcal conjugate vaccine; NVT, nonvaccine type

†Trend analysis showed that the proportion of PCV7 serotypes significantly decreased (P = 0.002) and the proportions of 3 PCV13−specific serotypes (3, 6A, and 19A) significantly increased (P = 0.016).

Table 2 shows serotype distribution, by age group. The proportions of PCV7, additional 3 serotypes in PCV10 (1, 5, and 7F), and 3 PCV13-specific serotypes (3, 6A, and 19A) were similar in aged < 2 years (43.6%, 1.8%, and 32.7%) and 2-4 years (41.8%, 0.0%, and 34.5%), but the proportions were different in aged ≥ 5 years (53.3%, 10.0%, and 13.3%).

Difference of the proportion of PCV7 serotypes between aged < 5 years and ≥ 5 years (42.7% vs. 53.3%, P = 0.301) was not statistically significant, but differences of additional 3 serotypes in PCV10 (0.9% vs. 10.0%, P = 0.031) or 3 PCV13-specific serotypes (33.6% vs. 13.3%, P = 0.030) were statistically significant.

Table 2. Distribution of serotypes among 140 invasive pneumococcal isolates in children in Korea by age group, 2006–2010*

Serotype	No. (%) isolates				
	< 5 years			≥ 5 years	Total
	< 2 years	2–4 years	Subtotal		
PCV7	24 (43.6)	23 (41.8)	47 (42.7)	16 (53.3)	63 (45.0)
4	1 (1.8)		1 (0.9)	1 (3.3)	2 (1.4)
6B	5 (9.1)	3 (5.5)	8 (7.3)	4 (13.3)	12 (8.6)
9V	2 (3.6)	4 (7.3)	6 (5.5)	2 (6.7)	8 (5.7)
14	2 (3.6)	6 (10.9)	8 (7.3)	2 (6.7)	10 (7.1)
18C				3 (10.0)	3 (2.1)
19F	9 (16.4)	7 (12.7)	16 (14.5)	1 (3.3)	17 (12.1)
23F	5 (9.1)	3 (5.5)	8 (7.3)	3 (10.0)	11 (7.9)
additional types in PCV10	1 (1.8)		1 (0.9)	3 (10.0)	4 (2.9)
1				3 (10.0)	3 (2.1)
7F	1 (1.8)		1 (0.9)		1 (0.7)
PCV13–specific serotypes	18 (32.7)	19 (34.5)	37 (33.6)	4 (13.3)	41 (29.3)
3	1 (1.8)	2 (3.6)	3 (2.7)		3 (2.1)
6A	3 (5.5)	2 (3.6)	5 (4.5)	1 (3.3)	6 (4.3)
19A	14 (25.5)	15 (27.3)	29 (26.4)	3 (10.0)	32 (22.9)
NVT	12 (21.8)	13 (23.6)	25 (22.7)	7 (23.3)	32 (22.9)

6C				1 (3.3)	1 (0.7)
6D				1 (3.3)	1 (0.7)
10A	3 (5.5)	1 (1.8)	4 (3.6)	1 (3.3)	5 (3.6)
11A	1 (1.8)	2 (3.6)	3 (2.7)	1 (3.3)	4 (2.9)
12F		1 (1.8)	1 (0.9)		1 (0.7)
15A/B/C	2 (3.6)	3 (5.5)	5 (4.5)	1 (3.3)	6 (4.3)
23A	2 (3.6)	1 (1.8)	3 (2.7)	1 (3.3)	4 (2.9)
24F		1 (1.8)	1 (0.9)		1 (0.7)
33F	1 (1.8)		1 (0.9)		1 (0.7)
34	2 (3.6)	1 (1.8)	3 (2.7)		3 (2.1)
35B		1 (1.8)	1 (0.9)	1 (3.3)	2 (1.4)
Untypeable[†]		1 (1.8)	1 (0.9)		1 (0.7)
Noncapsular	1 (1.8)	1 (1.8)	2 (1.8)		2 (1.4)
Total	55 (100.0)	55 (100.0)	110 (100.0)	30 (100.0)	140 (100.0)

[*] PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; NVT, nonvaccine type

[†] Untypeable; The isolate with positive *cpsA* gene but serotype could not be determined by 8 multiplex PCR reactions.

Table 3 shows distribution of serotypes, by underlying medical condition. Differences of the proportion of each vaccine serotypes between immunocompetent patients and patients with conditions at increased risk of pneumococcal infections were as follows; PCV7 (44.2% vs. 46.7%, P = 0.785), additional 3 serotypes in PCV10 (4.2% vs. 0.0%, P = 0.305), and 3 PCV13-specific serotypes (33.7% vs. 20.0%, P = 0.097) - all were statistically not significant. However, the proportions of NVT were larger in increased risk groups, compared to immunocompetent groups (17.9% vs. 33.3%, P = 0.042).

Table 3. Distribution of serotypes among 140 invasive pneumococcal isolates in children in Korea by underlying medical conditions, 2006–2010*

Serotype	No. (%) isolates					
	Immuno-competent	With conditions at increased risk of pneumococcal infections				Total
		Immuno-compromised†	With medical condition‡	Asplenia	Subtotal	
PCV7	42 (44.2)	13 (48.1)	6 (37.5)	2 (100.0)	21 (46.7)	63 (45.0)
4	2 (2.1)					2 (1.4)
6B	8 (8.4)	3 (11.1)	1 (6.3)		4 (8.9)	12 (8.6)
9V	3 (3.2)	3 (11.1)	1 (6.3)	1 (50.0)	5 (11.1)	8 (5.7)
14	8 (8.4)	1 (3.7)	1 (6.3)		2 (4.4)	10 (7.1)
18C	2 (2.1)			1 (50.0)	1 (2.2)	3 (2.1)
19F	11 (11.6)	4 (14.8)	2 (12.5)		6 (13.3)	17 (12.1)
23F	8 (8.4)	2 (7.4)	1 (6.3)		3 (6.7)	11 (7.9)
additional types in PCV10	4 (4.2)					4 (2.9)
1	3 (3.2)					3 (2.1)
7F	1 (1.1)					1 (0.7)
PCV13-specific serotypes	32 (33.7)	6 (22.2)	3 (18.8)		9 (20.0)	41 (29.3)
3	1 (1.1)	2 (7.4)			2 (4.4)	3 (2.1)
6A	4 (4.2)	1 (3.7)	1 (6.3)		2 (4.4)	6 (4.3)

19A	27 (28.4)	3 (11.1)	2 (12.5)		5 (11.1)	32 (22.9)
NVT	17 (17.9)	8 (29.6)	7 (43.8)		15 (33.3)	32 (22.9)
6C		1 (3.7)			1 (2.2)	1 (0.7)
6D			1 (6.3)		1 (2.2)	1 (0.7)
10A	2 (2.1)	1 (3.7)	2 (12.5)		3 (6.7)	5 (3.6)
11A	2 (2.1)	2 (7.4)			2 (4.4)	4 (2.9)
12F	1 (1.1)					1 (0.7)
15A/B/C	4 (4.2)	2 (7.4)			2 (4.4)	6 (4.3)
23A	3 (3.2)		1 (6.3)		1 (2.2)	4 (2.9)
24F	1 (1.1)					1 (0.7)
33F	1 (1.1)					1 (0.7)
34	1 (1.1)		2 (12.5)		2 (4.4)	3 (2.1)
35B	1 (1.1)	1 (3.7)			1 (2.2)	2 (1.4)
Untypeable[§]	1 (1.1)					1 (0.7)
Noncapsular		1 (3.7)	1 (6.3)		2 (4.4)	2 (1.4)
Total	95 (100.0)	27 (100.0)	16 (100.0)	2 (100.0)	45 (100.0)	140 (100.0)

[*] PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; NVT, nonvaccine type

†Immunocompromised group includes patients on immunosuppressive drugs, chronic renal failure or nephrotic syndrome, and congenital immunodeficiency.

‡Patients with medical conditions include patients with chronic heart disease, cerebrospinal fluid leaks, and cochlear implant.

§Untypeable; The isolate with positive *cpsA* gene but serotype could not be determined by 8 multiplex PCR reactions.

Serotype distribution according to clinical diagnosis is shown in Table 4. PCV7 serotypes showed constant proportion in each clinical diagnosis, but additional 6 serotypes in PCV13 were identified more often in pneumonia (bacteremic pneumonia or empyema) (46.0% in pneumonia vs. 24.4% in non-pneumonia, $P = 0.009$). On the other hand, NVT were identified more often in meningitis (39.3% in meningitis vs. 24.7% in non-meningitis, $P = 0.016$).

Table 4. Distribution of serotypes among 140 invasive pneumococcal isolates in children in Korea by clinical diagnosis, 2006–2010*

Serotype	No. (%) isolates				
	Bacteremia	Pneumonia	Meningitis	Others	Total
PCV7	29 (52.7)	19 (38.0)	11 (39.3)	4 (57.1)	63 (45.0)
4	2 (3.6)				2 (1.4)
6B	5 (9.1)	2 (4.0)	3 (10.7)	2 (28.6)	12 (8.6)
9V	3 (5.5)	2 (4.0)	3 (10.7)		8 (5.7)
14	4 (7.3)	5 (10.0)	1 (3.6)		10 (7.1)
18C	2 (3.6)		1 (3.6)		3 (2.1)
19F	9 (16.4)	4 (8.0)	3 (10.7)	1 (14.3)	17 (12.1)
23F	4 (7.3)	6 (12.0)		1 (14.3)	11 (7.9)
additional types in PCV10	1 (1.8)	2 (4.0)		1 (14.3)	4 (2.9)
1		2 (4.0)		1 (14.3)	3 (2.1)
7F	1 (1.8)				1 (0.7)
PCV13–specific serotypes	14 (25.5)	21 (42.0)	6 (21.4)		41 (29.3)
3	2 (3.6)	1 (2.0)			3 (2.1)
6A	4 (7.3)		2 (7.1)		6 (4.3)
19A	8 (14.5)	20 (40.0)	4 (14.3)		32 (22.9)
NVT	11 (20.0)	8 (16.0)	11 (39.3)	2 (28.6)	32 (22.9)
6C	1 (1.8)				1 (0.7)

6D			1 (3.6)		1 (0.7)
10A		1 (2.0)	4 (14.3)		5 (3.6)
11A	2 (3.6)	1 (2.0)	1 (3.6)		4 (2.9)
12F		1 (2.0)			1 (0.7)
15A/B/C	4 (7.3)	1 (2.0)	1 (3.6)		6 (4.3)
23A	1 (1.8)	1 (2.0)	1 (3.6)	1 (14.3)	4 (2.9)
24F	1 (1.8)				1 (0.7)
33F	1 (1.8)				1 (0.7)
34		1 (2.0)	1 (3.6)	1 (14.3)	3 (2.1)
35B			2 (7.1)		2 (1.4)
Untypeable†		1 (2.0)			1 (0.7)
Noncapsular	1 (1.8)	1 (2.0)			2 (1.4)
Total	55 (100.0)	50 (100.0)	28 (100.0)	7 (100.0)	140 (100.0)

* PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; NVT, nonvaccine type

† Untypeable; The isolate with positive *cpsA* gene but serotype could not be determined by 8 multiplex PCR reactions.

3. Antimicrobial drug susceptibility

The MIC was tested for 123 isolates, except 17 strains that failed to regrow. The susceptibility data are shown in Table 5. Nonsusceptibility rates of penicillin (according to 2003 CLSI breakpoints), cefotaxime, and erythromycin were 88.6% (109 isolates), 23.6% (29 isolates), and 87.0% (107 isolates), respectively.

Table 5. In vitro activity of antimicrobial agents against 123 invasive pneumococcal isolates in children in Korea, 2006–2010*

Antimicrobial agents	No. (%) isolates		
	Susceptible	Intermediate	Resistant
Penicillin	14 (11.4)	57 (46.3)	52 (42.3)
Cefotaxime	94 (76.4)	28 (22.8)	1 (0.8)
Levofloxacin	122 (99.2)		1 (0.8)
Tetracycline	18 (14.6)	1 (0.8)	104 (84.6)
Erythromycin	16 (13.0)		107 (87.0)
Clindamycin	33 (26.8)	1 (0.8)	89 (72.4)
Chloramphenicol	98 (79.7)	17 (13.8)	25 (20.3)
TMP/SMX†	44 (35.8)	23 (18.7)	56 (45.5)

*Susceptibility criteria of 2003 CLSI guideline were employed for epidemiological comparison.

†TMP/SMX, trimethoprim/sulfamethoxazole.

Nonsuseptiblity rates of penicillin, cefotaxime, and erythromycin according to each serotype are shown in Table 6. Penicillin nonsusceptibility rates of PCV7 serotypes, additional 3 serotypes in PCV10, 3 PCV13-specific serotypes, and NVT according to 2003 CLSI breakpoints were 91.2%, 0.0%, 94.4%, and 85.2%, respectively. Cefotaxime nonsusceptibility rates for each were 17.5%, 0.0%, 47.2%, and 7.4%; and erythromycin nonsusceptibility rates for each were 94.7%, 33.3%, 91.7%, and 70.4%, respectively. Serotypes 19A and 19F showed high nonsusceptibility rate of penicillin, cefotaxime and erythromycin.

As in Table 6, 102 (82.9%) isolates showed multidrug resistance (MDR), nonsusceptible to ≥ 3 antimicrobial drug classes. Among 102 MDR isolates, the most common serotypes were 19A (n = 29, 28.4%), 19F (n = 14, 13.7%), 6B (n = 12, 11.8%), and 23F (n = 10, 9.8%). The proportions of PCV7 serotypes, additional 3 serotypes in PCV10, 3 PCV13-specific serotypes, and NVT in MDR isolates were 51.0%, 0.0%, 32.4%, and 16.7%, respectively.

MDR rates of each vaccine serotypes were as follows; 91.2% (52/57) in PCV7 serotypes, 0.0% (0/3) in additional 3

serotypes in PCV10, 91.7% (33/36) in 3 PCV13-specific serotypes, and 63.0% (17/27) in NVT.

Table 6. Nonsusceptiblity rates of penicillin, cefotaxime, and erythromycin, and multidrug resistance, according to serotype, among 123 invasive pneumococcal isolates in children in Korea, 2006–2010[*,†]

Serotype	No. (%) nonsusceptible isolates			No. (%) of multidrug resistant isolates[‡]	Total
	Penicillin	Cefotaxime	Erythromycin		
PCV7	52 (91.2)	10 (17.5)	54 (94.7)	52 (51.0)	57
4			2 (100.0)		2
6B	12 (100.0)		12 (100.0)	12 (11.8)	12
9V	7 (100.0)		7 (100.0)	7 (6.9)	7
14	9 (100.0)		8 (88.9)	8 (7.8)	9
18C			1 (33.3)	1 (1.0)	3
19F	14 (100.0)	10 (71.4)	14 (100.0)	14 (13.7)	14
23F	10 (100.0)		10 (100.0)	10 (9.8)	10
additional types in PCV10			1 (33.3)		3
1					2
7F			1 (100.0)		1
PCV13–specific serotypes	34 (94.4)	17 (47.2)	33 (91.7)	33 (32.4)	36
3					2
6A	5 (100.0)		4 (80.0)	4 (3.9)	5
19A	29 (100.0)	17 (58.6)	29 (100.0)	29 (28.4)	29
NVT	23 (85.2)	2 (7.4)	19 (70.4)	17 (16.7)	27
6C	1 (100.0)				1

6D	1 (100.0)		1 (100.0)	1 (1.0)	1
10A	3 (100.0)	1 (33.3)	1 (33.3)	1 (1.0)	3
11A	3 (75.0)	1 (25.0)	4 (100.0)	3 (2.9)	4
12F					1
15A/B/C	4 (80.0)		5 (100.0)	4 (3.9)	5
23A	4 (100.0)		4 (100.0)	4 (3.9)	4
24F	1 (100.0)				1
33F	1 (100.0)		1 (100.0)	1 (1.0)	1
34	3 (100.0)		1 (33.3)	1 (1.0)	3
35B	2 (100.0)		2 (100.0)	2 (2.0)	2
Noncapsular					1
Total	109 (88.6)	29 (23.6)	107 (87.0)	102 (100.0)	123

* PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; NVT, nonvaccine type

† Susceptibility criteria of 2003 CLSI guideline were employed for epidemiological comparison.

‡ Multidrug resistance was defined as nonsusceptibility to ≥ 3 antimicrobial drug classes.

Table 7 shows serotype distribution of 102 MDR isolates during 2006–2010, by year. The proportion of PCV7 and additional 6 serotypes in PCV13 were 65.2% and 17.4% in 2006, and they changed to 21.7% and 47.8% in 2010, respectively. The trend analysis of proportions of each vaccine serotypes from 2006 to 2010 showed, PCV7 serotypes significantly decreased (from 65.2% to 21.7%, $P = 0.001$) and additional 6 serotypes in PCV13 increased over time (from 17.4% to 47.8%, $P = 0.008$).

Table 7. Distribution of serotypes among 102 multidrug-resistant invasive pneumococcal isolates in children in Korea by year, 2006-2010[*,†]

Serotype	No. (%) isolates by year						*P* for trend[‡]
	2006	2007	2008	2009	2010	Total	
PCV7	15 (65.2)	13 (76.5)	12 (52.2)	7 (43.8)	5 (21.7)	52	0.001
6B	2 (8.7)	5 (29.4)	2 (8.7)	2 (12.5)	1 (4.3)	12	
9V	2 (8.7)	2 (11.8)	1 (4.3)	1 (6.3)	1 (4.3)	7	
14	4 (17.4)	2 (11.8)	1 (4.3)		1 (4.3)	8	0.047
18C		1 (5.9)				1	
19F	4 (17.4)	1 (5.9)	5 (21.7)	2 (12.5)	2 (8.7)	14	
23F	3 (13.0)	2 (11.8)	3 (13.0)	2 (12.5)		10	
additional types in PCV13	4 (17.4)	3 (17.6)	8 (34.8)	7 (43.8)	11 (47.8)	33	0.008
6A			2 (8.7)		2 (8.7)	4	
19A	4 (17.4)	3 (17.6)	6 (26.1)	7 (43.8)	9 (39.1)	29	0.032
NVT	4 (17.4)	1 (5.9)	3 (13.0)	2 (12.5)	7 (30.4)	17	
6D			1 (4.3)			1	
10A					1 (4.3)	1	
11A				1 (6.3)	2 (8.7)	3	0.044
15A/B/C	2 (8.7)		1 (4.3)		1 (4.3)	4	
23A	2 (8.7)		1 (4.3)		1 (4.3)	4	
33F				1 (6.3)		1	

34					1 (4.3)	1
35B		1 (5.9)			1 (4.3)	2
Total	23 (100.0)	17 (100.0)	23 (100.0)	16 (100.0)	23 (100.0)	102

* PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; NVT, nonvaccine type

† Multidrug resistance was defined as nonsusceptibility to ≥ 3 antimicrobial drug classes, and susceptibility criteria of 2003 CLSI guideline were employed for epidemiological comparison.

‡ Statistically significant P value <0.05 by linear-by-linear analysis are shown.

Although penicillin susceptibility was mainly interpreted by 2003 CLSI guideline for epidemiological comparison, penicillin susceptibility data was also analysed by 2008 CLSI guideline (nonsusceptibility breakpoint defined as MIC of > 2.0 μg/mL). Among 123 isolates, 112 isolates (91.1%) were susceptible, 9 isolates (7.3%) were intermediate, and 2 isolates (1.6%) were resistant to penicillin according to 2008 CLSI breakpoints. Out of 11 isolates nonsusceptible to penicillin, 3 isolates were serotype 19F and 8 isolates were serotype 19A. For each vaccine serotypes, penicillin nonsusceptibility rates of PCV7 serotypes, additional 3 serotypes in PCV10, 3 PCV13-specific serotypes, and NVT were 5.3%, 0.0%, 22.2%, and 0.0%, respectively.

4. Outcome

Outcomes were available in 128 IPD patients. 84.4% (108 patients) recovered without any complications, 8.6% (11 patients) recovered with complications, and 7.0% (9 patients) died. Complications included 6 pulmonary complications and 5 neurologic complications.

In analysis of underlying medical condition and outcome, in 85 immunocompetent children, 82.4% (70 patients) recovered without any complications, 8.2% (7 patients) recovered with complications, and 9.4% (8 patients) died. All 26 immunocompromised patients recovered without any complications. In 15 patients with high-risk medical condition group, 73.3% (11 patients) recovered completely and 26.7% (4 patients) recovered with complications. 1 asplenia patient recovered without any complications but 1 asplenia patient died.

In analysis of clinical diagnosis and outcome, in 49 bacteremia patients, 93.9% (46 patients) recovered completely while 6.1% (3 patients) died. In 47 pneumonia (bacteremic pneumonia or empyema) patients, 85.1% (40 patients) recovered completely, 12.8% (6 patients) recovered with complications (3 patients had decortications by video-assisted thoracoscopic surgery, 2

had residual pleural change, and 1 had pulmonary hypertension), and 2.1% (1 patient) died. In 25 meningitis patients, 60.0% (15 patients) recovered without any complications, 20.0% (5 patients) had complications (3 patients had developmental delay, 1 had obstructive hydrocephalus, and 1 had hearing loss), and 20.0% (5 patients) died. All 7 patients with other infections with bacteremia recovered without any complications.

Among 11 patients with complications, the proportion of PCV7 serotypes, additional 3 serotypes in PCV10, and 3 PCV13-specific serotypes accounted for 18.2%, 9.1%, and 36.4%, respectively. Among 9 patients who died, the proportion of PCV7 serotypes, additional 3 serotypes in PCV10, and 3 PCV13-specific serotypes accounted for 44.4%, 0.0%, and 22.2%, respectively. Serotypes of deceased patients were as follows; 6B (2 patients), 6A, 11A, 15, 18C, 19A, 19F, and 33F.

Of 111 isolates with known outcome and antimicrobial susceptibilities, MDR rates were 84.0% (79/94) in complete recovery group, 77.8% (7/9) in complication group, and 62.5% (5/8) in deceased group. 7 of 9 deceased patients received appropriate antibiotics at the first day of admission.

DISCUSSION

This is the first multicenter study performed to determine serotypes and antibiotic susceptibility of *S. pneumoniae* responsible for IPD in children, after the introduction of PCV7 in Korea. PCV7 serotypes significantly decreased, while additional serotypes in PCV13 increased over time. Antimicrobial resistance rates were higher in PCV7 serotypes and additional serotypes in PCV13.

There have been several studies on serotype distribution of *S. pneumoniae* in Korea, only two of them were multicenter studies performed during the period of 2006–2010. In a study of 106 pediatric IPD isolates during 1996–2008, reported by Korea Centers for Disease Control and Prevention (17), the major serotypes were 23F, 14, 19A, 6A, 6B, and 19F. In children aged < 5 years, PCV7 serotypes accounted for 50.0% during 2007–2008. In a multicenter study of 12 hospitals which included 67 pediatric pneumococcal isolates (including noninvasive isolates) during 2008–2009 (18), PCV7 serotypes accounted for 52.2%, whereas PCV13 serotypes accounted for 83.6%. These results seem to be consistent with the results in

our study, but they included small numbers of patient and represented only short period.

However, compared to other regions of the world where PCVs were introduced, serotype changes in Korea are less prominent; Active Bacterial Core surveillance data indicated that in 2008, after 7 years of PCV7 use in U.S., PCV7 serotypes caused only <2% of IPD in children aged < 5 years (4). In England, PCV7 had been universally recommended since 2006, and the proportion of PCV7 serotypes in IPD in children aged ≤ 5 years decreased from 76.6% during 2000–2006 to 8.2% during 2008–2010 (19). These differences may be related to the fact that in Korea PCV7 has been used as an optional vaccine, not included in national immunization program, therefore, the impact of the vaccine may have been smaller than other regions where the vaccine was used as a routine vaccine.

In fact, in a recent nationwide immunization survey in Korea (20), estimated PCV7 coverage rate among children aged 7–83 months were 44.8% (3 infant series) and 31.3% (toddler dose) in 2006, and increased to 73.8% (infant series) and 50.8% (toddler dose) in 2010. This increase in PCV7 coverage rate may be responsible for the serotype changes in this study;

PCV7 serotypes decreased with increase of non-PCV7 serotypes over time.

This study showed surprisingly high rates of nonsusceptibility to penicillin (88.6%) and erythromycin (87.0%). In the U.S., the incidence of IPD caused by penicillin-resistant strains decreased by 57% overall and by 81% among children aged <2 years after routine PCV7 use, mainly due to the decline in the incidence of IPD due to penicillin-nonsusceptible PCV7 serotypes (4). However in Korea, as the previous study (17) has shown, penicillin and erythromycin nonsusceptibility rates reached over 80% among 80 pediatric IPD isolates in 1996-2008, and the antimicrobial susceptibility rates in this study are similar. This persistently high nonsusceptibility rates despite of the PCV7 use could be explained by the significant increase of multidrug resistant non-PCV7 serotypes (especially serotype 19A), since MDR PCV7 serotypes significantly decreased over time but MDR non-PCV7 serotypes had replaced them.

In this study, in 2010, the transitional time from PCV7 to PCV10 or PCV13, the proportion of PCV7, additional 3 serotypes in PCV10, and 3 PCV13-specific serotypes in

pediatric IPD were 21.4%, 3.6%, and 42.9%, respectively. Among the MDR isolates, the proportion of PCV7, additional 3 serotypes in PCV10, and 3 PCV13-specific serotypes in 2010 were 21.7%, 0.0%, and 47.8%, respectively. These findings suggest that PCV13 will provide more advantages in not only reducing invasive infections but also reducing multidrug-resistant pneumococcal infections than PCV7 or PCV10.

Since this study includes the data by the end of 2010, while PCV10 and PCV13 were introduced in July, direct or indirect effect of PCV10 and PCV13 might have influenced serotype distribution in the late 2010. When the isolates for the early 2010 were selectively considered, the proportion of PCV7, additional 3 serotypes in PCV10, and 3 PCV13-specific serotypes were 31.6%, 5.3%, and 36.8%, respectively, among 19 isolates collected from January to June 2010. When trend analysis of the proportions of serotypes by each vaccine were performed from 2006 to the first half of 2010, PCV7 serotypes significantly decreased (from 62.5% to 31.6%, $P = 0.030$) and additional 6 serotypes in PCV13 tended to increase over time, although statistically not significant (from 21.9% to 42.1%, $P = 0.087$).

As for outcome, high morbidity and case-fatality rate were observed; 8.6% of the patients had complications and 7.0% of the patients died due to IPD. The fatal cases were not limited to high-risk groups, 9.4% of previously healthy children died of IPD. The case-fatality rate has not significantly changed compared to the study of 1995-2005 (2), *S. pneumoniae* had 12.4% of case-fatality rate in invasive infections. These add more reasons to vaccinate pneumococcal conjugate vaccines to reduce IPD.

It is well known that IPD caused by non-PCV7 serotypes have increased in post-PCV7 period (4, 21), and there is the possibility of serotype replacement by non-PCV13 serotypes after introduction of PCV13. A 15-valent protein conjugate vaccine (includes PCV13 serotypes plus serotypes 22F and 33F) is undergoing clinical trials (22), but only 1 isolate of serotype 33F was discovered in the study. Also, there are more than 90 serotypes, it is also possible that vaccines with greater coverage may be needed in the future (23). There are many efforts to make vaccines with broad protection through the use of conserved antigens as well as through the inclusion of different variants of important virulence factors, either in

multicomponent vaccines or more complex formulations such as whole bacteria (24).

This study has several limitations. First, pneumococcal isolates were collected in retrospective manner, so there might have been some losses of IPD isolates, making it hard to present the exact data of the participating hospitals. Second, this study did not include the individual's vaccination status; the direct effectiveness of PCV7 could not be investigated. Third, this was not a population-based study, so the incidence of IPD could not be estimated; only the proportions were evaluated.

Despite these limitations, this study represent the largest study demonstrating the epidemiology of serotype distribution and antimicrobial susceptibilities of pediatric IPD isolates after optional use of the PCV7, by multicenter-based data collection.

This present study has several implications for future pneumococcal immunization programs. In particular, a large proportion of serotypes in PCV13 suggest possible changes of IPD in Korea after the introduction of PCV13. In addition, the high antimicrobial nonsusceptibility rate of pneumococci can provide treatment strategy of pneumococcal infections in children in Korea.

Future surveillance studies will be required to evaluate the impact of newly introduced vaccines on the prevalence of IPD, serotype distribution of pneumococcal isolates, and antimicrobial susceptibility patterns. Also, the efforts to develop more extended-valent and more effective vaccines need to be continued.

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국문 초록

서론: 폐구균은 소아청소년 침습성 세균 감염의 중요한 원인으로서, 조사시기 및 지역에 따라 그 혈청형의 분포가 다르며, 백신 사용 등의 의료 환경도 혈청형 분포에 영향을 미친다. 본 연구에서는 다기관 공동 연구를 통해 국내에 7 가 폐구균 단백결합 백신(PCV7)이 사용되고 PCV10 및 PCV13 백신이 갓 도입된 2006 년부터 2010 년까지, 침습성 폐구균 감염 소아청소년에서 분리된 폐구균의 혈청형과 항생제 감수성을 조사하였다.

방법: 2006 년 1 월부터 2010 년 12 월까지 국내 8 개 기관에서, 18 세 미만의 소아청소년의 침습성 감염 폐구균 140 균주에 대하여 Quellung reaction 및 Multiplex PCR 법을 통하여 혈청형을 결정하였다. 항생제 감수성은 E-test 법으로 검사한 후 역학적 비교를 위해 2003 년 CLSI 기준에 따라 감수성 여부를 판정하였고, 후향적으로 의무기록을 분석하였다.

결과: 총 140 균주의 주된 혈청형 분포는 19A (n=32, 22.9%), 19F (n= 17, 12.1%), 6B (n= 12, 8.6%), 23F (n=11, 7.9%) 순이었다. PCV7 혈청형은 45.0%이었으며 PCV10 에 추가된 3 가지 혈청형은 2.9%, PCV13 에 더 추가된 3 가지 혈청형은 29.3%이었다. 2006 년부터 2010 년까지 각 연도별 PCV7 혈청형의 비율은 62.5%에서 21.4%로 감소하였고 (P=0.002), PCV13 에 추가된 6

가지 혈청형은 21.9%에서 46.4%로 증가하였다 (P=0.031). 항생제 감수성 검사는 123 례에서 가능하였고, penicillin, cefotaxime 및 erythromycin 의 내성률이 각각 88.6%, 23.6%, 87.0%이었다. PCV7 혈청형, PCV10 에 추가된 3 가지 혈청형, PCV13 에 더 추가된 3 가지 혈청형, 비백신혈청형의 penicillin 내성률은 각각 91.2%, 0.0%, 94.4%, 85.2%이었으며 cefotaxime 내성률은 각각 17.5%, 0.0%, 47.2%, 7.4%이었고, erythromycin 내성률은 94.7%, 33.3%, 91.7%, 70.4%이었다.

결론: 2006 년부터 2010 년까지 국내 소아청소년의 침습성 감염증에서 분리된 폐구균을 분석한 결과, 시간이 경과함에 따라 PCV7 혈청형이 감소하고 PCV13 에 추가된 혈청형의 비율이 증가하였다. 또한 PCV7 및 PCV13 에 추가된 혈청형의 항생제 내성률이 더 높게 나타났다. 이러한 소견은 국내 폐구균 백신 정책 수립과 치료 방침을 결정하는 데 기초 자료가 될 것으로 생각된다.

주요어 : 폐구균, 혈청형, 항생제 내성, 7 가 폐구균 단백결합백신
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