

## Brief Communication



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# Changes in Serotype of *Streptococcus pneumoniae* After the Introduction of the 13-Valent Pneumococcal Vaccine in a Homogenous Population on Jeju Island

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## ABSTRACT

We compared the serotypes of *Streptococcus pneumoniae* between the pre-pneumococcal conjugate vaccine (PCV)13 era and post-PCV13 era among homogenous inhabitants of an isolated South Korean island. A total of 325 *S. pneumoniae* strains were isolated. In the pre-PCV13 era, 19A/F, 15A/F, 19B, and 23A serotypes were identified. In the post-PCV13 era, 15 serotypes were identified. The 19F and 23A serotypes showed the highest prevalence in the pre- and post-PCV13 era, respectively. After PCV13 introduction, the PCV 13 serotype coverage rate was decreased (80.0% and 30.5% in the pre- and post-PCV13 eras, respectively), while the proportion of non-PCV 13 serotypes increased.

**Keywords:** *Streptococcus pneumoniae*; vaccination; PCV 13 vaccine; Jeju Island; Korea

*Streptococcus pneumoniae* is the most common cause of community-acquired pneumonia in adults [1]. Pneumococcal vaccination is an important strategy for the prevention of pneumococcal infections. Since the introduction of polyconjugate vaccine (PCV) 7 in South Korea in 2003, PCV10 and PCV13 were introduced in 2010 as optional vaccines and included in the National Immunization Program in South Korea in 2014 [2]. Accordingly, healthy adults 65 years of age or older should be vaccinated with PCV13 or 23-valent pneumococcal polysaccharide vaccine (PPV23) [3-5]. However, after the introduction of PCV13, the proportion of non-PCV10/13 serotypes increased [2]. Pneumococcal disease continues to be a main public health concern despite the availability of effective vaccines. Because the prevalence of serotypes causing pneumococcal disease varies over time and geographically [6], the aim of this study was to assess the changes in the serotypes of *S. pneumoniae* after PCV13 was introduced to a homogenous population in South Korea.

We performed a cross-sectional study of patients with *S. pneumoniae* isolates who visited the Jeju National University Hospital to compare the serotypes of *S. pneumoniae* before and after administration of PCV13. The our hospital is located on Jeju Island in South Korea where


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**Conflicts of Interest**

No conflicts of interest.

**Author Contributions**

Conceptualization: JRY, STH. Data curation: KHL. Formal analysis: JRY, STH. Funding acquisition: JRY. Investigation: SHO, HJO, YRK. Methodology: KHL. Resources: YRK, KHL. Supervision: STH. Writing - original draft: JRY. Writing - review & editing: STH, KHL.

serves approximately 90% of the island's inhabitants. The study included patients who lived on the island, 680,000 people reside on the island. Jeju Island (33°0' N, 126°0' E) is the largest island off the coast of the Korean Peninsula.

The study period was divided to two groups (2009–2010 as the pre-PCV13 era and 2014–2017 as the post-PCV13 era). A total of 325 *S. pneumoniae* strains were isolated, with 190 strains from the pre-PCV13 era and 135 strains from the post-PCV13 era.

All *S. pneumoniae* isolates were recovered from the blood, respiratory samples (sputum, transtracheal aspirates, endotracheal aspirates, and bronchoalveolar lavage samples), ear canal discharge, cerebrospinal fluid, and wounds from patients of all ages in our in-patient clinic, out-patient clinic, and emergency room. The most common specimens were sputum and transtracheal aspirates (n = 230, 68.3%), followed by nasopharyngeal aspirates (n = 51, 15.1%), blood (n = 23, 6.8%), ear discharge (n = 9, 2.7%), and others (n = 13, 4.0%). Blood cultures were conducted using the Bact/Alert 3D system (bioMerieux, Inc., Marcy01'Etoile, France), and the cultures were incubated for 24–48 h and then inoculated onto blood agar. Colonies were collected from the plates for identification using an automated system (VITEK II, bioMerieux). We reviewed the medical records of the patients to retrieve epidemiological, clinical, and microbiological data. Clinical data from the patients were obtained using a structured case report form, which included demographics, underlying diseases, history of pneumococcal vaccination, diagnosis, results of laboratory test, drug susceptibility test of *S. pneumoniae*, results of pneumococcal urinary antigen, Pitts bacteremia score, and 30-day case mortality. The study protocol was approved by the Institutional Review Board of Jeju National University Hospital (JNUH 13-10-010).

All isolates collected during the study period were re-cultured for further testing. *S. pneumoniae* isolates were re-inoculated onto blood agar plates to identify the molecular serotypes. All isolates with appropriate properties were serotyped by the sequotyping method [7]. Genomic DNA was extracted from bacterial cells from a colony of pneumococci using the heat lysis method. The primers used to amplify and sequence the *cps* gene were as follows: *cps1*, 5'-GCA ATG CCA GAC AGT AAC CTC TAT-3', and *cps2*, 5'-CCT GCC TGC AAG TCT TGA TT-3'. PCR amplicons were analyzed by 1.5% agarose gel electrophoresis. A second primer pair targeting the 16S rRNA gene was used to identify the species. Amplicons with the expected *cps* band sizes (~1,000 base pairs) were purified using a PCR Purification Kit according to the manufacturer's instructions. Sequencing cycle was performed with BigDye Sequence Terminator v.3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol [7]. The amplicon nucleotide sequences were used to search the GenBank database, and the highest BLAST bit score (typically >98% identity) was identified as the serotype.

Data were analyzed using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). For categorical data, univariate analysis was performed using either the chi-square test or Fisher's exact test. The Mann-Whitney *U* test was used to compare ages between two groups and was expressed as a median (interquartile range). A *P*-value <0.05 was considered to be statistically significant.

The mean age of patients with *S. pneumoniae* was 40.3 ± 33.2 (range 1–94 years); 231 (68.5%) were male. Of all the isolates, 135 (40.1%) were obtained from elderly patients ≥65 years and 114 (35.0%) from children <5 years old; 226 (70%) cases were community-associated

**Table 1.** Baseline characteristics of patients with culture-proven *Streptococcus pneumoniae* (n = 325)

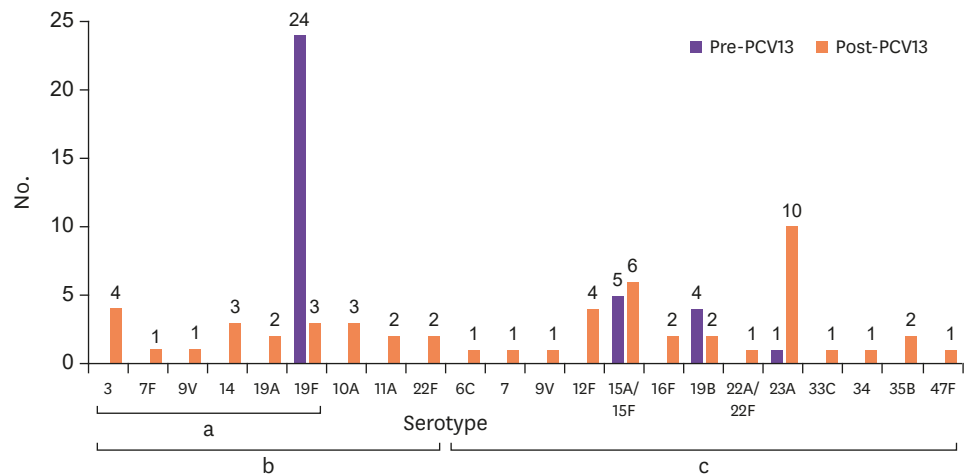
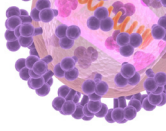
Variables	Result
Age mean $\pm$ SD	40.3 $\pm$ 33.2
Sex (male), No. (%)	231 (68.5)
Comorbidity, No. (%)	192 (57.0)
Hypertension	73 (21.7)
Diabetes	51 (15.1)
Liver disease	19 (5.6)
Renal disease	4 (1.2)
CVD disease	9 (2.7)
COPD	25 (7.4)
Solid Tumor	30 (8.9)
Hematologic malignancy	1 (0.3)
Splenic dysfunction	0
Diagnosis	
Pneumonia	173 (51.3)
Bacteremia	29 (8.6)
Meningitis	4 (1.2)
Otitis	10 (3.6)
Others	134 (39.8)
IPD, No. (%)	31 (9.2)
Pitt bacteremia score	0.6 $\pm$ 2.0
Community-onset, No. (%)	226 (70.0)
Initial adequate antibiotics	254 (78.2)
30 days mortality	20/308 (6.5)
Outcome (%)	
Improved	286/317 (90.2)

SD, standard deviation; no, number; CVD, cerebrovascular, COPD, chronic obstructive pulmonary disease; IPD, invasive pneumococcal disease.

and 99 (30%) cases were healthcare-associated. Pneumonia was the most common source of the pneumococcal disease (173, 51.3%), followed by 29 (8.6%) with bacteremia, 10 (3.6%) with otitis, 4 (1.2%) with meningitis, and 134 (39.8%) with other diseases including nasopharyngeal colonization (**Table 1**).

The most frequent serotypes were 19F, 15A/15F, 19B, and 23A, accounting for 62.5% of all isolates (n = 88) (**Fig. 1**). In the pre-PCV13 era (n = 33), the four serotypes 19A/F, 15A/F, 19B, and 23A were identified. In the post-PCV 13 era (n = 55), 15 serotypes were identified; the major serotypes were 23A, 15A/F, and 3. In children (<5 years of age), seven serotypes were identified, with 19F, 23A, and 15A/15F as the most common. In elderly patients ( $\geq 65$  years), 7 serotypes were identified; 19F, 15A/15F, 3, and 23A were the most common. There were no significant differences in the distribution of serotypes between children and elderly patients. In elderly patients, 15A/15F and 19F were common in the pre-PCV13 era. However, the 23A, 19A, 22F, and 34 serotypes were also identified in the post-PCV13 era. After PCV13 introduction, the percentage of non-vaccine types was increased compared to in the pre-PCV13 era (61.1% vs. 29.4%,  $P < 0.01$ ). Therefore, the PCV13 serotype coverage rate in the post-PCV13 era was reduced compared to in the pre-PCV13 era on Jeju Island (30.5% vs. 80.0%). The proportion of non-vaccine types, PPV23 serotypes, and PCV13 serotypes in patients with IPD were 13/33 (12F, 10A, 16F, 35B, 15A, 19B, 22F), 3/7 (11A, 22F), and 2/14 (19A), respectively.

This study was conducted to evaluate the changes in the serotype distribution after the introduction of PCV13 in a homogenous population of an isolated island. To evaluate the effectiveness of vaccination strategies, a homogenous population should be studied with respect to serotype distribution. Because geographic populations exhibit proportionally



**Figure 1.** Serotype distribution of *Streptococcus pneumoniae* between the pre-PCV13 vaccination period and post-PCV13 vaccination period.  
<sup>a</sup>PCV13 serotype.  
<sup>b</sup>PPV23 serotype.  
<sup>c</sup>Non-vaccination serotype.  
 Purple bars indicate the serotype of *S. pneumoniae* in the pre-PCV13 period, orange bars indicated the serotype of *S. pneumoniae* in the post-PCV13 period.  
 PCV, pneumococcal conjugated vaccine.

mixed homogenous and heterogeneous characteristics that may cause differences in vaccine effectiveness and population immunity [8]. As described in the Community-Acquired Pneumonia Immunization Trial in Adults study in a homogenous population [9], we evaluated the distribution of *S. pneumoniae* serotypes in a more homogenous population in an isolated region in South Korea.

Previous studies revealed an increased prevalence of non-PCV7 *S. pneumoniae* serotypes, 19F (9.8%), 23F (8.3%), 19A (7.8%), and 6A (7.5%), in the pre-PCV13 era in South Korea [10]. In the post-PCV13 era, the PCV13 serotype proportions decreased and non-PCV serotype proportions increased among nasopharyngeal carriage pneumococci in South Korea [2]. Meta-analysis revealed that the non-PCV13 serotypes contributed to 42.2% of childhood IPD cases, and non-PCV13 serotypes such as 22F, 12F, 33F, 24F, 15C, 15B, 23B, 10A, and 38 were predominant; regional differences in the serotype distribution were also observed [11]. In this study, an increase in IPD caused by *S. pneumoniae* of non-vaccine types was observed. The serotype distribution showed changes in the 19F and 19B serotypes in the post-PCV13 era. In other studies, the most common carriage isolates were 6C, 15B/C, 19A, and 23A, and a greater reduction in the prevalence of carriage was observed for serotype 19A in children [2, 12]. However, serotypes 19A and 6C were not common in our specific area. In South Korea, the national immunization program offers a 23-valent pneumococcal polysaccharide vaccine for the elderly (≥65 years), but PCV13 is not included. However, PCV13 or PPV23 are recommended for healthy adults aged 65 years or older in South Korea. PCV10 and PCV13 are included in the national immunization program for young children (aged <59 months) in South Korea. According to the changes in the serotype distributions in community outbreaks and nasopharyngeal carriage by country, changes in the serotypes of pneumococcus should be monitored to enable changes in the vaccination strategy to be made.

This study has several limitations. Of all isolates, the serotypes were identified in only 30%, and a high proportion of patients was not detected by sequotyping. This may have introduced

selection bias. However, the serotypes of *S. pneumoniae* in pre-PCV13 era in isolated regions in South Korea have never been examined. Second, our results may not represent the national data, and more serotypes should be examined to detect changes. Third, this study did not include each subject's vaccination status.

In conclusion, the proportion of non-PCV13 serotypes changed in the homogenous population after PCV13 introduction. This result is useful for developing pneumococcal vaccination strategies in the study area. Further epidemiological studies are needed to assess changes in the circulating serotypes in the post-PCV13 era.

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