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# Serotype Distribution and Antimicrobial Resistance of *Streptococcus pneumoniae* Causing Invasive Pneumococcal Disease in Korea Between 2017 and 2019 After Introduction of the 13-Valent Pneumococcal Conjugate Vaccine

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**Background:** *Streptococcus pneumoniae* is a serious pathogen causing various infections in humans. We evaluated the serotype distribution and antimicrobial resistance of *S. pneumoniae* causing invasive pneumococcal disease (IPD) after introduction of pneumococcal conjugate vaccine (PCV)13 in Korea and investigated the epidemiological characteristics of multidrug-resistant (MDR) isolates.

**Methods:** *S. pneumoniae* isolates causing IPD were collected from 16 hospitals in Korea between 2017 and 2019. Serotyping was performed using modified sequential multiplex PCR and the Quellung reaction. Antimicrobial susceptibility tests were performed using the broth microdilution method. Multilocus sequence typing was performed on MDR isolates for epidemiological investigations.

**Results:** Among the 411 *S. pneumoniae* isolates analyzed, the most prevalent serotype was 3 (12.2%), followed by 10A (9.5%), 34 (7.3%), 19A (6.8%), 23A (6.3%), 22F (6.1%), 35B (5.8%), 11A (5.1%), and others (40.9%). The coverage rates of PCV7, PCV10, PCV13, and pneumococcal polysaccharide vaccine (PPSV)23 were 7.8%, 7.8%, 28.7%, and 59.4%, respectively. Resistance rates to penicillin, ceftriaxone, erythromycin, and levofloxacin were 13.1%, 9.2%, 80.3%, and 4.1%, respectively. MDR isolates accounted for 23.4% of all isolates. Serotypes 23A, 11A, 19A, and 15B accounted for the highest proportions of total isolates at 18.8%, 16.7%, 14.6%, and 8.3%, respectively. Sequence type (ST)166 (43.8%) and ST320 (12.5%) were common among MDR isolates.

**Conclusions:** Non-PCV13 serotypes are increasing among invasive *S. pneumoniae* strains causing IPD. Differences in antimicrobial resistance were found according to the specific serotype. Continuous monitoring of serotypes and antimicrobial resistance is necessary for the appropriate management of *S. pneumoniae* infections.

**Key Words:** *Streptococcus pneumoniae*, Serotyping, Drug resistance, Multiple drug resistance, Bacterial, Mutlilocus sequence typing

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## INTRODUCTION

*Streptococcus pneumoniae* is one of the most serious pathogens of humans, causing acute otitis media, pneumonia, bacteremia, and meningitis [1]. Invasive pneumococcal diseases (IPDs) are more frequent in children and the elderly. To date, more than 90 capsular serotypes of pneumococci have been identified, and the serotype distribution differs according to patient age, geographic region, and period of vaccine availability [2]. The capsular polysaccharide of *S. pneumoniae* is a virulence factor, and the capsular serotype is closely related to IPDs [3].

A pneumococcal conjugate vaccine (PCV) including serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F was introduced for routine use in 2000, which dramatically reduced IPD prevalence in many countries [4]. After introduction of this 7-valent PCV (PCV7), serotype 19A was detected at a high rate and serotype 3 was detected mainly in adults [2, 5]. PCV10 (PCV7 plus serotypes 1, 5, and 7F) and PCV13 (PCV10 plus serotypes 3, 6A, and 19A) were introduced in 2010.

National immunization programs (NIPs) to prevent pneumococcal infections have been implemented in many countries. In Korea, PCV10 or PCV13 has been available for children since 2014, and pneumococcal polysaccharide vaccine (PPSV)23 for older adults (≥65 years old) has been available since 2013. The use of PCV13 is also recommended for older patients (≥65 years) in high-risk groups, such as immunocompromised patients [6]. The serotype distribution was reported immediately after implementation of NIPs in Korea between 2014 and 2016 [2]. In 2017, the vaccination rates of PCV13 and PPSV23 reached 95.0% for children and 60.0% for the elderly [7]. The stabilized serotype distribution reflecting the high vaccination rate after NIPs could be determined by investigating the serotype distribution between 2017 and 2019.

The prevalence of antimicrobial-resistant strains of *S. pneumoniae* has increased worldwide over the past few decades. The spread of multidrug-resistant (MDR) *S. pneumoniae* is a serious public health concern [2]. The aim of this study was to define the change in serotype distribution and antimicrobial resistance of *S. pneumoniae* causing IPDs after the introduction of PCV13 and to investigate the epidemiological characteristics of MDR *S. pneumoniae* isolates in Korea.

## MATERIALS AND METHODS

### Clinical isolates

In total, 411 *S. pneumoniae* isolates from patients with IPDs were collected prospectively from 16 hospitals in Korea between 2017 and 2019. All isolates were stored at −70°C in 10% skim milk. All *S. pneumoniae* isolates were identified by Gram staining, colony morphology, and the VITEK MS system (v3.0; BioMérieux, Marcy l'Etoile, France). This study was approved by the Institutional Review Board of Inje University Busan Paik Hospital (No.: 17-0147) with exemption for patient consent.

### Serotyping

Serotyping was performed using modified sequential multiplex (SM)-PCR, as previously described [8]. We carried out an additional multiplex PCR set for serotypes 2, 10F/10C/33C, 31, 35F/47F, and 38/25F/25A. Primer sequences provided by the US Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/streplab/pcr.html) were used to determine pneumococcal serotypes. The modified SM-PCR protocol consisted of seven multiplex PCR sets, and each reaction consisted of five primer pairs. If the serotype could not be determined using modified SM-PCR, the isolate was defined as non-typeable.

We applied the capsular Quellung reaction with factor antisera (Statens Serum Institute, Copenhagen, Denmark) to define specific serotypes 6A/6B/6C/6D, 11A/11D, 12F/12A/12B, 15F/15A/15B/15C, and 22F/22A [9].

We defined vaccine serotypes as those included in PCVs and non-vaccine serotypes as those that were not included in PCVs [10].

### Antimicrobial susceptibility

Antimicrobial susceptibility tests were performed using Microscan with the MICroSTREP plus Panel (Siemens Healthcare Diagnostics, Sacramento, CA, USA) for amoxicillin/clavulanate, cefotaxime, ceftriaxone, penicillin, clindamycin, erythromycin, levofloxacin, tetracycline, trimethoprim/sulfamethoxazole (SXT), and vancomycin. *S. pneumoniae* ATCC 49619 was used for quality control. The results were interpreted according to CLSI-recommended breakpoints [11]. We used the breakpoint of meningitis interpretation for isolates from the cerebrospinal fluid (CSF) and the breakpoint of non-meningitis interpretation for isolates collected from all other sources.

MDR was defined as resistance to three or more of the following four classes of antibiotics: β-lactams, macrolides, lincosamides, and fluoroquinolones [12]. Extensive drug resistance (XDR) was defined as resistance to five or more of the following six classes of antibiotics: β-lactams, macrolides, lincosamides, fluoroquinolones, tetracyclines, and folate-pathway inhibitors [12].


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### Multilocus sequence typing (MLST)

MLST was performed for MDR isolates according to a previously described MLST protocol for *S. pneumoniae* [13]. The sequences of the internal fragments from seven housekeeping genes (*aroE, gdh, gki, recP, spi, xpt,* and *ddl*) were amplified by PCR under the following conditions: 95°C for 4 minutes; followed by 30 amplification cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 7 minutes. Alleles and sequence types (STs) were assigned according to the PubMLST website (https://pubmlst.org/spneumoniae/).

Clonal complexes (CCs) were determined using sequence type analysis and recombination tests (START) [14]. For phylogenetic analysis, the sequences of the *aroE, gdh, gki, recP, spi,* and *xpt* gene fragments were concatenated using the PHYLOViZ online application (http://online.phyloviz.net/).

### Data analysis

Chi-square tests were used to determine significant differences in resistance and serotype distribution, as appropriate. Differences between groups were considered significant at $P<0.05$. IBM SPSS Statistics (v27.0; IBM Corp., Armonk, NY, USA) was used for statistical analysis.

## RESULTS

### Clinical characteristics of pneumococcal isolates

Of the 411 isolates, 265 (64.5%) were from male patients and 146 (35.5%) were from female patients. By period, 155 (37.7%), 156 (38.0%), and 100 (24.3%) isolates were obtained in 2017, 2018, and 2019, respectively. The most common source was blood (N=337; 82.0%), followed by abscess (N=25; 6.1%), CSF (N=25; 6.1%), other body fluids (N=21, 5.1%; ascitic fluid, N=12; pleural fluid, N=5; peritoneal fluid, N=4), and tissue (N=3, 0.7%). The median age of the patients was 57 (range, 0–104) years, and 46.7% (N=192) of the isolates were collected from patients ≥65 years old; 30 (7.3%) and 13 (3.2%) isolates were collected from patients aged ≤1 year and 1–5 years, respectively. Blood-obtained isolates were most common in all age groups, except for isolates derived from patients aged 6–20 years.

### Serotype distribution

Thirty-four serotypes were detected and four isolates were nontypeable (Table 1). The most prevalent serotype was 3 (12.2%), followed by 10A (9.5%), 34 (7.3%), 19A (6.8%), 23A (6.3%), 22F (6.1%), 35B (5.8%), 11A (5.1%), and others. The eight

**Table 1.** Serotype distribution of *Streptococcus pneumoniae* by patient age

| Serotype | Isolates, N (%) | | | | | |
|---|---|---|---|---|---|---|
| | Total (N=411) | Age (N) | | | | |
| | | ≤5 yr (43) | 6–18 yr (12) | 19–50 yr (63) | 51–64 yr (101) | ≥65 yr (192) |
| 3*,† | 50 (12.2) | 1 (2.3) | 1 (8.3) | 3 (4.8) | 14 (13.9) | 31 (16.1) |
| 10A† | 39 (9.5) | 14 (32.6) | 1 (8.3) | 4 (6.3) | 6 (5.9) | 14 (7.3) |
| 34 | 30 (7.3) | 1 (2.3) | 0 (0) | 4 (6.3) | 6 (5.9) | 19 (9.9) |
| 19A*,† | 28 (6.8) | 4 (9.3) | 0 (0) | 7 (11.1) | 5 (5.0) | 12 (6.3) |
| 23A | 26 (6.3) | 2 (4.7) | 1 (8.3) | 5 (7.9) | 9 (8.9) | 9 (4.7) |
| 22F† | 25 (6.1) | 1 (2.3) | 1 (8.3) | 4 (6.3) | 9 (8.9) | 10 (5.2) |
| 35B | 24 (5.8) | 2 (4.7) | 0 (0) | 1 (1.6) | 8 (7.9) | 13 (6.8) |
| 11A† | 21 (5.1) | 0 (0) | 0 (0) | 4 (6.3) | 2 (2.0) | 15 (7.8) |
| 15B† | 16 (3.9) | 4 (9.3) | 1 (8.3) | 4 (6.3) | 3 (3.0) | 4 (2.1) |
| 12F† | 15 (3.6) | 1 (2.3) | 0 (0) | 6 (9.5) | 3 (3.0) | 5 (2.6) |
| 19F*,†,‡ | 14 (3.4) | 0 (0) | 2 (16.7) | 1 (1.6) | 4 (4.0) | 7 (3.6) |
| 23B | 13 (3.2) | 4 (9.3) | 0 (0) | 3 (4.8) | 2 (2.0) | 4 (2.1) |
| 20† | 12 (2.9) | 0 (0) | 0 (0) | 3 (4.8) | 1 (1.0) | 8 (4.2) |
| 24F/24A/24B | 11 (2.7) | 3 (7) | 1 (8.3) | 1 (1.6) | 4 (4.0) | 2 (1.0) |
| 15A | 10 (2.4) | 0 (0) | 0 (0) | 2 (3.2) | 1 (1.0) | 7 (3.6) |
| 13 | 9 (2.2) | 0 (0) | 1 (8.3) | 2 (3.2) | 4 (4.0) | 2 (1.0) |
| 6A* | 8 (1.9) | 0 (0) | 0 (0) | 0 (0) | 2 (2.0) | 6 (3.1) |
| 14*,†,‡ | 8 (1.9) | 1 (2.3) | 0 (0) | 0 (0) | 2 (2.0) | 5 (2.6) |
| 6D | 7 (1.7) | 0 (0) | 0 (0) | 0 (0) | 2 (2.0) | 5 (2.6) |
| 6C | 6 (1.5) | 0 (0) | 0 (0) | 1 (1.6) | 2 (2.0) | 3 (1.6) |
| 6B*,†,‡ | 5 (1.2) | 0 (0) | 0 (0) | 1 (1.6) | 1 (1.0) | 3 (1.6) |
| 38/25F/25A | 5 (1.2) | 3 (7) | 0 (0) | 2 (3.2) | 0 (0) | 0 (0) |
| 15F | 4 (1.0) | 0 (0) | 1 (8.3) | 0 (0) | 2 (2.0) | 1 (0.5) |
| 23F*,†,‡ | 4 (1.0) | 0 (0) | 0 (0) | 0 (0) | 3 (3.0) | 1 (0.5) |
| 16F | 3 (0.7) | 0 (0) | 0 (0) | 1 (1.6) | 0 (0) | 2 (1.0) |
| 33F† | 3 (0.7) | 1 (2.3) | 0 (0) | 1 (1.6) | 0 (0) | 1 (0.5) |
| 9N/9L† | 2 (0.5) | 0 (0) | 0 (0) | 0 (0) | 2 (2.0) | 0 (0) |
| 15C | 2 (0.5) | 1 (2.3) | 0 (0) | 0 (0) | 0 (0) | 1 (0.5) |
| 31 | 2 (0.5) | 0 (0) | 1 (8.3) | 0 (0) | 1 (1.0) | 0 (0) |
| 2† | 1 (0.2) | 0 (0) | 0 (0) | 0 (0) | 1 (1.0) | 0 (0) |
| 7B | 1 (0.2) | 0 (0) | 0 (0) | 1 (1.6) | 0 (0) | 0 (0) |
| 9V*,†,‡ | 1 (0.2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.5) |
| 11D | 1 (0.2) | 0 (0) | 1 (8.3) | 0 (0) | 0 (0) | 0 (0) |
| 44/46 | 1 (0.2) | 0 (0) | 0 (0) | 0 (0) | 1 (1.0) | 0 (0) |
| Non-typeable | 4 (1.0) | 0 (0) | 0 (0) | 2 (3.2) | 1 (1.0) | 1 (0.5) |

*13-valent pneumococcal conjugate vaccine (PCV13) serotype; †Pneumococcal polysaccharide vaccine (PPSV23) serotype; ‡7-valent pneumococcal conjugate vaccine (PCV7) serotype.

most common serotypes accounted for 59.1% (N=243) of the isolates. In patients ≤5 years of age, serotypes 10A (N=14, 32.6%), 15B (N=4, 9.3%), 19A (N=4, 9.3%), and 23B (N=4, 9.3%) were the most prevalent. Among them, 12 of 14 serotypes 10A, three of four serotypes 19A, and all four serotypes 15B were isolated from children aged ≤1 year. In ≥65-year-old patients, serotypes 3 (N=31, 16.1%), 34 (N=19, 9.9%), 11A (N=15, 7.8%), 10A (N=14, 7.3%), and 35B (N=13, 6.8%) were prevalent.

Of the 411 isolates, 252 (61.3%) were vaccine serotypes. The

**Table 2.** Antimicrobial resistance of 411 *Streptococcus pneumoniae* isolates

| Antimicrobial agent | | Total (N=411) | | | ≤5 yr (N=43) | | | 6–64 yr (N=176) | | | ≥65 yr (N=192) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| β-Lactams | Penicillin | 62.8 | 24.1 | 13.1 | 58.1 | 23.3 | 18.6 | 61.9 | 22.7 | 15.3 | 64.6 | 25.5 | 9.9 |
| | Amoxicillin/clavulanate | 69.3 | 8.8 | 21.9 | 74.4 | 0 | 25.6 | 68.2 | 10.2 | 21.6 | 69.3 | 9.4 | 21.4 |
| | Cefotaxime | 70.8 | 19.7 | 9.5 | 67.4 | 23.3 | 9.3 | 71.6 | 20.5 | 8.0 | 70.8 | 18.2 | 10.9 |
| | Ceftriaxone | 70.3 | 20.4 | 9.2 | 67.4 | 25.6 | 7.0 | 68.2 | 21.6 | 10.2 | 72.9 | 18.2 | 8.9 |
| Macrolides | Erythromycin | 18.7 | 1.0 | 80.3 | 7.0 | 0 | 93.0 | 18.8 | 0 | 81.3 | 21.4 | 2.1 | 76.6 |
| Lincosamides | Clindamycin | 33.1 | 0.2 | 66.7 | 30.2 | 0 | 69.8 | 31.3 | 0.6 | 68.2 | 35.4 | 0 | 64.6 |
| Quinolones | Levofloxacin | 95.6 | 0.2 | 4.1 | 100 | 0 | 0 | 96.0 | 0 | 4.0 | 93.8 | 0.5 | 5.2 |
| Tetracyclines | Tetracycline | 22.6 | 1.5 | 75.9 | 14.0 | 0 | 86.0 | 22.2 | 1.7 | 76.1 | 25.0 | 1.6 | 73.4 |
| Folate-pathway inhibitors | Trimethoprim/ sulfamethoxazole | 58.6 | 13.6 | 27.7 | 72.1 | 14.0 | 14.0 | 58.0 | 14.2 | 27.8 | 56.3 | 13.0 | 30.7 |
| Glycopeptides | Vancomycin | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |

Abbreviations: S, susceptible; I, intermediate; R, resistant.

**Table 3.** Relation between serotype and antimicrobial resistance

| Serotype (N) | Resistance rate (%) and MDR, XDR in each serotype | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PEN (NS) | AMC | CTX (NS) | CRO (NS) | ERY | CLI | LEV | TET | SXT | VAN | MDR | XDR |
| 3*,† (50) | 4.0 (6.0) | 4.0 | 4.0 (4.0) | 4.0 (6.0) | 50.0 | 44.0 | 4.0 | 48.0 | 12.0 | 0 | 4.0 | 4.0 |
| 10A† (39) | 12.8 (41.0) | 0 | 5.1 (23.1) | 0.0 (28.2) | 89.7 | 87.2 | 0 | 89.7 | 2.6 | 0 | 12.8 | 0 |
| 34 (30) | 3.3 (10.0) | 0 | 3.3 (6.7) | 3.3 (3.3) | 43.3 | 40.0 | 6.7 | 40.0 | 3.3 | 0 | 6.7 | 3.3 |
| 19A*,† (28) | 35.7 (85.7) | 67.9 | 14.3 (67.9) | 14.3 (57.1) | 100 | 75.0 | 0 | 100 | 100 | 0 | 50.0 | 50.0 |
| 23A (26) | 30.8 (65.4) | 65.4 | 7.7 (61.5) | 23.1 (69.2) | 88.5 | 88.5 | 0 | 92.3 | 0 | 0 | 69.2 | 0 |
| 22F† (25) | 4.0 (12.0) | 0 | 4.0 (4.0) | 4.0 (4.0) | 68.0 | 40.0 | 0 | 40.0 | 4.0 | 0 | 4.0 | 4.0 |
| 35B (24) | 16.7 (25.0) | 12.5 | 20.8 (25.0) | 20.8 (25.0) | 100 | 95.8 | 20.8 | 87.5 | 20.8 | 0 | 20.8 | 20.8 |
| 11A† (21) | 23.8 (85.7) | 71.4 | 57.1 (95.2) | 38.1 (90.5) | 95.2 | 95.2 | 14.3 | 90.5 | 95.2 | 0 | 76.2 | 71.4 |
| 15B† (16) | 37.5 (62.5) | 43.8 | 6.3 (31.3) | 0 (43.8) | 100 | 56.3 | 0 | 100 | 43.8 | 0 | 50.0 | 25.0 |
| 12F† (15) | 0 (0) | 0 | 0 (0) | 0 (0) | 86.7 | 80.0 | 0 | 93.3 | 6.7 | 0 | 0 | 0 |
| 19F*,†,‡ (14) | 28.6 (92.9) | 64.3 | 7.1 (57.1) | 0 (57.1) | 100 | 78.6 | 14.3 | 78.6 | 100 | 0 | 50.0 | 50.0 |
| 23B (13) | 7.7 (61.5) | 53.8 | 0.0 (53.8) | 15.4 (61.5) | 69.2 | 61.5 | 0 | 61.5 | 0 | 0 | 53.8 | 0 |
| 20† (12) | 8.3 (16.7) | 8.3 | 16.7 (16.7) | 16.7 (16.7) | 83.3 | 83.3 | 16.7 | 83.3 | 16.7 | 0 | 16.7 | 16.7 |
| 24F/24A/24B (11) | 9.1 (9.1) | 0 | 0 (0) | 0 (0) | 100 | 100 | 0 | 100 | 0 | 0 | 9.1 | 0 |
| 15A (10) | 0 (50.0) | 0 | 0 (20.0) | 0 (20.0) | 100 | 70.0 | 0 | 100 | 70.0 | 0 | 0 | 0 |
| Others§ (77) | 6.5 (31.2) | 13.0 | 7.8 (27.3) | 9.1 (26.0) | 80.5 | 53.2 | 1.3 | 76.6 | 27.3 | 0 | 10.4 | 3.9 |

*13-valent pneumococcal conjugate vaccine (PCV13) serotype; †Pneumococcal polysaccharide vaccine (PPSV23) serotype; ‡7-valent pneumococcal conjugate vaccine (PCV7) serotype; §Other serotypes, including serotypes 2, 6A, 6B, 6C, 6D, 7B, 9N/9L, 9V, 11D, 13, 14, 15C, 15F, 16F, 23F, 31, 33F, 38/25F/25A, and 44/46, and non-typeable isolates.
Abbreviations: NS, non-susceptible; MDR, multidrug-resistant; XDR, extensively drug-resistant; AMC, amoxicillin/clavulanate; CTX, cefotaxime; CRO, ceftriaxone; CLI, clindamycin; ERY, erythromycin; LEV, levofloxacin; PEN, penicillin; TET, tetracycline; SXT, sulfamethoxazole/trimethoprim; VAN, vancomycin.


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coverage rates of PCV7, PCV10, PCV13, and PPSV23 were 7.8%, 7.8%, 28.7%, and 59.4%, respectively. The coverage rate for PCV13 (14.0%) was lower in patients ≤5 years than in those ≥65 years (33.9%, $P$=0.01).

### Antimicrobial resistance

Antimicrobial resistance of the isolates is presented in Table 2. Overall, 13.1%, 9.5%, and 9.2% of the isolates were resistant to penicillin, cefotaxime, and ceftriaxone, respectively. The proportion of intermediate resistance was high for penicillin (24.1%), cefotaxime (19.7%), and ceftriaxone (20.4%). Resistance rates to erythromycin, clindamycin, and tetracycline were high at 80.3%, 66.7%, and 75.9%, respectively. Resistance rates to SXT and levofloxacin were 27.7% and 4.1%, respectively.

There were some differences in antimicrobial resistance by age, although the difference was not significant. Resistance rates to penicillin (18.6% vs. 9.9%), erythromycin (93.0% vs. 76.6%), and tetracycline (86.0% vs. 73.4%) were higher in children ≤5 years of age than in adults ≥65 years of age. The rates of resistance to levofloxacin (0% vs. 5.2%) and SXT (14.0% vs. 30.7%) were lower in patients ≤5 years of age than in those ≥65 years of age.

The antimicrobial susceptibility results differed according to the serotype (Table 3). Resistance rates were higher in several specific serotypes. The resistance rates to penicillin of serotypes 15B, 19A, 23A, 19F, and 11A were 37.5%, 35.7%, 30.8%, 28.6%, and 23.8%, respectively ($P$<0.001). The resistance rate to cefotaxime was the highest in serotype 11A (57.1%, $P$<0.001), followed by 35B (20.8%, $P$<0.001) and 19A (14.3%, $P$<0.001). The resistance rates to SXT were high ($P$<0.001) in serotypes 19A (100%), 19F (100%), 11A (95.2%), 15A (70%), and 15B (43.8%). The resistance rates to levofloxacin of serotypes 35B, 11A, and 19F were 20.8%, 14.3%, and 14.3%, respectively ($P$<0.001).

MDR and XDR isolates accounted for 23.4% (N=96) and 13.1% (N=54) of all isolates, respectively (Fig. 1). Of the total MDR isolates, serotypes 23A, 11A, 19A, and 15B accounted for the highest proportions at 18.8%, 16.7%, 14.6%, and 8.3%, respectively. The percentage of MDR isolates was the highest in serotype 11A (76.2%, N=16; $P$<0.001), followed by 23A (69.2%, N=18; $P$<0.001), 23B (53.8%, N=7; $P$=0.026), 19A (50%, N=14; $P$=0.002), 15B (50%, N=8; $P$=0.031), and 19F (50%, N=7; $P$=0.048). XDR was common among serotypes 11A (71.4%, N=16; $P$<0.001), 19A (50%, N=14; $P$<0.001), and 19F (50%, N=14; $P$<0.001).

### MLST of MDR *S. pneumoniae*

The results of the MLST, CC, and eBURST tests are shown in Table 4 and Fig. 2. The major CCs were CC166 (N=59, 61.5%) and CC320 (N=20, 20.8%). Eleven singletons (N=17, 17.7%)

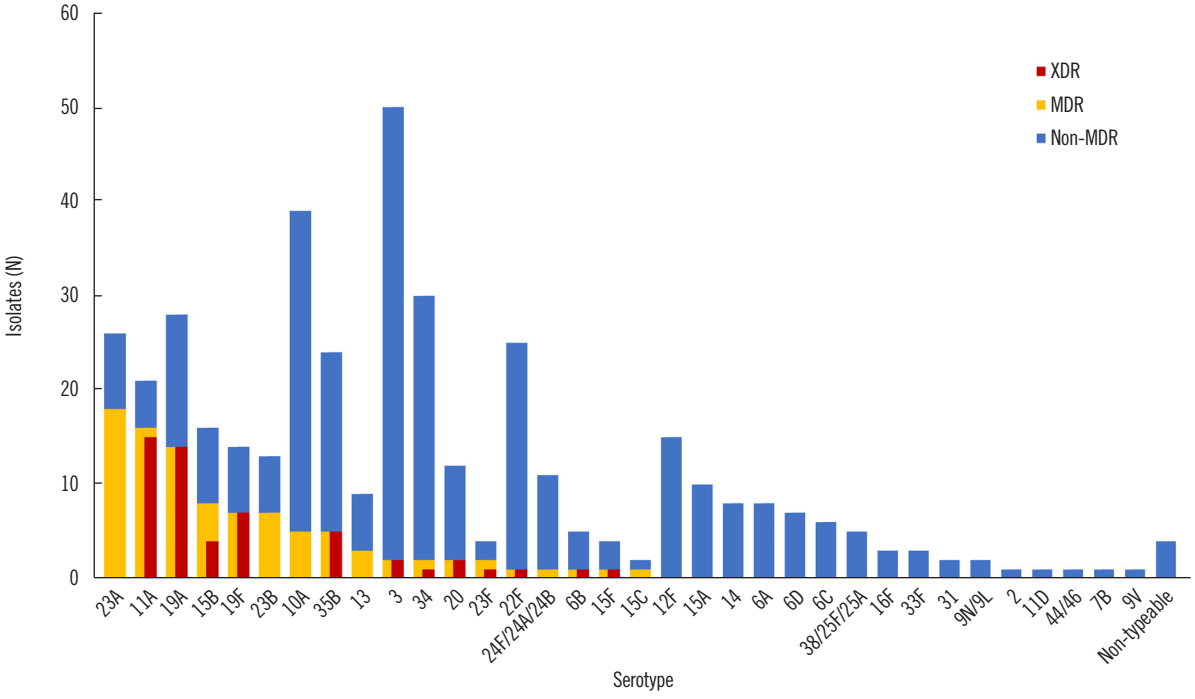


**Fig. 1.** Serotype distribution of multidrug-resistant (MDR) and extremely drug-resistant (XDR) *Streptococcus pneumoniae* isolates.

**Table 4.** MLST of 96 MDR *Streptococcus pneumoniae* isolates

| CC (N) | Sequence type | Serotype (N) | N (%) |
|---|---|---|---|
| CC166 (59) | 166 | 23A (13), 11A (9), 23B (7), 15B (6), 35B (3), 13 (2), 15C (1), 23F (1) | 42 (43.8) |
| | 10120 | 11A (3), 15B (1), 19A (1) | 5 (5.2) |
| | 13214 | 20 (2) | 2 (2.1) |
| | 16444* | 3 (2) | 2 (2.1) |
| | 8279 | 11A (1) | 1 (1.0) |
| | 9690 | 22F (1) | 1 (1.0) |
| | 9875 | 11A (1) | 1 (1.0) |
| | 16209 | 11A (1) | 1 (1.0) |
| | 16324 | 23A (1) | 1 (1.0) |
| | 16441* | 11A (1) | 1 (1.0) |
| | 16442* | 13 (1) | 1 (1.0) |
| | 16443* | 23A (1) | 1 (1.0) |
| CC320 (20) | 320 | 19A (10), 19F (2) | 12 (12.5) |
| | 1464 | 19F (5) | 5 (5.2) |
| | 6400 | 19A (2) | 2 (2.1) |
| | 2697 | 15B (1) | 1 (1.0) |
| Singleton (17) | 11189 | 10A (5) | 5 (5.2) |
| | 10272 | 23A (2) | 2 (2.1) |
| | 16202 | 35B (2) | 2 (2.1) |
| | 189 | 34 (1) | 1 (1.0) |
| | 338 | 23A (1) | 1 (1.0) |
| | 558 | 19A (1) | 1 (1.0) |
| | 1624 | 6B (1) | 1 (1.0) |
| | 3386 | 24F/24A/24B (1) | 1 (1.0) |
| | 9395 | 34 (1) | 1 (1.0) |
| | 16205 | 15F (1) | 1 (1.0) |
| | 16440* | 23F (1) | 1 (1.0) |
| Total | | | 96 (100) |

*Novel sequence types identified in our study.
Abbreviations: CC, clonal complex; MLST, multilocus sequence typing; MDR, multidrug-resistant.

were detected. The five novel STs belonged to CC166 (ST16441, ST16442, ST16443, and ST16444) and a singleton (ST16440). By ST, ST166 (N=42, 43.8%), ST320 (N=12, 12.5%), ST10120 (N=5, 5.2%), ST1464 (N=5, 5.2%), and ST11189 (N=5, 5.2%) were common. CC166 consisted of 12 STs, including ST166 (N=42, 43.8%), ST10120 (N=5, 5.2%), and ST13214 (N=2, 2.1%). CC320 consisted of four STs: ST320 (N=12, 12.5%), ST1464 (N=5, 5.2%), ST6400 (N=2, 2.1%), and ST2697 (N=1, 1.0%).

### Association between serotypes and MLST of 96 MDR *S. pneumoniae* isolates

Serotypes 23A (N=14 of 18), 11A (N=15 of 16), 15B (N=7 of 8), and 23B (N=7 of 7) were common in CC166. Serotypes 19A (N=12 of 14) and 19F (N=7 of 7) were more common in CC320. All serotype 10A isolates contained a singleton, ST11189. The six common CC-serotype combinations were CC166-23A, CC166-11A, CC166-23B, CC166-15B, CC320-19A, and CC320-19F.

## DISCUSSION

There are several reports showing the decrease of IPDs after the introduction of PCVs and a relative increase of the prevalence of non-vaccine serotypes [15, 16]. Recent reports have shown different serotype distributions in various countries, including the USA (35B, 3, 23A, 11A/11D, 15A/15F), Canada (19A, 3, 7F), Spain (12F, 8, 3, 14), and Japan (12F, 3, 23A, 19A) [17-20].

The serotype distribution of *S. pneumoniae* causing IPDs (N=386) collected between 1996 and 2008 in Korea was reported in the order of 19F (9.8%), 23F (8.3%), 19A (7.8%), 6A (7.5%), and 3 (7.3%) [21]. The serotype distribution between 2003 and 2014 in Korea was similar: 19F (12.0%), 19A (12.0%), 3A (12.0%), 4 (9.8%), and 14B (7.6%) [22]. The vaccine coverage rates were also similar in the previous two reports: PCV7 (40.9% vs. 40.7%), PCV10 (45.3% vs. 51.6%), PCV13 (69.9% vs. 78.0%), and PPSV23 (77.2% vs. 75.8%) [21, 22]. However, there have been many changes in the distribution of IPD serotypes since the introduction of the NIP in 2014 in Korea. The common serotypes between 2014 and 2016 were 3 (12.6%), 19A (7.8%), 34 (7.8%), 11A (6.8%), 10A (6.8%), and 12F (6.6%), with a decrease in serotypes 19F, 23F, 6A, 6B, and 9V [2]. In this study, serotypes 3, 10A, 34, 19A, 23A, 22F, and 35B were common, and the increase in rates of serotypes 10A (6.8% vs. 9.5%), 23A (4.6% vs. 6.3%), 22F (3.9% vs. 6.1%), and 35 B (3.7% vs. 5.8%) was remarkable when compared with the rates reported between 2014 and 2016 in Korea [2].

In our study, serotype 10A was the most common serotype in children (≤5 years old), and most of these isolates were collected from children ≤1 year old. This is completely different from the data of other countries, including the USA (19F, 14B, 6B), France (12F, 24F), and Japan (12F, 24F) [23-25]. There are few reports of an increase in serotype 10A. This serotype has been observed in Spain and Belgium [19, 26], and an increase was reported in pediatric patients with IPDs in Korea and Japan [27, 28]. Serotype 10A is not included among the serotypes targeted by PCV13; continuous surveillance is recommended because of


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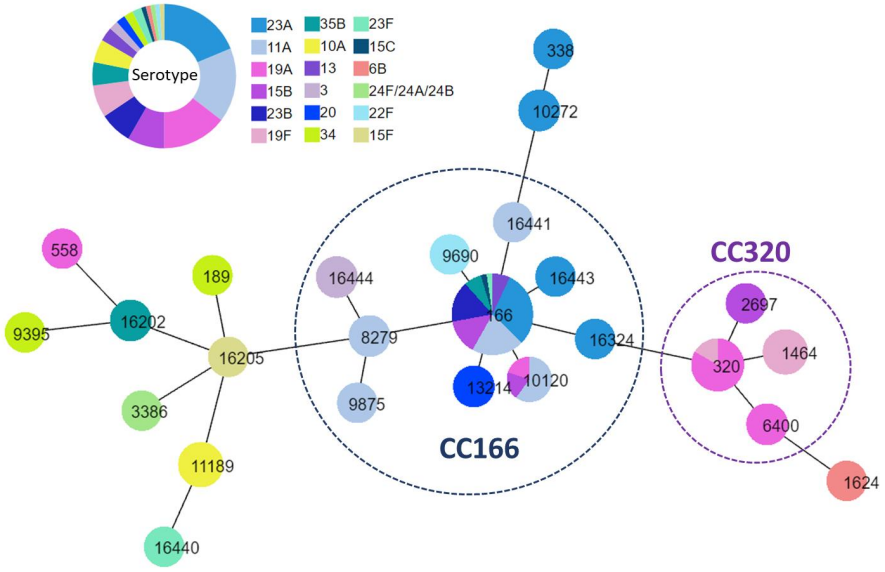


**Fig. 2.** Phylogenetic analysis using PHYLOViZ for 96 multidrug-resistant *Streptococcus pneumoniae* isolates.
Abbreviation: CC, clonal complex.

its high prevalence.

Serotype 3 is most prevalent in adults [29]. An increase in serotype 3 was observed in patients aged >50 years, especially in those ≥65 years old, whereas it was hardly ever found in children in this study. We surmise that this difference is attributable to the effectiveness of PCV13 in children, which supports the consideration of vaccination with PCV13 in the older population. Serotype 35B was common in those ≥65 years old in Korea, whereas this serotype is highly prevalent in children in the USA [30].

The coverage rate of PCV13 in Korea decreased to 28.7% compared to that in a previous report (34.5%) between 2014 and 2016 [2]. The coverage rate of PCV13 in children ≤5 years old was 14.0%, which is slightly higher than that observed in Japan (9.2%), although it is significantly lower than those in the USA (39.3%) and France (34.4%) [23-25]. There has been an increase in non-PCV13 serotypes after PCV13 vaccination, which has also been observed in other countries such as England and Wales (8, 12F, 9N, 22F, 15A, 33F, and 23A), the USA (15B/C, 22F, 33F, and 35B/D), Japan (22F, 15A, and 23A), and China (14, 19F, 19A, and 23F) [31-34]. In this study, the common non-PCV13 serotypes were 10A, 34, 23A, 22F, 35B, and 11A.

The rates of resistance and intermediate resistance to penicillin were 13.1% and 24.1%, respectively. Intermediate resistance rates (19.7% and 20.4%) to cefotaxime and ceftriaxone were higher than their resistance rates (9.5% and 9.2%, respectively). We previously reported that the rates of intermediate resistance to penicillin, cefotaxime, and ceftriaxone were 9.0%, 14.7%, and 11.3%, respectively, in Korea between 2014 and 2016 [2]. This increase in the intermediate resistance rate since 2016 warrants attention. High rates of intermediate resistance to penicillin, cefotaxime, and ceftriaxone have also been reported in Taiwan [35]. These results support the view that the resistance to β-lactam antimicrobial agents will increase in the near future. The resistance rate to levofloxacin was higher at 4.1% than that reported in Canada (1.0%) and Japan (1.0%), although it is lower than that in China (6.6%) [17, 25, 36].

MDR was common in serotypes 11A, 19A, and 19F in previous studies [5, 12, 37, 38]. Serotypes 23A and 23B were closely associated with MDR. Serotype 11A was closely related to levofloxacin resistance, as previously reported, which was highly prevalent in serotypes 35B, 19F, and 20 [5, 12, 37, 38].

CC166 and CC320 were the major clones of the MDR *S. pneumoniae* isolates in this study. CC166 was mainly confirmed in serotypes 11A, 23A, and 23B. The combinations 11A-CC166 and 15B-CC166 were previously reported in Korea [38, 39]; however, the newly emerging 23A-CC166 and 23B-CC166 combinations were identified in this study. The major components of CC166 are ST166 and ST10120. A high prevalence of 23A-ST166 was found among MDR *S. pneumoniae* isolates.

Although the relationship between 19F and CC320 was previously common, it has increased in recent years [12, 36, 37]. In this study, CC320 was common in both the 19A and 19F serotypes. Most serotype 19A isolates were ST320, whereas most

serotype 19F isolates were ST1464. The 19F-CC271 combination represents the major serotype-CC combination in China; however, ST271 was not identified in our study [40]. This demonstrates the epidemiological differences by country. We found an increase in the singleton 10A-ST11189, whereas 10A-ST3385 was common in a previous report [41].

We identified five new STs in MDR isolates (ST16440, ST16441, ST16442, ST16443, and ST16444), which all belong to CC166, except for ST16440. Three new STs, ST16441, ST16442, and ST16443, are single-locus variants of ST166. ST16444 is a single-locus variant of ST8279.

Baek, *et al.* [38] reported that serotype 11A was closely related to CC166, including ST166 and ST8279. However, ST166 was closely related to serotypes 23A and 11A among MDR *S. pneumoniae* isolates in this study. Consequently, CC166 is related to clonal dissemination and expansion of MDR in Korea.

In conclusion, we found a change in serotype distribution and a high rate of non-PCV13 serotypes after introduction of PCV13 vaccination in Korea. An increase in non-vaccine serotypes such as 23A, 23B, and 35B was noted. Differences in antimicrobial resistance according to the specific serotype were verified. These results highlight the need for continuous monitoring of serotypes and antimicrobial resistance to ensure the appropriate management of *S. pneumoniae* infections.

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## AUTHOR CONTRIBUTIONS

Kim GR and Kim EY: conceptualization, data curation, formal analysis, methodology, writing—original draft; Kim SH: data curation, validation, writing—review and editing; Lee HK, Lee J, Shin JH, Kim YR, Song SA, Jeong J, Uh Y, Kim YK, Yong D, Kim HS, Kim S, Kim YA, Shin KS, Jeong SH, and Ryoo N: resources, writing—review and editing; Shin JH: conceptualization, funding acquisition, project administration, resources, supervision, writing—review and editing. All authors reviewed and approved the manuscript.

## CONFLICTS OF INTEREST

None declared.

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## REFERENCES

1. Manoharan A, Manchanda V, Balasubramanian S, Lalwani S, Modak M, Bai S, et al. Invasive pneumococcal disease in children aged younger than 5 years in India: a surveillance study. Lancet Infect Dis 2017;17:305-12.
2. Park DC, Kim SH, Yong D, Suh IB, Kim YR, Yi J, et al. Serotype distribution and antimicrobial resistance of invasive and noninvasive *Streptococcus pneumoniae* isolates in Korea between 2014 and 2016. Ann Lab Med 2019;39:537-44.
3. Croney CM, Nahm MH, Juhn SK, Briles DE, Crain MJ. Invasive and noninvasive *Streptococcus pneumoniae* capsule and surface protein diversity following the use of a conjugate vaccine. Clin Vaccine Immunol 2013;20:1711-8.
4. Diawara I, Zerouali K, Katfy K, Zaki B, Belabbes H, Najib J, et al. Invasive pneumococcal disease among children younger than 5 years of age before and after introduction of pneumococcal conjugate vaccine in Casablanca, Morocco. Int J Infect Dis 2015;40:95-101.
5. Moreno J, Duarte C, Cassiolato AP, Chacón GC, Alarcon P, Sánchez J, et al. Molecular characterization of Latin American invasive *Streptococcus pneumoniae* serotype 19A isolates. Vaccine 2020;38:3524-30.

6. Kim CJ, Song JS, Choi SJ, Song KH, Choe PG, Park WB, et al. Serotype distribution and antimicrobial susceptibilities of invasive *Streptococcus pneumoniae* isolates from adults in Korea from 1997 to 2012. J Korean Med Sci 2016;31:715-23.
7. Kim SH, Chung DR, Song JH, Baek JY, Thamlikitkul V, Wang H, et al. Changes in serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates from adult patients in Asia: emergence of drug-resistant non-vaccine serotypes. Vaccine 2020;38:6065-73.
8. Park D, Kim SH, Bae IK, Kim NY, Kook JK, Park YH, et al. Evaluation of modified sequential multiplex PCR for *Streptococcus pneumoniae* serotyping. Jpn J Infect Dis 2019;72:224-7.
9. Habib M, Porter BD, Satzke C. Capsular serotyping of *Streptococcus pneumoniae* using the Quellung reaction. J Vis Exp 2014;(84):e51208.
10. Steenhoff AP, Shah SS, Ratner AJ, Patil SM, McGowan KL. Emergence of vaccine-related pneumococcal serotypes as a cause of bacteremia. Clin Infect Dis 2006;42:907-14.
11. CLSI. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2022.
12. Golden AR, Rosenthal M, Fultz B, Nichol KA, Adam HJ, Gilmour MW, et al. Characterization of MDR and XDR *Streptococcus pneumoniae* in Canada, 2007-13. J Antimicrob Chemother 2015;70:2199-202.
13. Enright MC and Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology (Reading) 1998;144:3049-60.
14. Jolley KA, Feil EJ, Chan MS, Maiden MC. Sequence type analysis and recombinational tests (START). Bioinformatics 2001;17:1230-1.
15. Wu CJ, Lai JF, Huang IW, Shiau YR, Wang HY, Lauderdale TL. Serotype distribution and antimicrobial susceptibility of *Streptococcus pneumoniae* in pre- and post- PCV7/13 eras, Taiwan, 2002-2018. Front Microbiol 2020;11:557404.
16. Harboe ZB, Dalby T, Weinberger DM, Benfield T, Mølbak K, Slotved HC, et al. Impact of 13-valent pneumococcal conjugate vaccination in invasive pneumococcal disease incidence and mortality. Clin Infect Dis 2014;59:1066-73.
17. Hink RK, Adam HJ, Golden AR, Baxter M, Martin I, Nichol KA, et al. Comparison of PCV-10 and PCV-13 vaccine coverage for invasive pneumococcal isolates obtained across Canadian geographic regions, SAVE 2011 to 2017. Diagn Microbiol Infect Dis 2021;99:115282.
18. Suaya JA, Mendes RE, Sings HL, Arguedas A, Reinert RR, Jodar L, et al. *Streptococcus pneumoniae* serotype distribution and antimicrobial nonsusceptibility trends among adults with pneumonia in the United States, 2009-2017. J Infect 2020;81:557-66.
19. Ludwig G, Garcia-Garcia S, Lanaspa M, Ciruela P, Esteva C, Fernandez de Sevilla M, et al. Serotype and clonal distribution dynamics of invasive pneumococcal strains after PCV13 introduction (2011-2016): Surveillance data from 23 sites in Catalonia, Spain. PLoS One 2020;15:e0228612.
20. Yanagihara K, Kosai K, Mikamo H, Mukae H, Takesue Y, Abe M, et al. Serotype distribution and antimicrobial susceptibility of *Streptococcus pneumoniae* associated with invasive pneumococcal disease among adults in Japan. Int J Infect Dis 2021;102:260-8.
21. Lee S, Bae S, Lee KJ, Yu JY, Kang Y. Changes in serotype prevalence and antimicrobial resistance among invasive *Streptococcus pneumoniae* isolates in Korea, 1996-2008. J Med Microbiol 2013;62:1204-10.
22. Park M, Kim HS, Shin KS, Kim HS, Park JY, Song W, et al. Changes in the incidence of *Streptococcus pneumoniae* bacteremia and its serotypes over 10 years in one hospital in South Korea. Vaccine 2014;32:6403-7.
23. Gaviria-Agudelo CL, Jordan-Villegas A, Garcia C, McCracken GH, Jr. The effect of 13-valent pneumococcal conjugate vaccine on the serotype distribution and antibiotic resistance profiles in children with invasive pneumococcal disease. J Pediatric Infect Dis Soc 2017;6:253-9.
24. Levy C, Varon E, Ouldali N, Béchet S, Bonacorsi S, Cohen R. Changes in invasive pneumococcal disease spectrum after 13-valent pneumococcal conjugate vaccine implementation. Clin Infect Dis 2020;70:446-54.
25. Nakano S, Fujisawa T, Ito Y, Chang B, Matsumura Y, Yamamoto M, et al. Nationwide surveillance of paediatric invasive and non-invasive pneumococcal disease in Japan after the introduction of the 13-valent conjugated vaccine, 2015-2017. Vaccine 2020;38:1818-24.
26. Desmet S, Wouters I, Heirstraeten LV, Beutels P, Van Damme P, Malhotra-Kumar S, et al. In-depth analysis of pneumococcal serotypes in Belgian children (2015-2018): diversity, invasive disease potential, and antimicrobial susceptibility in carriage and disease. Vaccine 2021;39:372-9.
27. Yun KW, Rhie K, Kang JH, Kim KH, Ahn JG, Kim YJ, et al. Emergence of serotype 10A-ST11189 among pediatric invasive pneumococcal diseases, South Korea, 2014-2019. Vaccine 2021;39:5787-93.
28. Ubukata K, Takata M, Morozumi M, Chiba N, Wajima T, Hanada S, et al. Effects of pneumococcal conjugate vaccine on genotypic penicillin resistance and serotype changes, Japan, 2010-2017. Emerg Infect Dis 2018;24:2010-20.
29. Imöhl M, Reinert RR, Ocklenburg C, van der Linden M. Association of serotypes of *Streptococcus pneumoniae* with age in invasive pneumococcal disease. J Clin Microbiol 2010;48:1291-6.
30. Olarte L, Kaplan SL, Barson WJ, Romero JR, Lin PL, Tan TQ, et al. Emergence of multidrug-resistant pneumococcal serotype 35B among children in the United States. J Clin Microbiol 2017;55:724-34.
31. Lo SW, Gladstone RA, van Tonder AJ, Lees JA, du Plessis M, Benisty R, et al. Pneumococcal lineages associated with serotype replacement and antibiotic resistance in childhood invasive pneumococcal disease in the post-PCV13 era: an international whole-genome sequencing study. Lancet Infect Dis 2019;19:759-69.
32. Suzuki S, Osato R, Wajima T, Hasebe T, Ishikawa H, Mitsumori H, et al. Impact of the introduction of a 13-valent pneumococcal vaccine on pneumococcal serotypes in non-invasive isolates from 2007 to 2016 at a teaching hospital in Japan. J Med Microbiol 2019;68:903-9.
33. Zhao C, Xie Y, Zhang F, Wang Z, Yang S, Wang Q, et al. Investigation of antibiotic resistance, serotype distribution, and genetic characteristics of 164 invasive *Streptococcus pneumoniae* from North China between April 2016 and October 2017. Infect Drug Resist 2020;13:2117-28.
34. Moore CE, Paul J, Foster D, Mahar SA, Griffiths D, Knox K, et al. Reduction of invasive pneumococcal disease 3 years after the introduction of the 13-valent conjugate vaccine in the Oxfordshire region of England. J Infect Dis 2014;210:1001-11.
35. Tsai YT, Lee YL, Lu MC, Shao PL, Lu PL, Cheng SH, et al. Nationwide surveillance of antimicrobial resistance in invasive isolates of *Streptococcus pneumoniae* in Taiwan from 2017 to 2019. J Microbiol Immunol Infect 2022;55:215-24.
36. Bao Y, Wang Q, Yao K, Xie G, Gao W, Huang L, et al. The changing phenotypes and genotypes of invasive pneumococcal isolates from children in Shenzhen during 2013-2017. Vaccine 2019;37:7248-55.
37. Wang Q, Shi W, Li Y, Gao W, Yuan L, Dong F, et al. Serotype distribution of *Streptococcus pneumoniae* isolated from children hospitalized in Beijing Children's Hospital (2013-2019). Vaccine 2020;38:7858-64.
38. Baek JY, Kim SH, Kang CI, Chung DR, Peck KR, Ko KS, et al. Prevalence of antimicrobial resistant *Streptococcus pneumoniae* serotype 11A isolates in Korea, during 2004-2013, due to the increase of multidrug-resistant clone, CC166. Infect Genet Evol 2016;38:122-5.
39. Park M, Kim HS, Kim HS, Park JY, Song W, Cho HC, et al. Novel levo-

floxacin-resistant multidrug-resistant *Streptococcus pneumoniae* serotype 11A isolates, South Korea. Emerg Infect Dis 2016;22:1978-80.
40. Wang X, Cong Z, Huang W, Li C. Molecular characterization of *Streptococcus pneumoniae* isolated from pediatric patients in Shanghai, China. Pediatr Pulmonol 2020;55:2135-41.
41. Yun KW, Choi EH, Lee HJ, Kang JH, Kim KH, Kim DS, et al. Genetic structures of invasive *Streptococcus pneumoniae* isolates from Korean children obtained between 1995 and 2013. BMC Infect Dis 2018;18:268.


https://doi.org/10.3343/alm.2023.43.1.45