Original Article
Clinical Microbiology

Ann Lab Med 2019;39:537-544
https://doi.org/10.3343/alm.2019.39.6.537
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Serotype Distribution and Antimicrobial Resistance of Invasive and Noninvasive *Streptococcus pneumoniae* Isolates in Korea between 2014 and 2016

Dong-Chul Park, Ph.D.[1]*, Si Hyun Kim, Ph.D.[2]*, Dongeun Yong, M.D.[3], In Bum Suh, M.D.[4], Young Ree Kim, M.D.[5], Jongyoun Yi, M.D.[6], Wonkeun Song, M.D.[7], Sae Am Song, M.D.[1], Hee-Won Moon, M.D.[8], Hae Kyung Lee, M.D.[9], Kyoung Un Park, M.D.[10], Sunjoo Kim, M.D.[11], Seok Hoon Jeong, M.D.[3], Jaehyeon Lee, M.D.[12], Joseph Jeong, M.D.[13], Yu Kyung Kim, M.D.[14], Miae Lee, M.D.[15], Jihyun Cho, M.D.[16], Jong-Wan Kim, M.D.[17], Kyeong Seob Shin, M.D.[18], Sang-Hyun Hwang, M.D.[19], Jae-Woo Chung, M.D.[20], Hye In Woo, M.D.[21], Chae Hoon Lee, M.D.[22], Namhee Ryoo, M.D.[23], Chulhun L. Chang, M.D.[6], Hyun Soo Kim, M.D.[7], Jayoung Kim, M.D.[24], Jong Hee Shin, M.D.[25], Soo Hyun Kim, M.D.[25], Mi-Kyung Lee, M.D.[26], Seong Gyu Lee, M.D.[27], Sook Jin Jang, M.D.[28], Kyutaeg Lee, M.D.[29], HunSuk Suh, M.D.[30], Yong-Hak Sohn, M.D.[31], Min-Jung Kwon, M.D.[21], Hee Joo Lee, M.D.[32], Ki Ho Hong, M.D.[33], Kwang-Sook Woo, M.D.[34], Chul Min Park, M.D.[35], and Jeong Hwan Shin, M.D.[1,36]

[1]Department of Laboratory Medicine, Inje University College of Medicine, Busan, Korea; [2]Department of Clinical Laboratory Science, Semyung University, Jecheon, Korea; [3]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea; [4]Department of Laboratory Medicine, Kangwon National University College of Medicine, Chuncheon, Korea; [5]Department of Laboratory Medicine, School of Medicine, Jeju National University, Jeju, Korea; [6]Department of Laboratory Medicine, Pusan National University School of Medicine, Busan, Korea; [7]Department of Laboratory Medicine, Hallym University College of Medicine, Chuncheon, Korea; [8]Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea; [9]Department of Laboratory Medicine, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea; [10]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea; [11]Department of Laboratory Medicine, Gyeongsang National University College of Medicine, Jinju, Korea; [12]Department of Laboratory Medicine, Chonbuk National University Medical School and Hospital, Jeonju, Korea; [13]Department of Laboratory Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Korea; [14]Department of Laboratory Medicine, School of Medicine, Kyungpook National University, Daegu, Korea; [15]Department of Laboratory Medicine, Ewha Womans University College of Medicine, Seoul, Korea; [16]Department of Laboratory Medicine, Wonkwang University College of Medicine, Iksan, Korea; [17]Department of Laboratory Medicine, Dankook University College of Medicine, Cheonan, Korea; [18]Department of Laboratory Medicine, Chungbuk National University College of Medicine, Cheongju, Korea; [19]Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; [20]Department of Laboratory Medicine, Dongguk University College of Medicine, Ilsan, Korea; [21]Department of Laboratory Medicine and Genetics, Samsung Medical Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; [22]Department of Laboratory Medicine, College of Medicine, Yeungnam University, Daegu, Korea; [23]Department of Laboratory Medicine, Keimyung University School of Medicine, Daegu, Korea; [24]Department of Laboratory Medicine, International St. Mary's Hospital, College of Medicine, Catholic Kwandong University, Incheon, Korea; [25]Department of Laboratory Medicine, Chonnam National University Medical School, Gwangju, Korea; [26]Department of Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Korea; [27]Department of Laboratory Medicine, Bundang Jesaeng General Hospital, Seongnam, Korea; [28]Department of Laboratory Medicine, College of Medicine, Chosun University, Gwangju, Korea; [29]Department of Laboratory Medicine, Cheju Halla General Hospital, Jeju, Korea; [30]Department of Laboratory Medicine, Daegu Catholic University School of Medicine, Daegu, Korea; [31]Department of Laboratory Medicine, Eulji University School of Medicine, Daejeon, Korea; [32]Department of Laboratory Medicine, School of Medicine, Kyung Hee University, Seoul, Korea; [33]Department of Laboratory Medicine, Seoul Medical Center, Seoul, Korea; [34]Department of Laboratory Medicine, Dong-A University College of Medicine, Busan, Korea; [35]Department of Laboratory Medicine, Dongnam Institute of Radiological & Medical Sciences, Busan, Korea; [36]Paik Institute for Clinical Research, Inje University College of Medicine, Busan, Korea

Background: Several factors contribute to differences in *Streptococcus pneumoniae* serotype distribution. We investigated the serotype distribution and antimicrobial resistance of *S. pneumoniae* isolated between 2014 and 2016 in Korea.

Methods: We collected a total of 1,855 *S. pneumoniae* isolates from 44 hospitals between May 2014 and May 2016, and analyzed the serotypes by sequential multiplex PCR. We investigated the distribution of each serotype by patient age, source of the clinical specimen, and antimicrobial resistance pattern.

Results: The most common serotypes were 11A (10.1%), followed by 19A (8.8%), 3 (8.5%), 34 (8.1%), 23A (7.3%), and 35B (6.2%). The major invasive serotypes were 3 (12.6%), 19A (7.8%), 34 (7.8%), 10A (6.8%), and 11A (6.8%). Serotypes 10A, 15B, 19A, and 12F were more common in patients ≤5 years old, while serotype 3 was more

Received: January 28, 2019
Revision received: April 14, 2019
Accepted: June 13, 2019

Corresponding author: Jeong Hwan Shin, M.D.
Department of Laboratory Medicine,
Busan Paik Hospital, Inje University College of Medicine, 75 Bokji-ro, Busanjin-gu,
Busan 47392, Korea
Tel: +82-51-890-6475
Fax: +82-51-890-8615
E-mail: jhsmile@inje.ac.kr

common in patients ≥65 years old compared with the other age groups. The coverage rates of pneumococcal conjugate vaccine (PCV)7, PCV10, PCV13, and pneumococcal polysaccharide vaccine 23 were 11.8%, 12.12%, 33.3%, and 53.6%, respectively. Of the 1,855 isolates, 857 (46.2%) were multi-drug resistant (MDR), with serotypes 11A and 19A predominant among the MDR strains. The resistance rates against penicillin, cefotaxime, and levofloxacin were 22.8%, 12.5%, and 9.4%, respectively.

Conclusions: There were significant changes in the major *S. pneumoniae* serotypes in the community. Non-PCV13 serotypes increased in patients ≤5 years old following the introduction of national immunization programs with the 10- and 13-polyvalent vaccines.

Key Words: *Streptococcus pneumoniae*, Serotype, Antimicrobial resistance, Pneumococcal vaccine

*These authors equally contributed to this study.

INTRODUCTION

Streptococcus pneumoniae is an important human pathogen that causes pneumonia, sepsis, and meningitis, especially in children [1-3]. This bacterium has more than 93 serotypes, but only a few cause the majority of pneumonias and invasive pneumococcal diseases (IPDs). The serotype distribution differs by patients' age, geographic region, and time of surveillance; these changes are affected by vaccination trends [4, 5].

Following the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7, targeting serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) in children, IPDs caused by PCV7 serotypes decreased dramatically in many countries [6-10]. However, the use of PCV7 led to an increase in infections with non-vaccine serotypes such as 19A [8, 11-13]. PCV7 has led to extensive changes in serotype distribution in Korea [14, 15]. Since 2010, PCV10 (includes PCV7 plus serotypes 1, 5, and 7F) and PCV13 (includes PCV10 plus serotypes 3, 6A, and 19A) have replaced PCV7 in Korea; national immunization programs (NIPs) have been provided for children since May 2014. Therefore, a survey of serotype distribution is necessary for the design of national strategies following the change in the type of pneumococcal vaccine used.

High rates of drug resistance and the spread of multi-drug resistant (MDR) strains of *S. pneumoniae* constitute serious public health concerns worldwide [14, 15]. In Korea, high resistance against most antimicrobial agents continues to be observed in pneumococcal diseases [16, 17], although the resistance rate to penicillin has decreased since the change in the CLSI breakpoints [18]. We aimed to investigate the serotype distribution and antimicrobial resistance of *S. pneumoniae* isolated between 2014 and 2016 in Korea

METHODS

Clinical isolates

A total of 1,855 *S. pneumoniae* isolates were prospectively collected from 44 hospitals in Korea between May 2014 and May 2016. All isolates were transported to the Inje University Busan Paik Hospital, Busan, Korea and stored until use at -70°C using 10% skim milk. This study was approved by the Institutional Review Board of Inje University Busan Paik Hospital (No. 14-0256).

Serotyping by sequential multiplex PCR assay

The serotype of all pneumococcal isolates was determined using sequential multiplex PCR (SM-PCR) according to the recommendations of the U.S. Centers for Disease Control and Prevention (CDC) [19]. For DNA extraction, colonies cultured on blood agar plates were mixed with 200 µL of Tris-EDTA buffer solution (Sigma-Aldrich Co., St Louis, MO, USA). This mixture was heated at 100°C for 10 minutes and then promptly placed on a frozen surface (-20°C) for 5 minutes, followed by centrifugation at 13,000 rpm. SM-PCR was performed with a PCR premix (AccuPower PCR PreMix, Bioneer Inc., Daejeon, Korea), 1 µL of each primer, 5 µL of DNA template, and distilled water in a final volume of 20 µL. Thermal cycling was conducted in a Veriti96-well thermal cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: 94°C for 5 minutes; 30 amplification cycles of 94°C for 30 seconds, 54°C for 30 sec-

https://doi.org/10.3343/alm.2019.39.6.537

onds, and 72°C for 30 seconds; and one cycle of 72°C for 7 minutes. The size of the amplification products was confirmed by electrophoresis on a 2% agarose gel. The Quellung reaction was additionally performed to differentiate serotype 6A from other serotype 6 subtypes using factor antisera (Statens Serum Institute, Copenhagen, Denmark).

Collection of antimicrobial resistance data

The drug resistant results of the pneumococcal isolates were collected from the participating hospitals; the assays were performed mainly by Microscan (Siemens Healthcare Diagnostics, Sacramento, CA, USA), the VITEK2 system (bioMérieux, Marcy-l'Étoile, France), and E-test (bioMérieux). The results were interpreted according to the CLSI guidelines [20]. Separate interpretive breakpoints were used to define the resistance of meningeal isolates to penicillin, cefotaxime, and ceftriaxone. An isolate resistant to three or more classes of antimicrobial agents was considered MDR. We analyzed serotype prevalence by age group, clinical source, and antimicrobial resistance.

RESULTS

Characteristics of *S. pneumoniae* isolates

Of the 1,855 isolates, 1,286 (69.3%) were from male patients and 438 (23.6%) were from patients with invasive disease. The most common source of invasive isolates was blood (N=372; 84.9%), followed by cerebrospinal fluid (N=21; 4.8%), pleural fluid (N=13; 3.0%), abscess (N=13; 3.0%), tissue (N=8, 1.8%), and others (N=11; 2.5%). Non-invasive isolates were recovered from respiratory specimens (N=1,253; 88.4%), wounds (N=127; 9.0%), catheter tips (N=16; 1.1%), urine (N=8; 0.6%), and other sites (N=13; 0.9%).

Distribution of pneumococcal serotypes

The most common serotype was 11A (10.1%), followed by 19A (8.8%), 3 (8.5%), 34 (8.1%), 23A (7.3%), 35B (6.2%), and 15A (5.1%); these serotypes accounted for 54.2% of the isolates (Table 1). Serotypes 23A, 15B, 19A, and 10A were more common in patients ≤5 years old (18.1%, 12.5%, 12.1%, and 8.1%, respectively). In contrast, serotypes 11A, 3, and 34 were much less common in patients ≤5 years old. The frequency of the major serotypes was very similar in patients ≥65 and 6–64 years old. The number of serotypes recovered from ≥65 years and 6–64 years age groups was 35 and 32, respectively, whereas only 20 serotypes were recovered from patients ≤5 years old. Non-typeable (NT) isolates that were not detected by SM-PCR accounted for 6.3% (N=117) of all isolates. These organisms were more common in children ≤5 years old (10.5%).

Table 1. Distribution of pneumococcal serotypes by patient age (N=1,855)

Serotype	N (%)	Age group (%)		
		≤5 years (N=248)	6–64 years (N=673)	≥65 years (N=934)
11A	188 (10.1)	12 (4.8)	77 (11.4)	99 (10.6)
19A	163 (8.8)	30 (12.1)	55 (8.2)	78 (8.4)
3	158 (8.5)	2 (0.8)	60 (8.9)	96 (10.3)
34	151 (8.1)	13 (5.2)	62 (9.2)	76 (8.1)
23A	136 (7.3)	45 (18.1)	47 (7.0)	44 (4.7)
35B	115 (6.2)	16 (6.5)	33 (4.9)	66 (7.1)
15A	94 (5.1)	12 (4.8)	38 (5.6)	44 (4.7)
15B	85 (4.6)	31 (12.5)	30 (4.5)	24 (2.6)
19F	79 (4.3)	7 (2.8)	25 (3.7)	47 (5.0)
6A	72 (3.9)	4 (1.6)	20 (3.0)	48 (5.1)
10A	63 (3.4)	20 (8.1)	29 (4.3)	14 (1.5)
13	59 (3.2)	4 (1.6)	18 (2.7)	37 (4.0)
23F	51 (2.7)	1 (0.4)	12 (1.8)	38 (4.1)
6C	43 (2.3)	6 (2.4)	21 (3.1)	16 (1.7)
12F	37 (2.0)	7 (2.8)	20 (3.0)	10 (1.1)
14	35 (1.9)		11 (1.6)	24 (2.6)
22F	35 (1.9)	6 (2.4)	14 (2.1)	15 (1.6)
6B	32 (1.7)		12 (1.8)	20 (2.1)
6D	25 (1.3)		9 (1.3)	16 (1.7)
20	21 (1.1)	1 (0.4)	8 (1.2)	12 (1.3)
9V	19 (1.0)		7 (1.0)	12 (1.3)
7B	15 (0.8)		6 (0.9)	9 (1.0)
16F	11 (0.6)	2 (0.8)	3 (0.4)	6 (0.6)
24F	9 (0.5)		3 (0.4)	6 (0.6)
33F	9 (0.5)		2 (0.3)	7 (0.7)
23B	7 (0.4)	2 (0.8)	2 (0.3)	3 (0.3)
9N	5 (0.3)		1 (0.1)	4 (0.4)
35A	4 (0.2)		2 (0.3)	2 (0.2)
7F	4 (0.2)		3 (0.4)	1 (0.1)
38	3 (0.2)	1 (0.4)	1 (0.1)	1 (0.1)
17F	3 (0.2)		2 (0.3)	1 (0.1)
5	2 (0.1)		2 (0.3)	
8	2 (0.1)		2 (0.3)	
18C	2 (0.1)		1 (0.1)	1 (0.1)
4	1 (0.1)		1 (0.1)	
Non-typeable	117 (6.3)	26 (10.5)	34 (5.1)	57 (6.1)

The most common serotype among the invasive isolates was 3 (12.6%), followed by 19A (7.8%), 34 (7.8%), 11A (6.8%), 10A (6.8%), and 12F (6.6%) (Table 2). However, serotypes 3, 10A, and 12F were more prevalent among invasive than noninvasive isolates (7.3%, 2.3%, and 0.6%, respectively). Serotypes 11A, 23A, and 35B were more common among noninvasive

Table 2. Comparison of invasive and noninvasive serotypes by patient age

Serotype	Total N (%)	Invasive				Noninvasive			
		Total N (%)	≤5 years	6–64 years	≥65 years	Total N (%)	≤5 years	6–64 years	≥65 years
11A	188 (10.1)	30 (6.8)		15	15	158 (11.2)	12	62	84
19A	163 (8.8)	34 (7.8)	6	11	17	129 (9.1)	24	44	61
3	158 (8.5)	55 (12.6)	1	18	36	103 (7.3)	1	42	60
34	151 (8.1)	34 (7.8)	3	13	18	117 (8.3)	10	49	58
23A	136 (7.3)	20 (4.6)	3	10	7	116 (8.2)	42	37	37
35B	115 (6.2)	16 (3.7)	2	4	10	99 (7.0)	14	29	56
15A	94 (5.1)	23 (5.3)	2	10	11	71 (5.0)	10	28	33
15B	85 (4.6)	20 (4.6)	7	8	5	65 (4.6)	24	22	19
19F	79 (4.3)	9 (2.1)		4	5	70 (4.9)	7	21	42
6A	72 (3.9)	13 (3.0)	1	7	5	59 (4.2)	3	13	43
10A	63 (3.4)	30 (6.8)	10	16	4	33 (2.3)	10	13	10
13	59 (3.2)	7 (1.6)	1	3	3	52 (3.7)	3	15	34
23F	51 (2.7)	8 (1.8)		2	6	43 (3.0)	1	10	32
6C	43 (2.3)	8 (1.8)		3	5	35 (2.5)	6	18	11
12F	37 (2.0)	29 (6.6)	5	18	6	8 (0.6)	2	2	4
14	35 (1.9)	16 (3.7)		4	12	19 (1.3)		7	12
22F	35 (1.9)	17 (3.9)	3	7	7	18 (1.3)	3	7	8
6B	32 (1.7)	7 (1.6)		2	5	25 (1.8)		10	15
6D	25 (1.3)	6 (1.4)		3	3	19 (1.3)		6	13
20	21 (1.1)	9 (2.1)		5	4	12 (0.8)	1	3	8
9V	19 (1.0)	6 (1.4)		4	2	13 (0.9)		3	10
7B	15 (0.8)	4 (0.9)		1	3	11 (0.8)		5	6
16F	11 (0.6)	2 (0.5)		1	1	9 (0.6)	2	2	5
24F	9 (0.5)	5 (1.1)		2	3	4 (0.3)		1	3
33F	9 (0.5)	3 (0.7)		1	2	6 (0.4)		1	5
23B	7 (0.4)	2 (0.5)	1		1	5 (0.4)	1	2	2
9N	5 (0.3)	2 (0.5)			2	3 (0.2)		1	2
35A	4 (0.2)	1 (0.2)		1		3 (0.2)		1	2
7F	4 (0.2)	1 (0.2)		1		3 (0.2)		2	1
38	3 (0.2)	3 (0.7)	1	1	1				
17F	3 (0.2)	1 (0.2)		1		2 (0.1)		1	1
5	2 (0.1)	1 (0.2)		1		1 (0.1)		1	
8	2 (0.1)	2 (0.5)		2					
18C	2 (0.1)					2 (0.1)		1	1
4	1 (0.1)	1 (0.2)		1					
Non-typeable	117	13 (3.0)	2	5	6	104 (7.3)	24	29	51
Total	1,855	438	48	185	205	1,417	200	488	729

https://doi.org/10.3343/alm.2019.39.6.537

isolates (11.2%, 8.2%, and 7.0%, respectively) than invasive isolates (6.8%, 4.6%, and 3.7%).

Serotypes 10A (20.8%), 15B (14.6%), 19A (12.5%), and 12F (10.4%) were common in patients ≤5 years old, whereas 11A and 3 were rarely observed (0% and 2.1%, respectively). Among the invasive isolates, serotypes 11A and 3 were common in patients ≥65 years old (7.3% and 17.6%, respectively) and 6–64 years old (8.1% and 9.7%, respectively), while serotypes 10A and 12F were less frequent in patients ≥65 years old (2.0% and 2.9%, respectively) than in those ≤5 years (20.8% and 10.4%, respectively) and 6–64 years old (8.6% and 9.7%, respectively).

The coverage rates for PCV7, PCV10, PCV13, pneumococcal polysaccharide vaccine 23 (PPSV23), and vaccine serotype (VT) were 11.8%, 12.1%, 33.3%, 53.6%, and 57.5%, respectively (Table 3). For invasive isolates, the coverage rates of PCV7, PCV10, PCV13, PPSV23, and VTs were 10.7%, 11.2%, 34.5%, 64.2%, and 67.1%, respectively. By age, the coverage rates of PCV7, PCV10, and PCV13 among the invasive isolates were 0%, 0%, and 16.7% in children ≤5 years old and 14.6%, 14.6%, and 42.9% in patients ≥65 years old.

Antimicrobial resistance

The antimicrobial resistance of the *S. pneumoniae* isolates is shown in Tables 4 and 5. The resistance rates against penicillin, cefotaxime and levofloxacin were 22.8%, 12.5% and 9.4%, respectively. Among the invasive isolates, the resistance rates against cefotaxime, ceftriaxone, and levofloxacin were higher in patients ≥65 years old (7.5%, 5.2%, and 4.9%, respectively) than in patients ≤5 years old (2.6%, 0%, and 0%, respectively).

Table 3. Prevalence of vaccine serotypes by patient age, specimen type, and period

Vaccine type	Total (N=1,855) (%)	Invasive (%)				Non-invasive (%)			
		Total (N=438)	Age group			Total (N=1,417)	Age group		
			≤5 years (N=48)	6–64 years (N=185)	≥65 years (N=205)		≤5 years (N=200)	6–64 years (N=488)	≥65 years (N=729)
PCV7	219 (11.8)	47 (10.7)	0 (0)	17 (9.2)	30 (14.6)	172 (12.1)	8 (4.0)	52 (10.7)	112 (15.4)
PCV10	225 (12.1)	49 (11.2)	0 (0)	19 (10.3)	30 (14.6)	176 (12.4)	8 (4.0)	55 (11.3)	113 (15.5)
PCV13	618 (33.3)	151 (34.5)	8 (16.7)	55 (29.7)	88 (42.9)	467 (33.0)	36 (18.0)	154 (31.6)	277 (38.0)
PPSV23	994 (53.6)	281 (64.2)	32 (66.7)	121 (65.4)	128 (62.4)	713 (50.3)	85 (42.5)	253 (51.8)	375 (51.4)
VTs	1,066 (57.5)	294 (67.1)	33 (68.8)	128 (69.2)	133 (64.9)	772 (54.5)	88 (44.0)	266 (54.5)	418 (57.3)
NVTs	672 (36.2)	131 (29.9)	13 (27.1)	52 (28.1)	66 (32.2)	541 (38.2)	88 (44.0)	193 (39.5)	260 (35.7)
Non-typeable	117 (6.3)	13 (3.0)	2 (4.2)	5 (2.7)	6 (2.9)	104 (7.3)	24 (12.0)	29 (5.9)	51 (7.0)

Abbreviations: PCV, pneumococcal conjugate vaccine; PPSV23, pneumococcal polysaccharide vaccine 23; VTs, vaccine serotypes; NVTs, non-vaccine serotypes.

Table 4. Resistance to antimicrobial agents by specimen type

Antimicrobial agent	Total (N=1,855)			Invasive (N=438)			Non-invasive (N=1,417)		
	I, N (%)	R, N (%)	S, N (%)	I, N (%)	R, N (%)	S, N (%)	I, N (%)	R, N (%)	S, N (%)
Cefotaxime	287 (18.8)	191 (12.5)	1,045 (68.6)	56 (14.7)	31 (8.1)	295 (77.2)	231 (20.2)	160 (14.0)	750 (65.7)
Ceftriaxone	142 (10.9)	157 (12.0)	1,006 (77.1)	36 (11.3)	16 (5.0)	268 (83.3)	106 (10.8)	141 (14.3)	738 (74.9)
Clindamycin	8 (0.5)	1,079 (69.2)	473 (30.3)	1 (0.3)	260 (66.0)	133 (33.8)	7 (0.6)	819 (70.2)	340 (29.2)
Erythromycin	13 (0.7)	1,507 (81.9)	319 (17.3)	2 (0.5)	336 (77.8)	94 (21.8)	11 (0.8)	1,171 (83.2)	225 (16.0)
Levofloxacin	12 (0.7)	168 (9.4)	1,604 (89.9)	3 (0.7)	18 (4.4)	390 (94.9)	9 (0.7)	150 (10.9)	1,214 (88.4)
Linezolid	0 (0)	3 (0.3)	1,162 (99.7)	0 (0)	3 (0.9)	325 (99.1)	0 (0)	0 (0)	837 (100)
Penicillin	227 (14.0)	370 (22.8)	1,024 (63.2)	31 (9.0)	73 (21.1)	242 (69.9)	196 (15.4)	297 (23.3)	782 (61.3)
Tetracycline	24 (1.4)	1,319 (76.7)	376 (21.9)	5 (1.3)	299 (75.9)	90 (22.8)	19 (1.4)	1,020 (77.0)	286 (21.6)
Trimethoprim-Sulfamethoxazole	174 (10.0)	812 (46.9)	746 (43.1)	36 (8.7)	158 (38.3)	219 (53.0)	138 (10.5)	654 (49.6)	527 (40.0)
Vancomycin	0 (0)	0 (0)	1,855 (100)	0 (0)	0 (0)	438 (100)	0 (0)	0 (0)	1,417 (100)

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

Table 5. Resistance to antimicrobial agents by patient age

Antimicrobial agent	≤5 years (N=248)			≥65 years (N=934)			6–64 years (N=673)		
	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)
Cefotaxime	38 (24.8)	18 (11.8)	97 (63.4)	105 (26.9)	74 (18.9)	391 (100)	144 (18.0)	99 (12.4)	557 (69.6)
Ceftriaxone	20 (14.6)	22 (16.1)	95 (69.3)	46 (9.3)	65 (13.2)	381 (77.4)	76 (11.2)	70 (10.4)	530 (78.4)
Clindamycin	1 (0.6)	127 (73.8)	44 (25.6)	2 (0.3)	394 (67.1)	191 (32.5)	5 (0.6)	558 (69.7)	238 (29.7)
Erythromycin	0 (0)	223 (91.8)	20 (8.2)	4 (0.6)	534 (80.1)	129 (19.3)	9 (1.0)	750 (80.7)	170 (18.3)
Levofloxacin	0 (0)	2 (0.9)	233 (99.1)	5 (0.8)	60 (9.2)	585 (90.0)	7 (0.8)	106 (11.8)	786 (87.4)
Linezolid	0 (0)	0 (0)	129 (100)	0 (0)	2 (0.4)	443 (99.6)	0 (0)	1 (0.2)	590 (99.8)
Penicillin	32 (14.3)	38 (17)	154 (68.8)	76 (12.9)	152 (25.8)	361 (61.3)	119 (14.7)	180 (22.3)	509 (63.0)
Tetracycline	1 (0.4)	200 (87.3)	28 (12.2)	6 (1.0)	464 (75.2)	147 (23.8)	17 (1.9)	655 (75.0)	201 (23.0)
Trimethoprim-Sulfamethoxazole	32 (14.6)	108 (49.3)	79 (36.1)	64 (10.1)	285 (45.0)	285 (45.0)	78 (8.9)	419 (47.7)	382 (43.5)
Vancomycin	0 (0)	0 (0)	248 (100)	0 (0)	0 (0)	673 (100)	0 (0)	0 (0)	934 (100)

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

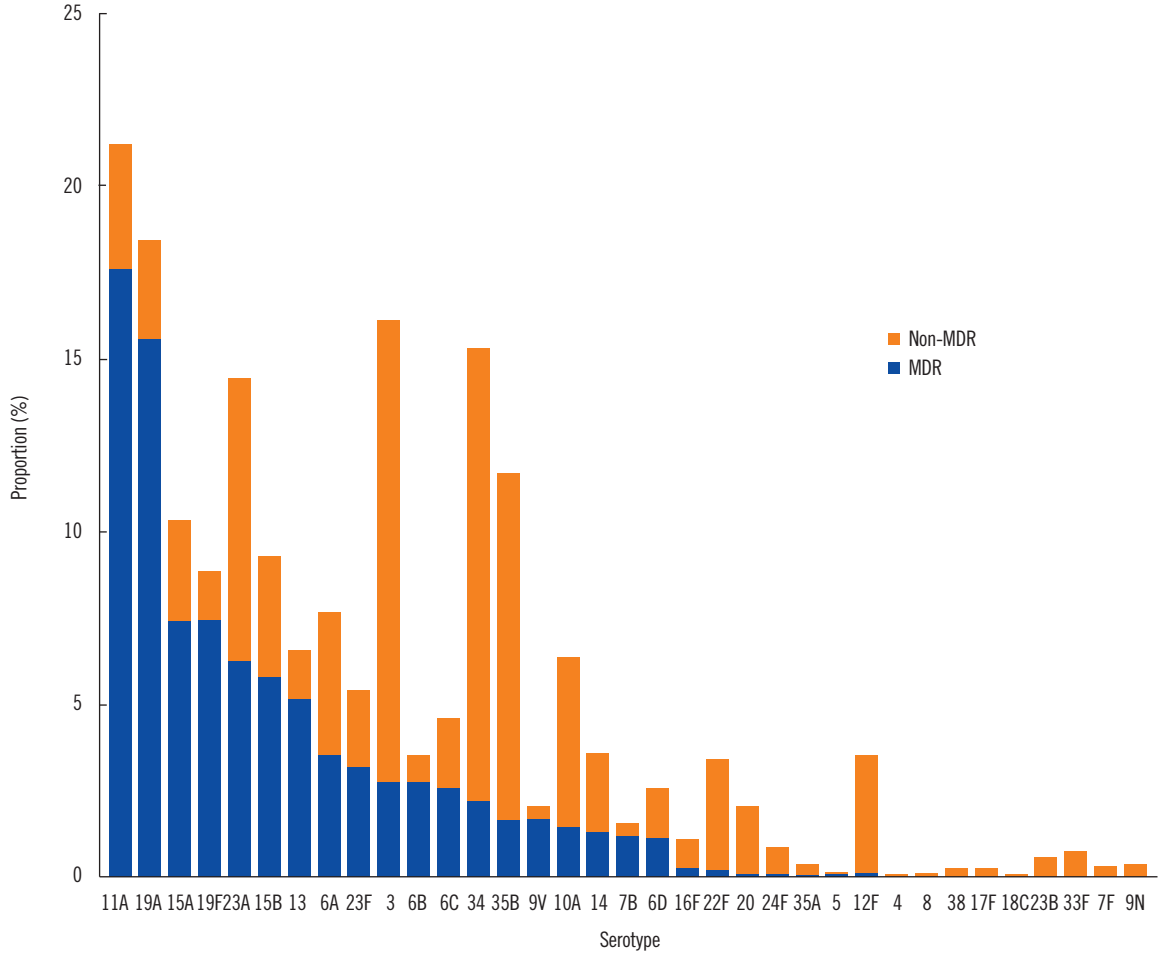

Fig. 1. Serotype distribution of MDR *S. pneumoniae* isolates.
Abbreviation: MDR, multi-drug resistant.

Of the 1,855 isolates, 857 (46.2%) were MDR, including 11A (17.7%), 19A (15.8%), 19F (7.6%), and 15A (7.6%) (Fig. 1). The proportion of MDR was extremely high in serotypes 11A (80.9%), 19A (82.8%), 19F (82.3%), 13 (78.0%), 6B (78.1%),

https://doi.org/10.3343/alm.2019.39.6.537

9V (84.2%), and 7B (80.0%). Serotypes 3, 34, and 6A expressed low-level resistance.

DISCUSSION

The prevalence of the common serotypes differed from that in our previous report [16]. Compared with the results from 2011 to 2014, the proportion of non-PCV13 serotypes, such as 11A, 23A, and 15A, remarkably increased. In addition, we confirmed that serotypes 3 and 6A are now less common, whereas there was no change in the prevalence rate of serotype 19A.

The coverage of PCV13 had decreased, whereas the coverage of PPSV23 had not changed since our previous results from 2011 to 2014 [16]. Surprisingly, the coverage rate of the PCV13 serotype among the invasive isolates was much lower in patients ≤5 years old (16.7%) than in the other age groups (6–64 years old [29.7%] and ≥65 years old [42.9%]). We hypothesize that this change resulted from PCV13 use in children ≤5 years old as the NIPs with PCV13 were provided only for children. In addition, this is associated with the high prevalence of serotype 3 in patients ≥65 years old. The 2014 Korean guidelines recommend the administration of PPSV23 or PCV13 to individuals ≥65 years old [21].

Richter, *et al.* [22] reported a decrease in the prevalence of the PCV13 serotypes in all isolates in the United States from 43.4% (2008–2009) to 27.1% (2012–2013) after the introduction of the PCV13 vaccine. In addition, the prevalence of non-PCV serotypes, such as 11A and 35B, increased among all isolates, while that of serotype 3 slightly increased. Interestingly, they observed a decrease in the prevalence of serotype 19A from 22% to 10% of all isolates, which differs from our results. However, Richter, *et al.* [23] reported that serotype 19A had not changed between 2010 and 2011. Therefore, we hypothesize that serotype 19A will shortly decrease in Korea. Galanis, *et al.* [24] and van der Linden, *et al.* [25] reported an increase in non-PCV13 serotypes in IPD. We confirmed the increase in non-PCV13 serotypes such as 11A, 23A, and 15A; however, there was no observed increase in serotype 23B. Thus, there is a need for a new pneumococcal vaccine, including non-PCV13 serotypes, to prevent IPDs in children.

Previously, we reported the resistance rate against penicillin as 9.0% from 2008 to 2014 [16] and 10.8% from four university hospitals in Busan and Gyeongnam in 2015 [17]. In this study, the resistance rate against penicillin among the isolates from 44 hospitals was 22.8%; thus, there was a striking tendency towards an increase in penicillin resistance. The resistance rates against cefotaxime, ceftriaxone, and levofloxacin were 12.5%, 12.0%, and 9.4%, respectively, which again are higher than those in a previous report [16]. Our findings suggest that resistance rates are increasing in Korea and elsewhere, highlighting the need to monitor antimicrobial resistance continually.

There was a strong association between serotype and antimicrobial resistance. The proportion of MDR *S. pneumoniae* was extremely high among serotypes 11A, 19A, 19F, 13, 6B, 9V, and 7B. Interestingly, the resistance rate against levofloxacin was quite low in serotypes 19A and 23A. Thus, serotypes showing high resistance should be controlled to diminish the risk of severe, even fatal, diseases caused by this organism.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgements

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number HI14C1005).

ORCID

Dong-Chul Park https://orcid.org/0000-0001-9392-420X
Si Hyun Kim https://orcid.org/0000-0003-0713-7985
Dongeun Yong https://orcid.org/0000-0002-1225-8477
In Bum Suh https://orcid.org/0000-0001-7012-0305
Young Ree Kim https://orcid.org/0000-0003-2454-8815
Jongyoun Yi https://orcid.org/0000-0001-9098-3765
Wonkeun Song https://orcid.org/0000-0001-5056-9033
Sae Am Song https://orcid.org/0000-0002-3574-1621
Hee-Won Moon https://orcid.org/0000-0001-9509-6073
Hae Kyung Lee https://orcid.org/0000-0001-8545-9272
Kyoung Un Park https://orcid.org/0000-0002-2402-7633
Sunjoo Kim https://orcid.org/0000-0001-8099-8891
Seok Hoon Jeong https://orcid.org/0000-0001-9290-897X
Jaehyeon Lee https://orcid.org/0000-0003-3211-8903
Joseph Jeong https://orcid.org/0000-0002-1407-9376
Yu Kyung Kim https://orcid.org/0000-0002-4699-8502
Miae Lee https://orcid.org/0000-0001-9140-3814
Jihyun Cho https://orcid.org/0000-0002-6504-7892

Jong-Wan Kim https://orcid.org/0000-0003-1995-4253
Kyeong Seob Shin https://orcid.org/0000-0002-1680-1510
Sang-Hyun Hwang https://orcid.org/0000-0003-3201-5728
Jae-Woo Chung https://orcid.org/0000-0002-4867-9708
Hye In Woo https://orcid.org/0000-0001-8851-7028
Chae Hoon Lee https://orcid.org/0000-0001-7722-9004
Namhee Ryoo https://orcid.org/0000-0001-8383-709X
Chulhun L. Chang https://orcid.org/0000-0001-9117-4919
Hyun Soo Kim https://orcid.org/0000-0002-7026-6715
Jayoung Kim https://orcid.org/0000-0003-2977-1813
Jong Hee Shin https://orcid.org/0000-0001-9593-476X
Soo Hyun Kim https://orcid.org/0000-0001-9739-711X
Mi-Kyung Lee https://orcid.org/0000-0003-1824-476X
Seong Gyu Lee https://orcid.org/0000-0002-7838-5065
Sook Jin Jang https://orcid.org/0000-0003-0286-2483
Kyutaeg Lee https://orcid.org/0000-0003-0729-688X
HunSuk Suh https://orcid.org/0000-0001-6364-5525
Yong-Hak Sohn https://orcid.org/0000-0002-1539-9304
Min-Jung Kwon https://orcid.org/0000-0002-2372-0700
Hee Joo Lee https://orcid.org/0000-0002-5527-7125
Ki Ho Hong https://orcid.org/0000-0002-5700-9036
Kwang-Sook Woo https://orcid.org/0000-0002-3674-8534
Chul Min Park https://orcid.org/0000-0002-9707-2216
Jeong Hwan Shin https://orcid.org/0000-0003-3960-6969

REFERENCES

1. Kalin M, Ortqvist A, Almela M, Aufwerber E, Dwyer R, Henriques B, et al. Prospective study of prognostic factors in community-acquired bacteremic pneumococcal disease in 5 countries. J Infect Dis 2000;182:840-7.
2. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet 2009;374:893-902.
3. Walker CLF, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. Global burden of childhood pneumonia and diarrhoea. Lancet 2013;381:1405-16.
4. Kim SH, Song JH, Chung DR, Thamlikitkul V, Yang Y, Wang H, et al. Changing trends in antimicrobial resistance and serotypes of *Streptococcus pneumoniae* isolates in Asian countries: an Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. Antimicrob Agents Chemother 2012;56:1418-26.
5. Konradsen HB and Kaltoft MS. Invasive pneumococcal infections in Denmark from 1995 to 1999: epidemiology, serotypes, and resistance. Clin Diagn Lab Immunol 2002;9:358-65.
6. Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med 2003;348:1737-46.
7. Kaplan SL, Barson WJ, Lin PL, Romero JR, Bradley JS, Tan TQ, et al. Early trends for invasive pneumococcal infections in children after the introduction of the 13-valent pneumococcal conjugate vaccine. Pediatr Infect Dis J 2013;32:203-7.
8. Moore MR, Gertz RE Jr, Woodbury RL, Barkocy-Gallagher GA, Schaffner W, Lexau C, et al. Population snapshot of emergent *Streptococcus pneumoniae* serotype 19A in the United States, 2005. J Infect Dis 2008;197:1016-27.
9. Centers for Disease Control and Prevention (CDC). Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease-United States, 1998-2003. MMWR Morb Mortal Wkly Rep 2005;54:893-7.
10. Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. N Engl J Med 2009;360:244-56.
11. Choi EH, Kim SH, Eun BW, Kim SJ, Kim NH, Lee J, et al. *Streptococcus pneumoniae* serotype 19A in children, South Korea. Emerg Infect Dis 2008;14:275-81.
12. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. Lancet Infect Dis 2011;11:760-8.
13. Lepoutre A, Varon E, Georges S, Dorléans F, Janoir C, Gutmann L, et al. Impact of the pneumococcal conjugate vaccines on invasive pneumococcal disease in France, 2001-2012. Vaccine 2015;33:359-66.
14. Klugman KP. Pneumococcal resistance to antibiotics. Clin Microbiol Rev 1990;3:171-96.
15. Jacobs MR, Koornhof HJ, Robins-Browne RM, Stevenson CM, Vermaak ZA, Freiman I, et al. Emergence of multiply resistant pneumococci. N Engl J Med 1978;299:735-40.
16. Kim SH, Bae IK, Park D, Lee K, Kim NY, Song SA, et al. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates causing invasive and noninvasive pneumococcal diseases in Korea from 2008 to 2014. Biomed Res Int 2016;2016:6950482.
17. Kim SH, Song SA, Yi J, Song D, Chang CL, Park DC, et al. Distribution and antimicrobial resistance of *Streptococcus pneumoniae* at four university hospitals in Busan and Gyeongnam. Ann Clin Microbiol 2016;19:48-53.
18. CLSI. Performance standards for antimicrobial susceptibility testing. 18th ed. Informational supplement M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.
19. Brito DA, Ramirez M, de Lencastre H. Serotyping *Streptococcus pneumoniae* by multiplex PCR. J Clin Microbiol 2003;41:2378-84.
20. CLSI. Performance standards for antimicrobial susceptibility testing M100, 27th ed. Wayne, PA: Clinical and Laboratory Standards Institute. 2017.
21. Choi WS, Choi JH, Kwon KT, Seo K, Kim MA, Lee SO, et al. Revised adult immunization guideline recommended by the Korean society of infectious diseases, 2014. Infect Chemother 2015;47:68-79.
22. Richter SS, Diekema DJ, Heilmann KP, Dohrn CL, Riahi F, Doern GV. Changes in pneumococcal serotypes and antimicrobial resistance after introduction of the 13-valent conjugate vaccine in the United States. Antimicrob Agents Chemother 2014;58:6484-9.
23. Richter SS, Heilmann KP, Dohrn CL, Riahi F, Diekema DJ, Doern GV. Evaluation of pneumococcal serotyping by multiplex PCR and quellung reactions. J Clin Microbiol 2013;51:4193-5.
24. Galanis I, Lindstrand A, Darenberg J, Browall S, Nannapaneni P, Sjöström K, et al. Effects of PCV7 and PCV13 on invasive pneumococcal disease and carriage in Stockholm, Sweden. Eur Respir J 2016;47:1208-18.
25. van der Linden M, Perniciaro S, Imöhl P. Increase of serotypes 15A and 23B in IPD in Germany in the PCV13 vaccination era. BMC Infect Dis 2015;15:207.