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Changes of serotype and genotype in *Streptococcus pneumoniae* isolates from a Korean hospital in 2007

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Abstract

We investigated the change in clones and serotypes of *Streptococcus pneumoniae* isolates in a Korean tertiary-care hospital. Serotypes of *S. pneumoniae* isolates were determined by the capsular quellung method, and in vitro susceptibility testing was performed by broth microdilution method. Multilocus sequence typing was performed to determine the genotypes of the *S. pneumoniae* isolates. The *erm*(B) and *mef*(A) genes in erythromycin-resistant isolates were also detected using the duplex polymerase chain reaction method. During the 2 periods assayed (1998–2000 and 2007), 7-valent pneumococcal conjugate vaccine (PCV7) serotypes decreased significantly from 58.3% to 30.9% ($P = 0.001$). Especially, serotypes 19F and 23F decreased significantly from 31.7% to 8.5% ($P < 0.0001$) and from 20.0% to 7.4% ($P = 0.021$), respectively. In contrast to the other PCV7 serotypes, serotype 14 coupled with CC554 emerged in 2007, which may indicate no effect of PCV7 against serotype 14 isolates from Korea and the possibility of a different subtype. Of the non- PCV7 serotypes, serotype 19A increased from 8.3% to 14.9% ($P = 0.227$) and serotype 15 increased from 0% to 8.5% ($P = 0.023$). The increase of serotype 19A was due to the expansion of a preexisting clone with serotype 19A, ST320. However, *S. pneumoniae* isolates of serotype 15 showed diverse STs. Our data may provide helpful information in local vaccine serotype expansion or replacement in Korea.

Keywords: *Streptococcus pneumoniae*; Serotype; 7-valent pneumococcal conjugate vaccine (PCV7); Antimicrobial resistance; Multilocus sequence typing (MLST)

1. Introduction

Streptococcus pneumoniae continues to be one of the most important bacterial pathogens, causing invasive infections worldwide especially in infants and children. High antimicrobial resistance rates in *S. pneumoniae* isolates, which may be due to the selection pressure by considerable use of antibiotics and the clonal dissemination of multidrug-resistant strains, are of great concern (Adam, 2002). As a preventive strategy with a significant public health impact, 7-valent pneumococcal conjugate vaccine (PCV7) against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F has recently been introduced in the United States and several other countries.

Since the introduction of PCV7 in the United States in 2000, a significant decrease in invasive pneumococcal disease (IPD) among children has been reported (CDC, 2005; Whitney et al., 2003). Instead, the incidence of IPD by nonvaccine serotypes increased in the United States and other countries since the introduction of PCV7 (Hicks et al., 2007; Muñoz-Almagro et al., 2008). Especially, the increase of serotype 19A is the main cause of this phenomenon (Hicks et al., 2009; Pai et al., 2005). Although serotype 19A belongs to the same serogroup with serotype 19F, a member of PCV7, PCV7 is not effective against IPD by serotype 19A (Whitney et al., 2006). Several hypotheses have been

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suggested for the increase in serotype 19A since the introduction of PCV7. First, a preexisting clone of serotype 19A might have expanded (Muñoz-Almagro et al., 2008). Second, capsular switching by recombination of serotype 19A might have occurred in successful clones associated with PCV7 serotypes (Brueggemann et al., 2007; Moore et al., 2008). Third, new clone(s) of serotype 19A may have been introduced into the population (Klugman, 2002).

Korea is one of the hot spots with regard to antimicrobial resistance of *S. pneumoniae* (Song et al., 2004). It has been reported that penicillin nonsusceptibility was more than 60% (in former criteria, that is, MIC ≥0.12 mg/L) and erythromycin resistance was more than 80% in Korea (Song et al., 2004). In Korea, CC81 (Spain^{23F}-1) and CC271 (Taiwan^{19F}-14) were known to be major multidrug-resistant pneumococcal clonal complexes (Ko and Song, 2004; Song et al., 2004).

PCV7 was introduced in November 2003 in Korea, although PCV7 was not introduced as a routine vaccination program. Although informally, some reports noted that 78% of newly born children in Korea have been vaccinated through the private market (http://www.equitybulls.com/admin/news2006/news_det.asp?id=22998) in 2006. To our knowledge, only limited reports have compared the serotype distribution change since the introduction of PCV7 in Korea (Choi et al., 2008). In that study, serotype 19A also increased in a Korean tertiary-care hospital even before the use of PCV7, unlike in the United States. They demonstrated that multidrug-resistant ST320 isolates were responsible for the expansion of serotype 19A (Choi et al., 2008). However, more investigations are required to evaluate the effect of pneumococcal vaccination and to draw an effective vaccination strategy in Korea.

In this article, we report the changes of pneumococcal serotype, clone distribution, and antimicrobial resistance between 1998 to 2000 and 2007 in Korea.

2. Materials and methods

2.1. Pneumococcal isolates

A total of 154 *S. pneumoniae* isolates from a tertiary-care hospital in Korea (Samsung Medical Center, Seoul, South Korea) were investigated. A total of 60 and 94 nonduplicate isolates were collected in 1998 to 2000 and 2007, respectively. More than half of the isolates were from sputum (83 isolates), followed by blood (23 isolates), tracheal aspirate (16 isolates), nasal swab (11 isolates), and pus (4 isolates). The other isolates were from broncho alveolar lavage (BAL) fluid, bile, bronchial aspirate, cerebrospinal fluid, ear discharge, eye discharge, joint fluid, peritoneal fluid, and throat swabs. Thirteen isolates were from children (5 years or younger), with 5 isolates (8.3%) obtained in 1998 to 2000 and 8 isolates in 2007 (8.5%). Although all 60 isolates in 1998 to 2000 were invasive, 44 isolates (46.8%) were invasive among 94 pneumococcal isolates in 2007.

2.2. Serotyping

Serotypes were determined via the capsular quellung reaction with commercial antisera (Statens Serum Institute, Copenhagen, Denmark), as recommended by the manufacturer.

2.3. In vitro susceptibility testing

Minimum inhibitory concentration (MIC) was determined by the method of broth microdilution, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008). In vitro susceptibility was tested for 8 antimicrobial agents including penicillin, erythromycin, amoxicillin–clavulanate, levofloxacin, ciprofloxacin, clarithromycin, clindamycin, and trimethoprim–sulfamethoxazole.

2.4. Multilocus sequence typing and detection of macrolide resistance determinants

Multilocus sequence typing (MLST) was performed for all isolates as described previously (Enright and Spratt, 1998; Moore et al., 2008). New alleles and STs were submitted to the *S. pneumoniae* MLST database (http://spneumoniae.mlst.net/). Clonal complexes were determined using the eBURST program (Feil et al., 2004). The *erm* (B) and *mef* (A) genes in erythromycin-resistant isolates were detected by the duplex polymerase chain reaction method, as previously described (Ko and Song, 2004).

2.5. Statistical analysis

Fisher's exact *t* test was used to determine the significant differences in resistance using SPSS for Windows (version 11.5 software package; SPSS, Chicago, IL).

3. Results

3.1. Serotypes

Among the 60 *S. pneumoniae* isolates obtained in 1998 to 2000, 58.3% showed PCV7 serotypes. These serotypes decreased to 30.9% in 2007 ($P = 0.001$) (Table 1). Serotype 19F decreased from 31.7% to 8.5%, and serotype 23F decreased significantly from 20.0% to 7.4% ($P < 0.0001$ and $P = 0.021$, respectively). In contrast, serotype 14 was found in 6 isolates in 2007, whereas no isolates with serotype 14 were identified in 1998 to 2000 ($P = 0.082$). Accompanying the decrease in pneumococcal isolates with PCV7 serotypes, serotypes not included in PCV7 (non-PCV7 serotypes) increased to 69.1%. Representatively, serotype 19A increased from 8.3% to 14.9%, although not significant ($P = 0.227$). Although serotype 15 was not found in any pneumococcal isolates in 1998 to 2000, it was found in 8 isolates (8.5%) in 2007 ($P = 0.023$). Besides serotype 15, serotypes 1, 4, 9A, 13/28, 17, 18C, 22, 23A, and 33 were newly found among the pneumococcal isolates in 2007.

Table 1
Change of serotypes in *S. pneumoniae* isolates between 1998 to 2000 and 2007

Serotype	No. (%) of isolates in 1998–2000 (n = 60)	No. (%) of isolates in 2007			
		Total (n = 94)	P	Invasive (n = 44)	P
PCV7 serotypes	35 (58.3)	29 (30.9)	0.001	13 (29.6)	0.004
19F	19 (31.7)	8 (8.5)	<0.0001	2 (4.6)	<0.001
23F	12 (20.0)	7 (7.4)	0.021	7 (15.9)	0.059
6B	3 (5.0)	4 (4.3)	1.000	2 (4.6)	1.000
14	–	6 (6.4)	0.082	4 (9.1)	0.030
9V	1 (1.7)	2 (2.1)	1.000	1 (2.3)	1.000
4	–	1 (1.1)	1.000	–	–
18C	–	1 (1.1)	1.000	1 (2.3)	0.423
Non-PCV7 serotypes	25 (41.7)	65 (69.1)	0.001	31 (70.4)	0.004
1	–	2 (2.1)	0.521	2 (4.6)	0.177
3	4 (6.7)	3 (3.2)	0.432	3 (6.8)	1.000
5	1 (1.7)	–	0.390	–	1.000
6A	3 (5.0)	7 (7.4)	0.741	2 (4.6)	1.000
9A	–	1 (1.1)	1.000	1 (2.3)	0.423
9N	1 (1.7)	–	0.390	–	1.000
10	1 (1.7)	2 (2.1)	1.000	1 (2.3)	1.000
11	3 (5.0)	9 (9.6)	0.369	–	0.261
13/28	–	2 (2.1)	0.521	–	–
15	–	8 (8.5)	0.023	5 (11.4)	0.012
17	–	1 (1.1)	1.000	1 (2.3)	0.423
19A	5 (8.3)	14 (14.9)	0.227	6 (13.6)	0.521
22	–	3 (3.2)	0.282	–	–
23A	–	1 (1.1)	1.000	1 (2.3)	0.423
23B	1 (1.7)	–	0.390	–	1.000
27.32.41	1 (1.7)	–	0.390	–	1.000
33	–	1 (1.1)	1.000	1 (2.3)	0.423
NT	5 (8.3)	11 (11.3)	0.504	8 (18.2)	0.134

NT = nontypeable.

When only invasive isolates (60 and 44 isolates in 1998–2000 and 2007, respectively) were compared, PCV7 serotypes decreased significantly (from 58.3% to 29.6%) (P = 0.004). Although serotype 19F decreased significantly (4.6%, P < 0.001), decrease of serotype 23F was not significant (7.4%, P = 0.059). Of note, all 23F isolates in 2007 were invasive. Of 6 isolates of serotype 14 in 2007, 4 were invasive isolates (P = 0.030). Among 31 isolates of non-PCV7 serotypes, 19A increased to 13.6% in 2007, which was not significant (P = 0.521). Of 8 serotype 15, which was newly found in 2007, 5 were invasive (P = 0.012).

3.2. Multilocus sequence typing analysis

Although 19 STs were identified among the 60 *S. pneumoniae* isolates obtained in 1998 to 2000, 48 STs were identified among the 94 *S. pneumoniae* isolates obtained in 2007 (Table 2). In both periods, CC271 was the most prevalent clonal complex, and CC81 was the 2nd most prevalent. However, both clonal complexes decreased during the periods, 36.7% to 21.3% (P = 0.037) and 25.0% to 14.9% (P = 0.118), respectively. Particularly, ST271 and ST81, which were founders or ancestors of the corresponding CCs, decreased greatly during these periods (Table 2). In CC271, ST271 was identified in 10 isolates in 1998 to 2000 (16.7%), but only 1 ST271 isolate (1.1%) was found in 2007 (P < 0.0001). Instead, ST320 (an ST of CC271) increased from 10.0% to 12.8%, which was not significant (Fig. 1A). ST81 decreased from 23.3% to 3.2% (P < 0.0001). Instead, its single- or double-locus variants such as ST189, ST282, ST932, ST1591, and ST3170 emerged within CC81 in 2007 (Table 2). CC554, including 3 STs, was identified in 8 *S. pneumoniae* isolates in 2007, whereas no CC554 isolates were found in 1998 to 2000. Of these 8 CC554 isolates, 6 isolates showed serotype 14, which is one of the PCV7 serotypes (Table 1).

Among 44 invasive isolates in 2007, each 6 isolates (13.6%) belonged to CC271, CC81, CC180, and CC554. Thus, most isolates of CC180 and CC554 were invasive, but only 30.0% were invasive in CC271. Of 6 isolates of CC271, 5 isolates belonged to ST320 with serotype 19A. Thus, invasive CC271 isolates decreased more significantly in 2007, but ST320 coupled with serotype 19A increased relatively among CC271.

3.3. Antimicrobial resistances

Antimicrobial resistance rates were not significantly different between the 2 periods for most antimicrobial agents except trimethoprim–sulfamethoxazole (Table 3). Trimethoprim–sulfamethoxazole resistance decreased from 71.7% to

Table 2
Distribution of STs among *S. pneumoniae* isolates in 1998 to 2000 and 2007

CC/ST	No. (%) of isolates in 1998–2000 (n = 60)	No. (%) of isolates in 2007 (n = 94)	P
CC271	22 (36.7)	20 (21.3)	.037
ST236	3 (5.0)	–	.057
ST271	10 (16.7)	1 (1.1)	<.0001
ST283	2 (3.3)	–	.150
ST320	6 (10.0)	12 (12.8)	0.602
ST1464	–	3 (3.2)	.282
ST2477	–	1 (1.1)	1.000
ST2533	–	1 (1.1)	1.000
ST2697	–	2 (2.1)	.521
ST3587[a]	1 (1.7)	–	.390
CC81	15 (25.0)	14 (14.9)	.118
ST81	14 (23.3)	3 (3.2)	<.0001
ST83	1 (1.7)	4 (4.3)	.649
ST189	–	1 (1.1)	1.000
ST282	–	3 (3.2)	.282
ST932	–	1 (1.1)	1.000
ST1591	–	1 (1.1)	1.000
ST3170	–	1 (1.1)	1.000
CC166	3 (5.0)	8 (8.5)	.530
ST166	3 (5.0)	6 (6.4)	1.000
ST3597	–	1 (1.1)	1.000
ST3598[a]	–	1 (1.1)	1.000
CC180	5 (8.3)	7 (7.4)	1.000
ST180	5 (8.3)	6 (6.4)	.751
ST3599[a]	–	1 (1.1)	1.000
CC554	–	8 (8.5)	.023
ST554	–	2 (2.1)	.521
ST3600[a]	–	1 (1.1)	1.000
ST3601[a]	–	5 (5.3)	.157
CC880	6 (10.0)	5 (5.3)	.340
ST880	4 (6.7)	–	.022
ST881	1 (1.7)	–	.390
ST3176	1 (1.7)	1 (1.1)	1.000
ST3416	–	1 (1.1)	1.000
ST3602[a]	–	1 (1.1)	1.000
ST3588[a]	–	1 (1.1)	1.000
ST3589[a]	–	1 (1.1)	1.000
CC558-907	2 (3.3)	4 (4.3)	1.000
ST558	1 (1.7)	4 (4.3)	.649
ST907	1 (1.7)	–	.390
CC90-3387	1 (1.7)	3 (3.2)	1.000
ST90	1 (1.7)	2 (2.1)	1.000
ST3387[a]	–	1 (1.1)	1.000
CC99-3596	–	4 (4.3)	.157
ST99	–	3 (3.2)	.282
ST3596[a]	–	1 (1.1)	1.000
Singletons			
ST179	–	1 (1.1)	1.000
ST338	–	2 (2.1)	.521
ST392	–	1 (1.1)	1.000
ST433	–	2 (2.1)	.521
ST447	–	2 (2.1)	.521
ST615	–	2 (2.1)	.521
ST855	–	1 (1.1)	1.000
ST877	2 (3.3)	1 (1.1)	.561
ST899	–	1 (1.1)	1.000
ST1540	–	1 (1.1)	1.000
ST2754	–	1 (1.1)	1.000
ST3181	–	1 (1.1)	1.000
ST3280	–	1 (1.1)	1.000
ST3590[a]	1 (1.7)	1 (1.1)	1.000
ST3591[a]	1 (1.7)	–	.390
ST3592[a]	1 (1.7)	–	.390
ST3593[a]	–	1 (1.1)	1.000
ST3594[a]	–	1 (1.1)	1.000
ST3595[a]	–	1 (1.1)	1.000

[a] Newly identified STs in this study.

48.9% (P = 0.005), but its MIC_{90} increased. Although not significant statistically, 4 levofloxacin-resistant isolates were found only among the *S. pneumoniae* isolates in 2007 (P = 0.157), and MIC_{90} of levofloxacin was also increased from 1 to 2 mg/L. Applying the new breakpoint of CLSI for penicillin susceptibility in *S. pneumoniae* (CLSI, 2008), only 1 isolate in 2007 was resistant to penicillin. Penicillin-nonsusceptible *S. pneumoniae* isolates (MIC, 4 mg/L) were found in 15.0% and 13.8% in 1998 to 2000 and 2007, respectively. Of 22 penicillin-nonsusceptible isolates, 16 isolates (72.7%) belonged to CC271. That is, 38.1% of 42 CC271 isolates were penicillin nonsusceptible, and the MIC_{90} of penicillin in CC271 isolates was 4 mg/L (Table 3). Within CC271, most STs except ST1464 showed non-susceptibility to penicillin (Fig. 1C). *S. pneumoniae* isolates of CC81 showed rather low antimicrobial resistance rates to penicillin as opposed to those of CC271 (Table 4). In addition, antimicrobial resistance rates for amoxicillin–clavulanate and clindamycin were significantly higher in CC271 than in CC81 (Table 4).

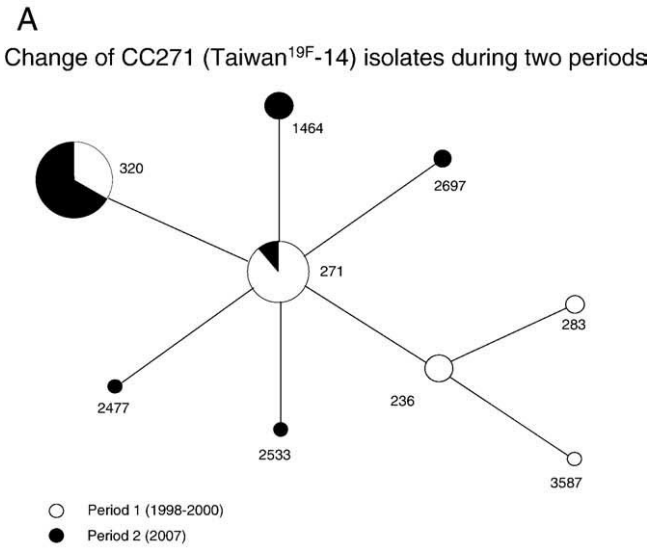
Antimicrobial resistances among 44 invasive pneumococcal isolates in 2007 were not different significantly both from isolates in 1998 to 2000 and from 94 clinical isolates in 2007 (data not shown).

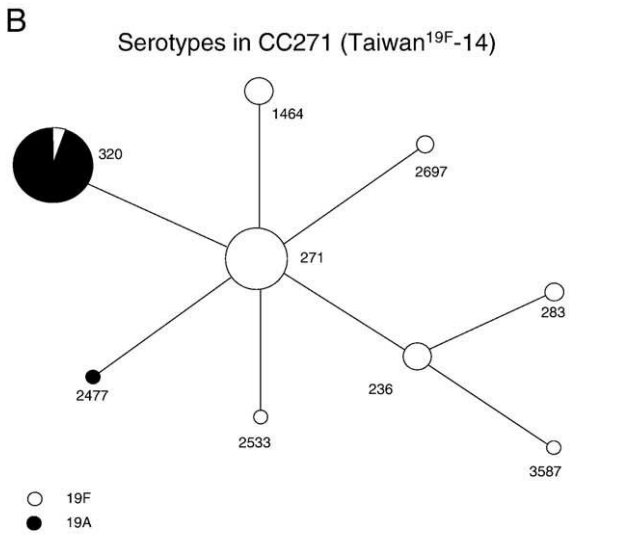
3.4. Macrolide resistance determinants

Overall, 83.1% of *S. pneumoniae* isolates from the 2 periods were resistant to erythromycin (Table 3). Of these, 39.8% contained both the *erm*(B) and *mef*(A) genes (Table 5). The distribution of the *erm*(B) and *mef*(A) genes was not different between the 2 periods. However, it was greatly different between the CC271 and CC81 isolates. Although most CC271 isolates simultaneously contained both genes, only 24.1% of CC81 isolates possessed both genes (Table 5). Only 2 isolates of ST236 contained the *erm*(B) and *mef*(A) genes, and 1 isolate of ST320 was susceptible to erythromycin. Besides the CC271 isolates, *S. pneumoniae* isolates with both the *erm*(B) and *mef*(A) genes were found in 7 CC81, 2 CC166, and 3 CC558-907 isolates, all of which were collected in 2007, except for 2 of the CC81 isolates.

4. Discussion

Most reports from the United States and other countries have reported a decrease of IPD due to *S. pneumoniae* but a





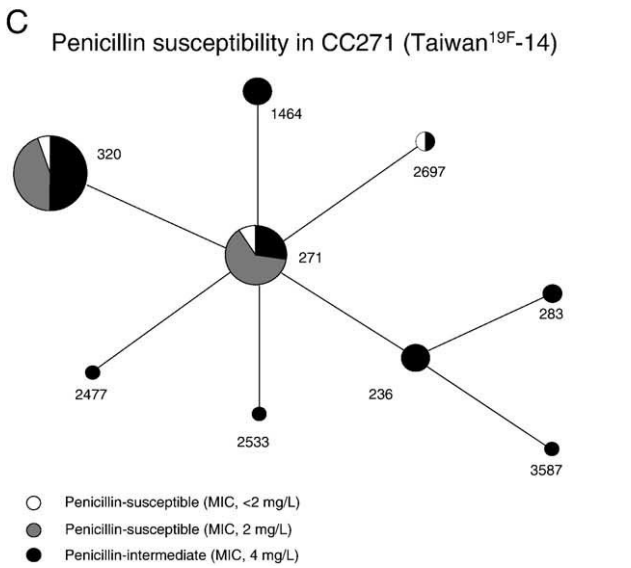


Table 3
Comparison of antimicrobial resistance in *S. pneumoniae* isolates between 1998 to 2000 and 2007

Antimicrobials	1998–2000 (n = 60) (%)		2007 (n = 94) (%)		P
	R	MIC_{90} (mg/L)	R	MIC_{90} (mg/L)	
Penicillin	9 (15.0)[a]	4	13 (13.8)[a]	4	.840
Erythromycin	52 (86.7)	>128	76 (80.9)	>128	.347
Amoxicillin–clavulanate	10 (16.7)	8/4	19 (20.2)	8/4	.583
Levofloxacin	–	1	4 (4.3)	2	.157
Ciprofloxacin	3 (5.0)	2	9 (9.6)	2	.369
Clarithromycin	52 (86.7)	>32	73 (77.7)	>32	.163
Clindamycin	41 (68.3)	>32	62 (66.0)	>32	.760
Trimethoprim–sulfamethoxazole	43 (71.7)	8/152	46 (48.9)	16/304	.005

[a] Nonsusceptible (MIC, ≥4 mg/L).

relative increase of non-PCV7 serotypes, especially 19A, after the introduction of PCV7 (Hanage et al., 2007; Hicks et al., 2007; Muñoz-Almagro et al., 2008; Pai et al., 2005; Pichichero and Casey, 2007). In Korea, a recent study reported an increase of serotype 19A, even before the introduction of PCV7 (Choi et al., 2008). In this study, we found an increase of serotype 19A in 2007, although this was not significant because of the limited number of isolates included. When only invasive isolates were included in the analysis, similar results were shown. The increase of serotype 19A was accompanied with the increase of ST320. All 12 pneumococcal isolates of ST320 in 2007 showed serotype 19A. Most ST320 isolates in 1998 to 2000 also showed serotype 19A, except one. In contrast, the most prevalent clone in 1998 to 2000, ST271, was combined exclusively with serotype 19F (Fig. 1B), and it decreased from 16.7% in 1998 to 2000 to 1.1% in 2007 (Table 2). ST236, which is also combined with serotype 19F, was also not found in 2007. Thus, we did not find that the strain with serotype 19F evolved into serotype 19A (Table 2, Fig. 1B). That is, there is no evidence of capsular switching by recombination of serotype 19A in pneumococcal isolates with serotype 19F, at least in this study. Instead, the increase of serotype 19A after the introduction of PCV7 in Korea could be explained mainly by the expansion of a preexisting clone with serotype 19A, ST320. Although PCV7 is not included in the routine vaccination program in Korea, many children, especially in urban areas, have been vaccinated.

Fig. 1. Diagrams of clonal complex 271 (CC271), or Taiwan^{19F}-1, as defined by eBURST analysis. The size of each circle is related to the number of isolates of corresponding STs. Panel A shows the distribution of STs in the 2 periods (1998–2000 and 2007). Panel B shows the distribution of serotypes (19F and 19A) in each ST. Panel C shows the penicillin susceptibility in CC271. "Penicillin susceptible" is divided into MIC less than 2 mg/L and MIC of 2 mg/L, which is the former breakpoint for penicillin resistance (CLSI, 2008).

Table 4
Comparison of antimicrobial resistance in 2 major clonal complexes of *S. pneumoniae* (CC271 and CC81)

Antimicrobials	CC271 (n = 42)		CC81 (n = 29)		P
	R (%)	MIC_{90} (mg/L)	R (%)	MIC_{90} (mg/L)	
Penicillin[a]	16 (38.1)	4	2 (6.9)	2	.003
Erythromycin	41 (97.6)	>128	29 (100)	>128	1.000
Amoxicillin–lavulanate c	24 (57.1)	8/4	1 (3.4)	4/2	<.0001
Levofloxacin	–	1	1 (3.4)	2	.408
Ciprofloxacin	2 (4.8)	2	3 (10.3)	4	.393
Clarithromycin	42 (100)	>32	26 (89.7)	>32	.064
Clindamycin	41 (97.6)	>32	14 (48.3)	>32	≤.0001
Trimethoprim–sulfamethoxazole	41 (97.6)	8/152	22 (75.9)	8/152	.007

[a] Nonsusceptible (MIC, ≥4 mg/L).

Thus, the decrease of PCV7 serotypes, especially 19F and 23F, can be explained mainly by the effect of PCV7.

According to eBURST analysis, ST320 belongs to CC271. CC271 including ST236, an original ST of the Taiwan^{19F}-14 clone, is characterized as multidrug resistant and has both the *erm*(B) and *mef*(A) genes (Ko and Song, 2004; McGee et al., 2001). As shown in Fig. 1A, ST320 evolved from ST271, a presumed founder in CC271. ST320 differs from ST271 only in the *ddl* gene, which is located close to the *pbp2b* gene. The *pbp2b* gene often showed high sequence divergences in penicillin-resistant pneumococcal isolates because a recombination event conferring penicillin resistance includes all or part of the flanking *ddl* gene (a hitchhiking effect) (Enright and Spratt, 1999). Although the serotype conversion from 19F to 19A might be combined with recombination including the *ddl* gene in the ST320 clone, the capsular locus is not near the *ddl* and *pbp2b* genes (Brueggemann et al., 2007). The serotype conversion in ST320 from 19F to 19A might not be due to vaccine pressure because ST320 isolates with serotype 19A existed before the introduction of PCV7. In addition, recombination including the *ddl* and *pbp2b* genes in ST320 might also not be due to antibiotic pressure because most isolates of both ST271 and ST320 were nonsusceptible to penicillin. Recombination of the *pbp2b* gene did not affect antimicrobial resistance in these pneumococcal clones. However, the penicillin resistance of the ST320 isolates may confer a great advantage for survival even before the era of PCV7. In addition, high virulence of serotype 19A may make it advantageous during survival (Pelton et al., 2007). Thus, the ST320 clone with serotype 19A (which existed before the introduction of PCV7 in Korea), because of several advantageous features, is now expanding because it escapes from PCV7 treatment.

In the United States, ST320 was the 2nd most frequently identified clone among serotype 19A isolates in 2005 (Moore et al., 2008). ST320 was also found in Australia, Norway, Germany, mainland China, and Hong Kong (http://spneumoniae.mlst.net/) (Ko and Song, 2004). Thus, it may be one of the clones spreading worldwide, although it is not yet included in the Pneumococcal Molecular Epidemiology Network (PMEN) clones (http://www.sph.emory.edu/PMEN; McGee et al., 2001). It is expected that the ST320 clone with serotype 19A will become more prevalent worldwide because PCV7 is introduced universally. Its characteristics such as penicillin resistance, high virulence, and high macrolide resistance make it more prone to worldwide dissemination. However, although CC199 including ST199, ST667, and others was the most frequently found clonal complex among pneumococcal isolates with serotype 19A in the United States in 2005 (Hanage et al., 2007; Moore et al., 2008), no isolates belonging to CC199 were found in Korea. This is in contrast to the surveys done in the United States.

In addition to serotype 19A, the emergence of serotype 15 in 2007 should be paid further attention. In our study, serotype 15 was not found in any pneumococcal isolates in 1998 to 2000, but it appeared as one of the most frequently found serotypes in 2007 (8 isolates in total isolates and 5 isolates in invasive isolates) (Table 1). Among the 8 isolates of serotype 15, 5 belonged to CC81 (3 isolates of ST83 and 1 each of ST3170 and ST1591), and the others showed singletons such as ST1540, ST3280, and ST3590. All isolates of serotype 15 were susceptible to amoxicillin–clavulanate, levofloxacin, and ciprofloxacin. However, 5 isolates of CC81 showed a penicillin MIC of 2 mg/L (resistant to penicillin in the former breakpoint of CLSI) and resistance to clarithromycin, trimethoprim–sulfamethoxazole, and erythromycin. Increase in serotype 15, especially 15A, since the introduction of PCV7 was also reported in the United States (Hanage et al., 2007; Moore et al., 2008). In Hanage et al. (2007), pneumococcal isolates of serotype 15A showed ST63, which is the Sweden^{15A}-25 clone. Thus, isolates of serotype 15 from Korea differ from those from the United States. ST83 might be derived from ST81 (Spain^{23F}-1 clone), which is different at the *spi* locus. The *spi* locus is near the capsular locus (Brueggemann et al., 2007). Thus, it might be that recombination at the *spi* locus was accompanied with a change of serotype from 23F to 15, probably under pressure from the vaccine. However, the serotype 15 in singletons such as ST1540, ST3280, and ST3590 indicates that the emergence of serotype 15 in Korea occurred in several ways.

Table 5
Distribution of macrolide resistance determinants

	Macrolide resistance determinants		
	erm(B)	*mef*(A)	*erm*(B) and *mef*(A)
Total (n = 128)	52 (40.6)	25 (19.5)	51 (39.8)
Period			
1998–2000 (n = 52)	19 (36.5)	12 (23.1)	21 (40.4)
2007 (n = 76)	33 (43.4)	13 (17.1)	30 (39.5)
Clonal complex			
CC271 (n = 41)	1 (2.4)	1 (2.4)	39 (95.1)
CC81 (n = 29)	8 (27.6)	14 (48.3)	7 (24.1)

One of the emerging clones in 2007 found in this study was CC554 with serotype 14. Although no isolates of CC554 were identified in 1998 to 2000, 8 isolates (8.5%) belonged to CC554 ($P = 0.023$) (Table 2). Six isolates of CC554 showed serotype 14 (Table 1). All isolates of CC554 were resistant to clarithromycin, clindamycin, and erythromycin but were susceptible to other antimicrobials. Penicillin MICs were between 0.5 and 2.0 mg/L. All isolates contained the *erm*(B) gene. Because serotype 14 is included in the PCV7, the increase of serotype 14 was unexpected. Like the other PCV7 serotypes, serotype 14 decreased in the United States since the introduction of PCV7 (Moore et al., 2008; Pelton et al., 2007; Whitney et al., 2003). Genotypes of pneumococcal isolates showing serotype 14 from Korea were different from the worldwide clones with serotype 14 such as England^{14}-9 (ST9), Spain^{14}-5 (ST18), and CSR^{14}-10 (ST20) (http://spneumoniae.mlst.net/pmen/). Only 1 isolate of ST554 with serotype 14, which originated from Sweden and was isolated in 1997, was submitted to the MLST Web site. However, several isolates of ST3177, a single locus variant of ST554 differing from ST554 at the *xpt* locus, were identified in Korea between 2000 and 2005 (http://spneumoniae.mlst.net/). The other newly identified STs of CC554, ST3600, and ST3601 might also be restricted to Korea. Thus, it can be speculated that the pneumococcal clones with serotype 14 from Korea may differ from those from the United States and other regions. That is, serotype 14 isolates from Korea may represent a different subtype against which PCV7 is not effective, such as in serotype 19A. Further investigation as to whether serotype 14, among the *S. pneumoniae* isolates from Korea, represents a new serotype is required.

This study had a few limitations. First, it did not include only invasive pneumococcal isolates from children, especially in 2007. However, many studies have shown that the introduction of PCV7 in children reduce the colonization of pneumococci in adults as well as in children possibly by the herd immunity (McGee, 2007). Thus, the results of this study may be extended to the invasive pneumococcal isolates in children, although further investigations should be pursued. Another limitation is that this study was restricted to a single tertiary-care hospital in Korea. Thus, this study may not represent the complete trend of change in pneumococcal isolates since the introduction of PCV7 in Korea. However, supporting the previous study by Choi et al. ,(2008), which was also performed in a single (though different) tertiary-care hospital in Korea, our study may be helpful in vaccine serotype expansion or replacement in Korea.

In summary, we investigated the change in serotypes and genotypes of *S. pneumoniae* isolates in a Korean tertiary-care hospital. We found a recent increase in non-PCV7 serotypes such as 19A and 15. The unexpected increase of a PCV7 serotype, serotype 14, was also found. Further nationwide surveillance of antimicrobial resistance, change of serotypes, and distribution of pneumococcal clones, including the investigation of centers with diverse locations, is warranted to obtain concrete information on the effect of PCV7 in Korea.

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