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Multidrug-Resistant *Streptococcus pneumoniae* Serotype 6D Clones in South Korea

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To investigate the characteristics of main *Streptococcus pneumoniae* clones of serotype 6D (ST282 and ST3171) in South Korea, antimicrobial susceptibility testing was performed, and 11 genes around the *cps* locus were sequenced on ST282^{6D}, ST3171^{6D}, and ST81^{6A} isolates. The antimicrobial susceptibility patterns were very similar between clones belonging to the same clonal complex, ST81^{6A} and ST282^{6D}; nonsusceptibilities to penicillin and cefuroxime, high MICs of ceftriaxone, and high resistance rates to trimethoprim-sulfamethoxazole. However, ST3171^{6D} isolates showed resistance to only macrolides and clindamycin. The sequences of 11 genes around the *cps* locus indicated the same genetic backgrounds between the ST81^{6A} and ST282^{6D} isolates. On the other hand, ST3171^{6D} isolates showed nucleotide and amino acid differences from ST81^{6A} and ST282^{6D} isolates in most genes, indicating a different genetic background. The mosaic structure of *dexB* gene in ST282^{6D} isolates indicated that recombination might occur in the *dexB* gene. Our results suggest that the multidrug-resistant ST282^{6D} pneumococcal clone has emerged by serial genetic recombination, including capsular switch.

Streptococcus pneumoniae is a common and important pathogen that causes invasive and noninvasive bacterial diseases in infants, children, and adults. *S. pneumoniae* comprises more than 90 serotypes. Although serogroup 6 was classically considered to consist of serotypes 6A and 6B, another two serotypes, 6C and 6D, have recently been described (14, 22). It has been postulated that serotypes 6C and 6D emerged by the replacement of $wciN_{\beta}$ in the capsular loci of serotypes 6A and 6B, respectively (3, 22). However, two recent papers reported that the serotypes 6C and 6D emerged just by the replacement of the $wciN_{\beta}$ and *wciP* mutation (5, 23). Bratcher et al. (5) proposed that serotype 6C has been produced by introduction of a DNA fragment spanning the capsular polysaccharide synthesis (*cps*) locus irrespective of serotypes 6A and serotype 6D has resulted from a recombination between the *cps* loci of serotypes 6B and 6C. In addition, we also showed the emergence of *cps* loci of serotypes 6C and 6D by complicated recombination (23).

To date, *S. pneumoniae* serotype 6D isolates have been reported in several regions including Fiji, South Korea, China, Japan, Hong Kong, Denmark, Poland, Australia, and Peru (3, 4, 7, 12, 16, 18, 20, 21, 25). Especially, serotype 6D is relatively prevalent in South Korea, comprising >10% of serogroup 6 isolates (1, 8). Choi et al. (8) reported that serotype 6D isolates from South Korea consist of three sequence types (STs) in multilocus sequence typing (MLST) analysis: ST189, ST282, and ST3171. Interestingly, ST189 and ST282 are single-locus variants (SLVs) of ST81, differing in the *aroE* locus. Although ST81 belongs to the Spain^{23F}-1 clone, a globally disseminated multidrug-resistant (MDR) pneumococcal clone (15), it was also found in serotype 6A from South Korea (2). However, relationships between ST282 showing serotype 6D (ST282^{6D}) and ST81^{6A} isolates have not been investigated.

In the present study, we sequenced the region around the *cps* locus to identify the evolutionary scenario resulting in the newly found serotype 6D. In addition, we compared the antimicrobial resistances among serotypes and genotypes.

MATERIALS AND METHODS

***S. pneumoniae* isolates.** Forty-five *S. pneumoniae* isolates from South Korea were investigated: 26 ST81^{6A} isolates, 16 ST282^{6D} isolates (including ST3595 and ST4672, which are SLVs of ST282), and 3 ST3171^{6D} isolates (Table 1). All isolates were collected as part of several surveillance studies of ANSORP (Asian Network for Surveillance of Resistant Pathogens) or KONSID (Korean Network for Studies of Infectious Diseases). They were isolated from seven tertiary-care hospitals from 1997 to 2009. Most of them (31 isolates [68.9%]) were isolated from sputum, and three and one isolates were obtained from cerebrospinal fluid and blood, respectively (Table 1). Isolates obtained between 2008 and 2009 were identified to be invasive, but the clinical significance for the others is not known. Serotyping was done using the capsular Quellung reaction with commercial antisera (Statens Serum Institute, Copenhagen, Denmark), as recommended by the manufacturer. In addition, a previously described serotype-specific PCR method (13) was used to identify serotype 6D. MLST was performed as described previously (11).

Antimicrobial susceptibility testing. MIC was determined for all *S. pneumoniae* isolates by the broth microdilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (9). *In vitro* susceptibility was tested for 14 antimicrobial agents: penicillin, amoxicillin, amoxicillin-clavulanate, ceftriaxone, cefuroxime, erythromycin, azithromycin, clarithromycin, levofloxacin, moxifloxacin, gatifloxacin, ciprofloxacin, clindamycin, and trimethoprim-sulfamethoxazole. Penicillin resistance was evaluated by the current CLSI breakpoints for oral therapy (i.e., intermediate, 0.12 to 1 mg/liter; resis-

Received 23 September 2011 Returned for modification 2 November 2011
Accepted 7 December 2011
Published ahead of print 14 December 2011
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Supplemental material for this article may be found at http://jcm.asm.org/.

doi:10.1128/JCM.05895-11

TABLE 1 *S. pneumoniae* serotype 6D isolates used in this study, their MLST data, and antimicrobial susceptibilities

Serotype	ST[a]	Isolate	Yr	Specimen[b]	Penicillin resistance[c]	Resistance profile[d]
6A	81 (4-4-2-4-4-1-1)	Kor 146	1998	Transtracheal aspirate	I	FUR-ERY-AZI-CLA-CD
		420	1999	ND	I	FUR-ERY-SXT
		K10-25	2000	Eye discharge	I	FUR-ERY-AZI-CLA
		Kor 14	2000	Sputum	I	FUR-ERY
		K08-52	2008	Sputum	R	FUR-ERY-AZI-CLA-CD
		K01-39	2008	CSF	R	FUR-ERY
		K07-13	2008	Sputum	I	FUR-SXT
		K08-9	2008	CSF	I	FUR
		K08-12	2008	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-1	2008	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-4	2008	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-17	2008	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-50	2008	Sputum	I	FUR-ERY-AZI-CLA-CD
		K16-94	2008	CSF	I	FUR-ERY-AZI-CLA-CD-SXT
		K13-75	2009	Sputum	R	FUR-ERY-AZI-CLA-CD
		K13-59	2009	Sputum	R	FUR-ERY-AZI-CLA-CD
		K13-70	2009	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-72	2009	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-78	2009	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-85	2009	Sputum	I	FUR-ERY-AZI-CLA-CD-SXT
		K13-87	2009	Sputum	I	FUR-ERY-AZI-CLA-CD
		K13-98	2009	Sputum	I	FUR-ERY-AZI-CLA-CD-SXT
		K13-102	2009	Sputum	S	ERY
		K13-120	2009	Sputum	I	ERY-AZI-CLA-CD
		K13-130	2009	Sputum	I	FUR-ERY-AZI-CLA-CD
		K15-64	2009	Sputum	I	FUR-ERY
6D	282 (30-4-2-4-4-1-1)	04-8	2004	Sputum	R	FUR-ERY-AZI-CLA
		05-246	2005	Sputum	R	FUR-ERY-AZI-CLA
		05-387	2005	Pus	R	FUR-ERY -CLA
		06-265	2006	Sputum	I	FUR-ERY -CLA
		07-056	2007	Sputum	R	FUR-ERY-AZI
		07-077	2007	Sputum	R	FUR-ERY-AZI-CLA-CIP
		07-107	2007	Transtracheal aspirate	I	FUR-ERY-AZI-LEV-GAT-CIP
		K15-99	2008	ND	I	FUR-ERY-AZI-CLA-CD-SXT
		K15-129	2008	ND	I	FUR-ERY-AZI-CLA
		K15-115	2009	ND	I	FUR-ERY-AZI-CLA-SXT
		K15-17	2009	Sputum	I	FUR-ERY
		K13-108	2009	Sputum	I	FUR-LEV-GAT-CIP
		K13-109	2009	Sputum	I	LEV-GAT-CIP
		K13-110	2009	Sputum	I	FUR-LEV-GAT-CIP
	4762 (30-4-2-4-30-1-1)	K15-60	2008	Sputum	I	FUR
	3595 (30-4-2-1-4-4-1)	B0704-047	2007	Blood	I	FUR-ERY-AZI
	3171 (8-13-9-6-78-119-14)	Kor 74	1997	Throat swab	S	ERY-AZI-CLA-CD
		05-447	2005	Transtracheal aspirate	S	ERY-AZI-CLA-CD
		K13-22	2009	Sputum	S	ERY-AZI-CLA-CD

[a] *aroE-gdh-gki-recP-spi-xpt-ddl*.
[b] ND, not described; CSF, cerebrospinal fluid.
[c] MIC breakpoints of penicillin susceptibility: susceptible (S), ≤0.06 mg/liter; intermediate (I), 0.12 to 1 mg/liter; and resistant (R), ≥2 mg/liter.
[d] FUR, cefuroxime; AMX, amoxicillin; A/C, amoxicillin-clavulanate; AXO, ceftriaxone; ERY, erythromycin; AZI, azithromycin; CLA, clarithromycin; CD, clindamycin; LEV, levofloxacin; MOX, moxifloxacin; GAT, gatifloxacin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole.

tance, ≥2 mg/liter) (9). *S. pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 were used as control strains.

Sequencing. Eleven genes around the *cps* locus (*mraW*, *ftsL*, *pbp2x*, *mraY*, *clpL*, *luxS*, *dexB*, *aliA*, cell wall surface-anchored protein gene [SPN23F_03400 in ATCC 700669], *pbp1a*, and *recU*) of four ST81^{6A}, three ST282^{6D}, and three ST3171^{6D} isolates (Fig. 1) were sequenced using the primers listed in Table 2. The isolates were selected according to locality and isolation year.

GenBank accession numbers. The sequences have been deposited in the GenBank database under accession numbers JN645687 to JN645796.

RESULTS

Antimicrobial susceptibility. The results of antimicrobial susceptibility testing for the 45 *S. pneumoniae* isolates belonging to three groups are shown in Table 1. Notably, nearly all ST81^{6A} and ST282^{6D} isolates were susceptible to penicillin, but three isolates

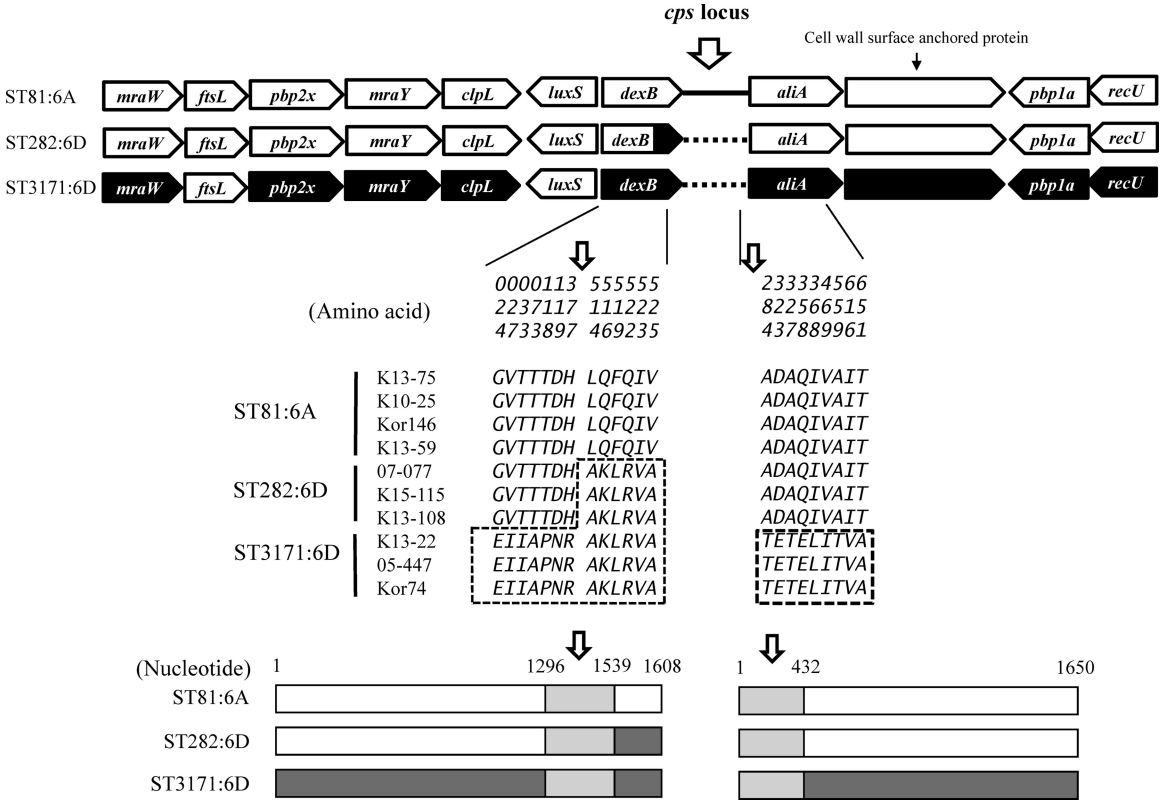

FIG 1 Structure of regions around *cps* locus in ST81^{6A}, ST282^{6D}, and ST3171^{6D} isolates. The amino acid and nucleotide variations in *dexB* and *aliA* genes are indicated. Filled arrows indicate the tentative recombination site in ST282^{6D} isolates.

of ST3171^{6D} were susceptible (Table 1), which was also demonstrated in the penicillin MIC distribution (see the supplemental material). MIC distribution for amoxicillin, amoxicillin/clavulanate, and ceftriaxone also differed among groups (see the supplemental material). Unlike penicillins and cephalosporins, all ST3171^{6D} isolates showed resistance to erythromycin, azithromycin, and clarithromycin (Table 1). Fluoroquinolone-resistant isolates were found only in ST282^{6D}. Four or five ST282^{6D} isolates from two different hospitals were resistant to fluoroquinolones. Although 24 (92.3%) and 9 (56.3%) of the ST81^{6A} and ST282^{6D} isolates, respectively, were nonsusceptible to trimethoprim-sulfamethoxazole, all isolates of ST3171^{6D} were susceptible to it (Table 1; see also the supplemental material).

TABLE 2 Primers used in this study

Gene(s)	Primer	Sequence (5′–3′)
mraW and *ftsL*	mraW-F	GACAAGTGCAAGCTGGTCG
	ftsL-R	GAGTTGCTCTCTTACATAGGA
mraY	mraY-F	GTTGTTCAGAAGCAAGATGTTC
	mraY-R	GAGCAGCCTAAAGTTAGCTTTC
	mraY-R1	CCC ATC AAT TGG TTA AAT AAA TC
clpL	clpL-F1	TAGGGTAGCGGTTTAACTAGTC
	clpL-R1	GCGACGAACACGTTCTGTTAA
	clpL-F2	CTTGCTAAGCAATTGGCACTC
	clpL-R2	AGGACCACAGCCTCTTCTCT
luxS	luxS-F	GCCTATATGTGTAAATCACGAGA
	luxS-R	CCAATACGACCGCTTATATCG
Cell wall surface-anchored protein gene[a]	SPN23-F1	CTTGGAACTTCAAGACAAGGC
	SPN23-R1	ATGATCATCTCCGCTGACATG
	SPN23-F2	GAGACAACAGCAGAGTACTTG
	SPN23-R2	ACGTCCGTTGAGCGTTACTG
	SPN23-F3	GCTAGGTGGCTACAGCATGA
	SPN23-R3	ATTTCCACCAGAATCACCACG
	SPN23-F4	CTCAAGCAAGCAACTTGGAAA
	SPN23-R4	TGTATTCAAAAATGGAGCTCGC
	SPN23-R4-1	CAA AAT CAG AAT CCT CAA CC
pbp1A	P1A-F2	GAGCTCCAAGTTGGGCGAT
recU	recU-R	CCAAAACCAAAGCTGTAGCCA

[a] SPN23F_03400 in ATCC 700669.

Structure of regions around *cps* locus. The structure of the 11 genes around the *cps* locus in the three groups (ST81^{6A}, ST282^{6D}, and ST3171^{6D}) is shown in Fig. 1. Most genes, except *dexB*, were nearly identical between ST81^{6A} and ST282^{6D} isolates at both the amino acid and nucleotide levels. On the other hand, ST3171^{6D} isolates showed amino acid differences from ST81^{6A} and ST282^{6D} isolates in most genes except *ftsL* and *luxS*. Amino acid variations were found: 2 in *mraW*, 62 in *pbp2x*, 15 in *mraY*, 13 in *clpL*, 9 in *aliA*, 28 in the cell wall surface-anchored protein gene, 57 in *pbp1a*, and 5 in *recU*. In addition, inserted sequences of 9 bp in the cell wall surface-anchored protein gene were found in only three ST3171^{6D} isolates.

Unlike the rest of the genes, *dexB* of ST282^{6D} isolates showed a mosaic structure (Fig. 1). By amino acid 377 (nucleotide 1296) of *dexB*, ST282^{6D} showed the same sequences with ST81^{6A}. However, from amino acid 514 (nucleotide 1539) onward, it shared the same sequences with ST3171^{6D}, which were clearly different from ST81^{6A}. For the *aliA* gene, all isolates of three groups showed the same sequences, but ST3171^{6D} showed sequence variations from amino acid 284 (nucleotide 432).

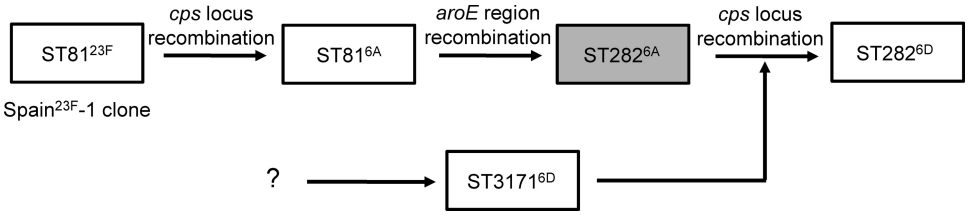

FIG 2 Hypothesis on the emergence of ST282^{6D} clone.

DISCUSSION

S. pneumoniae serotype 6D isolates have been found in several countries and are relatively abundant in South Korea (8, 23). In our study, two main clones (ST282 and ST3171) have been identified among *S. pneumoniae* serotype 6D isolates from Korea. In addition to these two clones, ST189, a SLV of ST81, was also identified previously (8). Besides these STs from Korea, very diverse STs have been identified worldwide, including Australia (ST4241), Finland (ST5163), Poland (ST948, ST2181, ST1612, and ST4734), Peru (ST6148), Japan (ST2924), Fiji (ST639, ST473, and ST4240), China (ST982 and ST4190), and Hong Kong (ST5085 and ST5086) (7, 12, 16, 18, 20, 24, 25). Of these, only four STs from Australia, China, and Hong Kong, ST4241 (7-13-9-6-10-6-14), ST982 (8-13-9-60-78-119-6), ST5085 (8-13-241-60-78-119-6), and ST5086 (8-13-9-60-78-1-6), might belong to the same clonal complex with ST3171 (8-13-9-6-78-119-14), differing at two or three alleles. Thus, it could be inferred that serotype 6D isolates might occur independently worldwide.

In a previous study, another 6D clone, ST189, was reported in South Korea (8). ST282 and ST189 belong to the same clonal complex, CC81, as does the representative resistant pneumococcal clone, Spain^{23F}-1. Both ST282 and ST189 differ from ST81 at *aroE*. Since the ST189^{6D} isolate was not found in our collections, we could not include it in our analysis. Two different clones of 6D pneumococci (ST282 and ST3171) from South Korea showed different antimicrobial susceptibility profiles, which was partially suggested in a previous study (8). Presently, we identified different resistance profiles in most antimicrobial agents tested between two main 6D pneumococcal isolates from South Korea, which may indicate different genetic and evolutionary backgrounds. In addition, the antimicrobial susceptibility profiles of ST282^{6D} isolates were very similar to those of the ST81^{6A} isolates. With respect to antimicrobial resistance, ST282^{6D} may be closer to ST81^{6A} than ST3171^{6D}.

The structure of the 11 genes around *cps* locus evidenced the close relatedness between ST282^{6A} and ST81^{6A}. The complete sequence identity of four genes of the *cps* locus (*wchA*, *wciN*, *wciO*, and *wciP*) between ST282^{6D} and ST3171^{6D} isolates was described in our previous study (23). ST282^{6D} is thought to have originated as a result of capsular switch at ST695^{19A} in the United States (6). Sequence comparison suggests that the genetic recombinant site is located between nucleotides 1296 and 1539 of *dexB* at the left side. Although the genetic recombinant site at the right side could not be identified, it is thought to be any site between *rmlA* and *rmlC*, which are the right and low divergent genes of the *cps* locus of serogroup 6 (17).

Figure 2 illustrates the evolutionary scenario on the emergence of ST282^{6D} isolates in South Korea. ST81 is known as a genotype of the Spain^{23F} pneumococcal clone, the most globally prevalent clone (15, 19). However, ST81 isolates showing serotype 6A (ST81^{6A}) are prevalent in South Korea (2). According to a recent study of genome sequencing of large ST81 pneumococcal collections worldwide, ST81^{6A} is a result of genetic recombination of *cps* locus, that is, a capsular switch (10). Since ST81 and ST282 were different only at the *aroE* locus, a genetic recombination of regions including *aroE* might explain the emergence of ST282^{6A} from ST81^{6A}. Although the ST282^{6A} isolate was not identified in South Korea, an ST282^{6A} isolate has been found in Japan (23). Finally, ST282^{6D} might have emerged by a genetic recombination of the *cps* locus of ST3171^{6D}, which has existed in South Korea since at least 1996 (Table 1) (8). A clonal complex including ST3171 might have represented serotype 6D long before that, because it shares four or five alleles with ST4241, ST982, ST5085, and ST5086, which also represent serotype 6D. Thus, serial recombination may have led to the emergence of high antimicrobial-resistant serotype 6D pneumococcal clone in South Korea.

In the present study, we demonstrate by the sequencing of genes around the *cps* locus and antimicrobial susceptibility testing that the ST282^{6D} clone has emerged by genetic recombination of the *cps* locus from the ST3171^{6D} clone.

ACKNOWLEDGMENTS

The *S. pneumoniae* isolates used in this study were obtained from the Asian Bacterial Bank of the Asia Pacific Foundation for Infectious Diseases (Seoul, Korea).

This study was supported by a Samsung Biomedical Research Institute grant.

REFERENCES

1. **Baek JY, Ko KS, Song J-H.** 2011. *Streptococcus pneumoniae* serotype 6D cross-reacting with serotype 6A, 6B, and 6C factor sera. J. Clin. Microbiol. **49**:765–766.
2. **Baek JY, et al.** 2011. Comparison of genotypes of *Streptococcus pneumoniae* serotypes 6A and 6B before and after the introduction of PCV7 vaccination in Korea. Diagn. Microbiol. Infect. Dis. **69**:370–375.
3. **Bratcher PE, Park IH, Hollingshead SK, Nahm MH.** 2009. Production of a unique pneumococcal capsule serotype belonging to serogroup 6. Microbiology **155**:576–583.
4. **Bratcher PE, Kim KH, King JH, Hong JY, Moon MH.** 2010. Identification of natural pneumococcal isolates expressing serotype 6D by genetic, biochemical and serological characterization. Microbiol. **156**:555–560.
5. **Bratcher PE, et al.** 2011. Evolution of the capsular gene locus of *Streptococcus pneumoniae* serogroup 6. Microbiology **157**:189–198.
6. **Brueggemann AB, Pai R, Crook DW, Beall B.** 2007. Vaccine escape recombinants emerge after pneumococcal vaccination in the United States. PLoS Pathog. **3**:e168.
7. **Chang B, et al.** 2010. Isolation of *Streptococcus pneumoniae* serotypes 6C and 6D from the nasopharyngeal mucosa of healthy Japanese children. Jpn. J. Infect. Dis. **63**:381–383.
8. **Choi EH, et al.** 2010. Prevalence and genetic structures of *Streptococcus pneumoniae* serotype 6D, South Korea. Emerg. Infect. Dis. **16**:1751–1753.

9. **Clinical Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing, 21st informational supplement, M100–S21. CLSI, Wayne, PA.
10. **Croucher NJ, et al.** 2011. Rapid pneumococcal evolution in response to clinical interventions. Science **331**:430–434.
11. **Enright MC, Spratt BG.** 1998. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology **144**:3049–3069.
12. **Ho P-L, Ang I, Chow K-H, Lai EL, Chiu SS.** 2010. The prevalence and characteristics of *Streptococcus pneumoniae* isolates expressing serotypes 6C and 6D in Hong Kong prior to the introduction of the 7-valent pneumococcal conjugate vaccine. Diagn. Microbiol. Infect. Dis. **68**:439–444.
13. **Jacobs MR, et al.** 2009. Occurrence, distribution, and origins of *Streptococcus pneumoniae* serotype 6C, a recently recognized serotype. J. Clin. Microbiol. **47**:64–72.
14. **Jin P, et al.** 2009. First report of putative *Streptococcus pneumoniae* serotype 6D among nasopharyngeal isolates from Fijian children. J. Infect. Dis. **200**:1375–1480.
15. **Klugman KP.** 2002. The successful clone: the vector of dissemination of resistance in *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **50**(S2):1–5.
16. **Kuch A, Sadowy E, Skoczyńska A, Hryniewicz W.** 2010. First report of *Streptococcus pneumoniae* serotype 6D isolates from invasive infections. Vaccine **28**:6406–6407.
17. **Mavroidi A, et al.** 2004. Evolutionary genetics of the capsular locus of serogroup 6 pneumococci. J. Bacteriol. **186**:8181–8192.
18. **Mercado E, et al.** 2011. First report of *Streptococcus pneumoniae* serotype 6D in South America. J. Clin. Microbiol. **49**:2080–2081.
19. **McGee L, et al.** 2001. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. J. Clin. Microbiol. **39**:2565–2571.
20. **Nahm MH, et al.** 2011. A report of *Streptococcus pneumoniae* serotype 6D in Europe. J. Med. Microbiol. **60**:46–48.
21. **Oftadeh S, Satzke C, Gilbert GL.** 2010. Identification of newly described *Streptococcus pneumoniae* serotype 6D by use of the Quellung reaction and PCR. J. Clin. Microbiol. **48**:3378–3379.
22. **Park IH, et al.** 2007. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. J. Clin. Microbiol. **45**:1225–1233.
23. **Song JH, Baek JY, Ko KS.** 2011. Comparison of capsular genes of *Streptococcus pneumoniae* serotype 6A, 6B, 6C, and 6D isolates. J. Clin. Microbiol. **49**:1758–1764.
24. **Satzke C, et al.** 2010. Molecular epidemiology of *Streptococcus pneumoniae* serogroup 6 isolates from Fijian children, including newly identified serotypes 6C and 6D. J. Clin. Microbiol. **48**:4298–4300.
25. **Yao K, et al.** 2011. Type distribution of serogroup 6 *Streptococcus pneumoniae* and molecular epidemiology of newly identified serotypes 6C and 6D in China. Diagn. Microbiol. Infect. Dis. **70**:291–298.