Increase in Serotype 6C Pneumococcal Carriage, United Kingdom

To the Editor: *Streptococcus pneumoniae* is a major human pathogen. In 2007, Park et al. identified a novel serotype, 6C (*1*), which emerged from serotype 6A. A study of children in the Netherlands who had not previously received a pneumococcal vaccine found low prevalence of this newly identified serotype before the heptavalent pneumococcal conjugate vaccine Prevnar/Prevenar (PCV7) (Wyeth, Taplow, UK) was introduced (*2*). Studies have shown cross-protection between vaccine serotype 6B and vaccine-related serotype 6A. However, PCV7 elicits no cross-protection against serotype 6C.

The potential exists for the emergence of nonvaccine serotypes or novel clones. These serotypes and clones may be better adapted to colonize the nasopharynx, evade the human immune response, and cause disease. A recent study showed an increase in prevalence of serotype 6C pneumococci in children and a corresponding decrease in serotype 6A after introduction of PCV7 (*3*). We studied the underlying genetic basis for expansion of serotype 6C. Initial data from an ongoing study of pneumococcal carriage are presented.

This study was reviewed and approved by the Southampton and South West Hampshire Research Ethics Committee (B) (reference 06/Q1704/105). A total of 697 nasopharyngeal swab specimens were collected from unselected (not selected by a method) children ≤4 years of age in the pediatric outpatient department of a large teaching hospital in the United Kingdom. Samples were obtained during October 2006–March 2007, during implementation of PCV7 in the infant immunization schedule of the United Kingdom. During October 2007–March 2008, a total of 202 pneumococci were isolated. All pneumococci were characterized by serotype and genotype.

In the first year of this study, we identified 3 (3.1%) serotype 6C pneumococci belonging to 3 sequence types (STs): ST65, ST1714, and ST1692 (online Appendix Figure, available from www.cdc.gov/EID/content/16/1/154-appF.htm). ST1714 and ST 1692 shared a common clonal complex. Only ST 65 was shared between serotype 6C and serotype 6A. In the second year, we identified 14 (13.6%) serotype 6C pneumococci belonging to 6 STs (online Appendix Figure). Two of these STs, of the same ST, were from siblings. Three of them (ST1692 [n = 8], ST1714 [n = 2], and ST395 [n = 1]) were members of a common clonal complex with a predicted founder of ST395. Each of the remaining 3 STs (ST398, ST1862, and ST3460) was isolated only once. One serotype 6A isolate of ST1692 was also observed.

No serotype 6C ST65 was observed in the second year. We isolated more serotype 6C pneumococci in year 2 than in year 1 ($p \leq 0.01$), which was explained mostly by a large increase in ST1692 ($p \leq 0.03$) (online Appendix Figure). A recent study by Nunes et al. reported serotype 6C ST1692 within a clonal complex that also included ST395 and ST1714 (*4*), and we identified the same clonal complex in year 2 of our study.

Our study showed a large increase in ST1692 in serotype 6C pneumococci during the implementation of PCV7 and an increase in serotype 6C. Depending on the extent of cross-protection between vaccine-related serotypes, introduction of conjugate vaccines could induce clearance or emergence of vaccine-related serotypes. This introduction could also contribute to their substitution with novel or existing serotypes that are better adapted to the ecologic niche. However, our data may only be relevant to carried pneumococci and not reflected in pneumococcal disease epidemiology. Nevertheless, the increase in serotype 6C pneumococci in the United Kingdom, which is supported by a similar observation in the United States (*3*), highlights the potential for emergence of serotypes not included in the current study and newly developed pneumococcal conjugate vaccines.

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Oseltamivir- and Amantadine-Resistant Influenza Virus A (H1N1)

To the Editor: We previously reported detection of double resistance to oseltamivir and amantadine of influenza virus A (H1N1) in Hong Kong during the first half of 2008 (*1*). Three different strains of A/Hong Kong/2652/2006-like (clade 2C) viruses that carried the S31N mutation in the matrix (M2) gene associated with amantadine resistance acquired a neuraminidase (NA) gene with CAT→TAT change at position 274 through either reassortment with an oseltamivir-resistant A/Brisbane/59/2007-like (clade 2B) virus or spontaneous H274Y mutation in the NA gene. A clade 2C strain resistant to both oseltamivir and amantadine also was detected in Cambodia in 2007 (*2*).

From July 2008 through December 2008, we continued to monitor antiviral susceptibility of all influenza A (H1N1) viruses in our laboratory, using previously described methods (*1*). Resistance to oseltamivir increased from 16.9% in July to 97.8% in December (Table). Sequencing of the hemagglutinin (HA) gene showed that, beginning in October, A/Brisbane/59/2007-like clade 2B virus had overtaken A/Hong Kong/2652/2006-like clade 2C virus to become the predominating circulating influenza A virus (H1N1) in Hong Kong. Of 916 isolates, 6 (0.7%), isolated from July through September 2008, were resistant to both amantadine and oseltamivir. Genetic analysis showed that 5 were similar to those we described previously, 4 were A/Hong Kong/2652/2006-like clade 2C viruses with spontaneous H274Y mutation in the NA gene, and 1 was a clade 2C virus but acquired a clade 2B NA gene carrying the H274Y mutation. The sixth double-resistant virus was an A/Brisbane/59/2007-like clade 2B virus with a spontaneous S31N mutation in the M2 gene. No epidemiologic link was detectable between these viruses. From October through December 2008, no double-resistant viruses were detected.

From January through June 2009, A/Brisbane/59/2007-like clade 2B virus continued to be the predominating strain. Of the total 1,537 influenza virus A (H1N1) isolates tested during the period, 1,509 (98.2%) were resistant to oseltamivir. Of the 1,509 oseltamivir-resistant isolates tested from April through June 2009, 50 (3.3%) also were resistant to amantadine (Table). Nucleotide sequencing of the HA, NA, and M2 genes was performed on all 50 oseltamivir- and amantadine-resistant viruses. All were A/Brisbane/59/2007-like clade 2B viruses that had acquired an M2 gene carrying the S31N mutation by reassortment with an amantadine-resistant A/Hong Kong/2652/2006-like clade 2C virus. Nucleotide sequencing of the other 5 internal genes (nonstructural, nucleoprotein, polymerase acidic, polymerase basic 1, and polymerase basic 2 proteins) was performed on 2 double-resistant strains isolated in April and on 3 isolated in June. Sequence comparison showed that 1 virus in April, in addition to acquiring an M2 gene, acquired a nonstructural protein gene from an A/Hong Kong/2652/2006-like clade 2C virus. All the viruses were susceptible to zanamivir and were not associated with unusual severity of disease.

Along with pandemic (H1N1) 2009, seasonal influenza viruses continue to circulate in Hong Kong (*3*). An alarming proportion of the circulating seasonal influenza A virus

Table. Prevalence of oseltamivir-resistant and amantadine- and oseltamivir-resistant influenza A virus (H1N1) detected in Hong Kong, with clade designations, July 2008–June 2009

Date detected	No. isolates tested	No. (%) isolates oseltamivir resistant	No. (%) isolates oseltamivir and amantadine resistant	Predominating influenza A virus (H1N1) clade, no. identified/total no. sequenced (%)
2008 Jul	462	78 (16.9)	4 (5.1)	Clade 2C, 104/182 (57.1)
2008 Aug	313	45 (14.4)	1 (2.2)	Clade 2C, 51/95 (53.7)
2008 Sep	61	21 (34.4)	1 (4.8)	Clade 2B, 20/39 (51.3)
2008 Oct	19	13 (68.4)	0	Clade 2B, 13/16 (81.3)
2008 Nov	16	15 (93.8)	0	Clade 2B, 16/16 (100)
2008 Dec	45	44 (97.8)	0	Clade 2B, 41/41 (100)
2009 Jan	327	313 (95.7)	0	Clade 2B, 90/104 (86.5)
2009 Feb	769	755 (98.2)	0	Clade 2B, 138/153 (90.2)
2009 Mar	279	279 (100)	0	Clade 2B, 61/61 (100)
2009 Apr	63	63 (100)	2 (3.2)	Clade 2B, 18/18 (100)
2009 May	22	22 (100)	2 (9.1)	Clade 2B, 7/7 (100)
2009 Jun	77	77 (100)	46 (59.7)	Clade 2B, 55/55 (100)