Background. Because classic pneumococcal serotyping methods cannot distinguish between serotypes 6A and 6C, the effects of pneumococcal vaccines against serotype 6C are unknown. Pneumococcal vaccines contain serotype 6B but not serotypes 6A and 6C.

Methods. We used a phagocytic killing assay to estimate the immunogenicity of the 7-valent conjugate vaccine (PCV7) in children and the 23-valent polysaccharide vaccine (PPV23) in adults against serotypes 6A and 6C. We evaluated trends in invasive pneumococcal disease (IPD) caused by serotypes 6A and 6C in the United States, using active surveillance.

Results. Serum specimens from PCV7-immunized children had median opsonization indices of 150 and 20 for serotypes 6A and 6C, respectively. Similarly, only 52% of adults (25 of 48) vaccinated with PPV23 showed opsonic indices of ≥20 against serotype 6C. During 1999–2006, the incidence of serotype 6A IPD decreased by 91% (from 4.9 to 0.46 cases per 100,000 persons; \( P < .05 \)) among individuals aged <5 years and by 58% (from 0.86 to 0.36 cases per 100,000 persons; \( P < .05 \)) among those aged ≥5 years. Although the incidence of 6C IPD showed no consistent trend (range, 0–0.6 cases per 100,000 persons) among individuals aged <5 years, it increased from 0.25 to 0.62 cases per 100,000 persons (\( P < .05 \)) among those aged ≥5 years.

Conclusions. PCV7 introduction has led to reductions in serotype 6A IPD but not serotype 6C IPD in the United States.

Streptococcus pneumoniae is a common cause of pneumonia, bacteremia, meningitis, and otitis media. Two different pneumococcal vaccines are currently used in the United States: a 23-valent polysaccharide vaccine (PPV23) has been used in adults since 1983, and a 7-valent pneumococcal conjugate vaccine (PCV7; Prevnar) has been used for young children since 2000 [1]. These vaccines elicit polysaccharide capsule–specific antibodies that provide serotype-specific protection by opsonizing pneumococci for phagocytes. PCV7 has been highly effective in young children, for whom it has dramatically reduced the incidence of invasive pneumococcal disease (IPD) [2–4]. PCV7 has also provided strong herd immunity by reducing the incidence of IPD among unvaccinated children and adults [3, 5, 6]. Although recently observed reductions in IPD are largely attributable to reductions in IPD caused by the serotypes included in PCV7, it was hoped that PCV7 would also reduce the incidence of IPD caused by serologically related pneumococcal serotypes. For instance, PCV7 contains the 6B and 19F capsular polysaccharides but not the related 6A and 19A polysaccharides. However, studies have shown that PCV7 does not provide cross-protection against serotype 19A [6, 7] and that the incidence of IPD caused by serotype 19A has increased dramatically since 2000 [6, 8, 9]. In contrast, most [10–12] but not all [13] studies have found that PCV7 and similar conjugate vaccines do provide cross-protection against serotype 6A.
Recently, we described a new pneumococcal serotype, which we named 6C for its serologic similarities to serotype 6A [14]. Classic serotyping methods do not distinguish between 6A and 6C [15], but recent retrospective work has shown that serotype 6C has circulated for >25 years [16]. Serotype 6C is both genetically and biochemically different from serotypes 6A and 6B [14, 16]. In view of these findings, we investigated the impact of pneumococcal vaccines on the 6A/6C serotypes by determining the prevalence of the 6A and 6C serotypes among IPD isolates and the capacity of serum specimens from vaccinees to opsonize 6A and 6C serotypes in vitro.

**SUBJECTS, MATERIALS, AND METHODS**

**Serum specimens from children.** Serum samples used in this analysis were obtained from 19 children (from 1 of 2 large private pediatric practices in southeastern Massachusetts) who were born between 3 July 1999 and 2 July 2003, participated in a prospective study of nasopharyngeal colonization, were identified as having been colonized with *S. pneumoniae* on at least 1 occasion without having had invasive pneumococcal disease, and received 3 or 4 doses of PCV7. The children’s vaccination status was confirmed from pediatric provider records. The children were 39–86 months old at the time of serum collection. The study was approved by the Boston University Medical Campus institutional review board, and written informed consent was obtained from a parent or guardian of each subject.

**Serum specimens from adults.** Adult subjects (*n* = 48) were medically stable, community dwellers aged ≥65 years of age who were participants in an institutional review board–approved clinical trial comparing 9-valent pneumococcal conjugate vaccine (Wyeth) with PPV23 (Merck). This report describes subjects immunized with PPV23, all of whom were recruited in Rochester, New York, and Cincinnati, Ohio. Peripheral blood samples were obtained from subjects 1 month after their PPV23 vaccinations. Approximately half of the subjects had previously received PPV23. Serum was obtained from blood samples and kept frozen until analysis.

**Opsonophagocytosis assay.** The opsonic capacity of the serum samples was determined using the killing-type opsonization assay, which is currently accepted as the reference method [17] and was previously described in detail [18]. Differentiated HL-60 cells (ATCC) were used as phagocytes, and baby rabbit serum (Pel-Freez) was used as the complement source. To eliminate clonal differences, isogenic target bacteria were prepared by inserting 6A, 6B, and 6C capsule gene loci into a TIGR4 background, as previously described [16]. The opsonization index was defined as the serum dilution that kills 50% of the target bacteria. Because all serum samples were diluted 5-fold before the assay and diluted 4-fold during the assay, the minimum detectable opsonization index was 20.

**Population surveillance for invasive pneumococcal disease.** Cases of IPD were defined by the isolation of pneumococci from a normally sterile site among residents of the Centers for Disease Control and Prevention’s (CDC’s) Active Bacterial Core surveillance (ABCs) areas [19]. Trends over time in the incidence of IPD caused by serotype 6C were evaluated by comparing rates in 2006 with rates in 1999 (the pre-PCV7 baseline period) for areas under continuous surveillance, which included all of Connecticut and selected counties in California, Georgia, Maryland, New York, Oregon, and Tennessee. The total population in 2006 for these continuous-surveillance areas was 17,922,545 persons (1,189,369 children <5 years old), according to 2006 postcensus population estimates.

**Serotyping.** Pneumococcal isolates available from IPD cases occurring in 1999 and 2003–2006 in ABCs sites were initially serotyped by the CDC, using the quellung reaction, which classifies serotype 6C as 6A. All available isolates serotyped as 6A were sent to the University of Alabama at Birmingham to distinguish between the 6A and 6C serotypes, using 2 different antibodies, Hyp6AG1 and Hyp6AM3 [14].

**Susceptibility testing.** Susceptibility testing was performed using broth microdilution according to 2007 Clinical and Laboratory Standards Institute guidelines [20]. Susceptibility testing was performed for penicillin G, erythromycin, cefotaxime, and levofloxacin at the University of Texas Health Sciences Center, San Antonio, and the CDC.

**Statistical analysis.** Because not all cases identified by the ABCs in 1999 and 2003–2006 had isolates available for serotyping, we assumed that the serotype distribution among cases without isolates was similar to that among cases with isolates, adjusting for age. We evaluated trends over time in the incidence of IPD caused by serotype 6C by comparing rates in 2006 with rates in 1999, using the χ² method or the Fisher exact test, as appropriate. For all analyses, *P* values of <.05 were considered statistically significant.

**RESULTS**

**Trends in the incidence of IPD due to serotypes 6A and 6C following PCV7 introduction.** During 1999 and 2003–2006, a total of 13,907 cases of IPD were identified through the ABCs; isolates from 12,219 (88%) were available for serotyping. Classic serotyping methods showed that 778 (6.4%) were serotype 6A, of which 752 (97%) were available for further characterization. Of these 752 isolates, monoclonal antibody typing confirmed that 486 (65%) were serotype 6A and that the remaining 266 (35%) were serotype 6C.

For children aged <5 years, we found no serotype 6C cases among the 42 cases characterized as serotype 6A by means of classic methods during 1999, before PCV7 was introduced (table 1). During 2003–2006, the incidence of serotype 6A IPD among children aged <5 years decreased steadily, and, by 2006, it had decreased by 91% (95% CI, 78%–97%) (table 1). Among children <5 years of
Table 1. Incidence of invasive pneumococcal disease (IPD) caused by serotypes 6A and 6C in selected US sites during 1999 and 2003–2006.

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<tr>
<td>&lt;5 years</td>
<td>4.9 (42)</td>
<td>2.0 (19)</td>
<td>0.23 (2)</td>
<td>0.34 (3)</td>
<td>0.46 (5)</td>
<td>0</td>
<td>0.10 (1)</td>
<td>0.70 (6)</td>
<td>0</td>
<td>0.55 (6)</td>
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<tr>
<td>&gt;5 years</td>
<td>0.86 (111)</td>
<td>0.88 (118)</td>
<td>0.57 (80)</td>
<td>0.40 (57)</td>
<td>0.36 (49)</td>
<td>0.25 (32)</td>
<td>0.29 (40)</td>
<td>0.29 (41)</td>
<td>0.37 (53)</td>
<td>0.62 (87)</td>
</tr>
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**NOTE.** Data indicate the number of new serotype-specific IPD cases per 100,000 persons. Values in parentheses indicate the actual number of serotype-specific IPD cases observed.


<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Serotype 6A, % (no.) of isolates (n = 486)</th>
<th>Serotype 6C, % (no.) of isolates (n = 265)</th>
<th>P*</th>
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<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
<td>Resistant</td>
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<tr>
<td>Penicillin</td>
<td>51 (246)</td>
<td>33 (162)</td>
<td>16 (78)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50 (245)</td>
<td>0</td>
<td>50 (241)</td>
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<tr>
<td>Cefotaxime</td>
<td>97 (474)</td>
<td>2 (10)</td>
<td>0.4 (2)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>99 (484)</td>
<td>. . .</td>
<td>0.4 (2)</td>
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* By the \( \chi^2 \) test for difference in the distribution of susceptible, intermediate, and resistant strains among serotype 6A versus 6C cases.
children <5 years old (the population targeted for PCV7 vaccination), rates of 6A IPD decreased markedly. Few cases of 6C IPD occurred among these younger children, indicating no clear trend after PCV7 introduction. Among older children and adults, we observed both a reduction in the incidence of serotype 6A IPD and an increase in the incidence of serotype 6C IPD of similar magnitude (table 1). These findings suggest that, at the population level, PCV7 provides cross-protection against 6A but not against 6C.

This conclusion is further supported by results of the in vitro opsonization studies. The protective effect of pneumococcal vaccine has been associated with the capacity of anticapsule antibodies to opsonize pneumococci in vitro [21]. We found that high percentages (70% and 95%) of PCV7-vaccinated children have opsonic indices of >20 against serotypes 6A and 6B. These opsonic indices should be sufficient to protect young children [21]. However, against serotype 6C, only 26% of the vaccinated children (5 of 19) had opsonic indices of >20. Furthermore, their median opsonic index against serotype 6C was 10–100 fold less than their median opsonic indices against serotypes 6A and 6B. Similar observations were made of adults in response to a routinely used PPV23. Thus, the in vitro opsonization studies also suggest that currently available pneumococcal vaccines provide little or no protection against serotype 6C.

Since the introduction of PCV7, the incidences of IPD caused by a few non-PCV7 serotypes have increased significantly [6, 22, 23]. Such increases have been most evident for serotype 19A, which accounted for 2.5% of IPD cases among children before 2000 and 36% of IPD cases in 2005 [24]. Among children <5 years old, rates of IPD caused by serotype 19A increased from 2.6 cases per 100,000 persons before PCV7 introduction to 8.9 cases per 100,000 persons in 2005 [6, 9]. The incidence of serotype 6C IPD among children <5 years old and among older children and adults is less than that for serotype 19A, but, similar to serotype 19A, the incidence of serotype 6C IPD has increased 2.5-fold between 1999 and 2006 among persons ≥5 years old (table 1). Whether this increase in serotype 6C IPD is causally associated with the introduction of PCV7 is unclear. A trend toward an increase in the incidence of serotype 19A IPD has been observed in association with antibiotic-resistant and antibiotic-susceptible pneumococcal clones [9, 25].

The difference in vaccine-induced cross-protection against serotypes 6A and 6C may reflect the presence of just 1 structural difference (i.e., rhamnose-ribitol linkage) between 6A and 6B polysaccharide but of 2 structural differences (i.e., rhamnose-ribitol linkage and glucose/galactose substitution) between 6C and 6B polysaccharide. However, the induction of cross-protective antibodies depends on the exact methods used to conjugate polysaccharide to protein [27]. Also, a pneumococcal vaccine already under development that contains 6A polysaccharide [28] may provide sufficient protection against serotype 6C as 6A polysaccharide elicits antibodies cross-reactive with 6C in animals [15]. Consequently, pneumococcal conjugate vaccines may vary in their abilities to protect against serotypes 6A, 6B, and 6C.

Another striking observation with PCV7 is its indirect protective effect on unvaccinated populations. Presumably, this occurs because PCV7 reduces nasopharyngeal colonization with pneumococci and subsequent transmission. This indirect effect, or herd immunity, has not been evident for serotype 6A [6, 29]. Our results suggest that this apparent lack of indirect cross-protection against serotype 6A among unvaccinated persons can be explained by the inability of classic serotyping methods to distinguish serotypes 6A and 6C. Using our monoclonal antibody assay, we observed a reduction in the incidence of serotype 6A IPD among older children and adults concomitant with a nearly equivalent increase in the incidence of serotype 6C IPD, the net effect of which was no change in the incidence of IPD caused by isolates characterized as serotype 6A by means of classic serotyping methods.
In considering cross-protection, one generally evaluates the production of cross-reactive antibodies but does not evaluate the presence of unidentified subtypes among the pneumococci of the cross-reactive serotype. Our experience with serotype 6C shows that the latter possibility must be considered. The presence of 6C might explain inconsistent findings by investigators evaluating the cross-protection to 6A among young children. Vaccine trials from Israel and Finland reported cross-protection [10–12], but a study from South Africa did not find such cross-protection [13]. A recent study performed with children (age, <18 years) in Philadelphia, Pennsylvania, suggested an increase in the incidence of serotype 6A IPD since the introduction of PCV7 [8]. The ambiguity may have arisen from the relatively small number of children evaluated in these studies. Alternatively, we speculate that serotype 6C might have been more prevalent in South Africa than in Europe (or in Philadelphia than in other parts of the United States) and that these studies identified serotype 6C as 6A, resulting in ambiguous cross-protection findings, owing to the failure to distinguish between 6C and 6A. Indeed, a recent abstract reported the presence of 6C in South Africa [30]. Distinguishing serotype 6C from 6A is essential if cross-protection is to be measured precisely.

Acknowledgments

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References