The Effect of 13-Valent Pneumococcal Conjugate Vaccine on the Serotype Distribution and Antibiotic Resistance Profiles in Children With Invasive Pneumococcal Disease

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Background. Invasive pneumococcal disease (IPD) continues to be a significant burden in children despite the implementation of two generations of conjugate vaccines. Serotype replacement by nonvaccine serotypes is reported in multiple areas around the world. This study is a continuation of previous studies and describes the incidence, serotype distribution, and antibiotic resistance pattern of Streptococcus pneumoniae serotypes causing IPD at Children’s Medical Center Dallas after introduction of 13-valent pneumococcal conjugate vaccine (PCV13).

Methods. Streptococcus pneumoniae isolates from normally sterile sites were collected from January 1, 1999 to June 30, 2014. Demographic and clinical information was extracted for analysis. Incidence of IPD was calculated using inpatient and emergency center admissions to Children’s Medical Center of Dallas as the denominator. Isolates were serotyped and penicillin/cefotaxime susceptibilities were determined. Selected nontypeable isolates were further characterized by multilocus sequence typing. A χ² test and the Cochran–Armitage Trend Test for trend analysis were used to evaluate change in serotype and antibiotic susceptibility patterns over time.

Results. Comparison of the different study periods showed a significant reduction in the incidence of IPD in PCV13 era compared with prevaccine era and PCV7 era (P < .05). Children younger than 24 months showed the largest reduction of disease incidence. More than 40% of patients with IPD had a documented comorbidity. Cases of pneumonia continued to decrease in the PCV13 era (P < .002). The most common non-PCV13 serotypes after vaccine introduction were as follows: 23B, 6C, 23A, 9N/L, and 12. Penicillin resistance by meningitis breakpoint decreased significantly in the PCV13 era.

Conclusions. After introduction of PCV13 in Dallas, incidence of IPD caused by strains contained in the vaccine and penicillin resistance continued to decrease. Serotype replacement phenomena and persistence of PCV7 serotypes were documented. Patients with comorbidities represented a large percentage of patients with IPD. Concerns for geographic variation in serotype replacement phenomena arise from the present study.

Keywords. Streptococcus pneumoniae; IPD; pneumococcal conjugate vaccine; serotype distribution.
Decline in the incidence of IPD by PCV13-related serotypes is a reasonable expectation based on epidemiologic data after PCV7 introduction. Multiple studies from different areas in the United States and in European countries have documented early effectiveness of PCV13 in the reduction of nasopharyngeal carriage and incidence of IPD caused by vaccine serotypes [3, 10–15].

Our previous reports about the epidemiology and evolution of IPD in Children’s Medical Center Dallas (CMCD) after PCV7 introduction documented a sustained IPD incidence reduction [1, 9]. The objective of the present study was to assess the incidence, serotype distribution, antibiotic resistance pattern, comorbidities, and viral coinfection of S pneumoniae serotypes causing IPD at CMCD after introduction of both PCV7 and PCV13 vaccines.

METHODS

Identification of Invasive Pneumococcal Disease
Streptococcus pneumoniae isolates causing IPD from January 1999 to June 2014 were collected from the CMCD microbiology laboratory by the same method described in previous report [9]. Invasive pneumococcal disease cases were defined as the isolation of S pneumoniae from a normally sterile site (eg, blood, pleural fluid, cerebrospinal fluid, joint fluid). The evidence of chest radiograph infiltrates and isolation of the bacteria from at least 1 of the following sites confirmed the diagnosis of pneumococcal pneumonia: blood, pleural fluid, or empyema. Because some cases of pneumonia can present with bacteremia, clinical syndromes were divided into isolated pneumonia, bacteremic pneumonia, and isolated bacteremia. Medical records of patients with culture-confirmed IPD at CMCD were reviewed for demographic and clinical data extraction. If a subject had recurrent IPD or multiple serotypes isolated, it was included in the analysis only once. All patient information was obtained in accordance with the Health Information Protection and Portability Act guidelines.

Study Time Definition
Three study periods were defined according to the time of introduction of PCV7 and PCV13 vaccines in Dallas: pre-PCV era (January 1999 to December 2000), PCV7 era (January 2001 to December 2010), and PCV13 era (January 2011 to June 2014).

Specimen Processing/Serotyping/Antimicrobial Susceptibility Testing
Pneumococcal isolates were serotyped by the capsular swelling method using commercially available rabbit antipneumococcal antisera (Statens Serum Institute, Copenhagen, Denmark). The pneumococcal strains that did not react with all pooled antisera were classified as nontypeable. Selected nontypeable isolates were analyzed by multilocus sequence typing (MLST) from 2000 to 2010, and then they were serotyped by using the antipneumococcal antisera based on the serotypes in the MLST database.

Antimicrobial susceptibility information was obtained from the CMCD microbiology laboratory using the standard breakpoints established by the Clinical and Laboratory Standards Institute. In January 2008, the Clinical and Laboratory Standards Institute published new nonmeningitis breakpoints for parenteral penicillin therapy [16]. Penicillin and cefotaxime breakpoints used in this study are the 2008 breakpoints by isolate location and meningitis breakpoint. From 1999 to 2002, susceptibilities to penicillin and cefotaxime were performed by the epsilometric test. Starting in 2003, antibiotic susceptibilities were determined by MICROSTREP microtiter method (Dade MicroScan Inc., West Sacramento, California), a microbroth dilution method using Mueller-Hinton media with 3% lysed horse blood.

Genetic Analysis of Nontypeable Isolates
Genetic identification of some nontypeable serotypes was determined by MLST. Multilocus sequence typing analysis was carried out according to previously published methods [17]. Internal fragments of the 7 housekeeping genes, aroE, gdh, gki, recP, spi, xpt, and ddl, were amplified by polymerase chain reaction (PCR) from chromosomal DNA obtained from these S pneumoniae isolates. The amplified PCR gene products were purified using the QIAGEN QIAquick PCR purification kit (QIAGEN Inc., Valencia, California), and then sequenced in both the 5’ and 3’ directions using the ABI Big Dye Terminator 3.1 Sequencer (Applied Biosystems Inc, Foster City, California), and analyzed on ABI capillary instruments by the University of Texas Southwestern Medical School’s McDermott Center Sequencing Core Facility.

Allele and sequence type assignments were made using the MLST Web site (http://spneumoniae.mlst.net). All alleles not already present in the pneumococcal MLST database were verified by resequencing the gene fragment on both strands.

Respiratory Viruses Detection
Polymerase chain reaction-based methods were used for identification of viruses. The Multi-Code Respiratory Virus Panel (Eragen Biosciences, Inc., Madison, Wisconsin) test was used from 2009 until February 2010 (influenza A, influenza B, respiratory syncytial virus [RSV], parainfluenza virus, adenovirus, human metapneumovirus, and rhinovirus). Then, the panel was changed to the xTAG Respiratory Viral Panel (Luminex Molecular Diagnostics, Austin, Texas) for detection of the following 8 viruses and subtypes: influenza A, influenza A subtype H1, influenza A subtype H3, influenza B, RSV, human metapneumovirus, rhinovirus, and adenovirus. After September 2012, the xTAG RVP was replaced by the FilmArray Respiratory Panel (Idaho Technologies, Salt Lake City, Utah), and the test was able to identify viruses (adenovirus, coronavirus HKU1, coronavirus
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NL63, coronavirus 229E, coronavirus OC43, human metapneumovirus, rhinovirus/enterovirus, influenza A, influenza A/H1, influenza A/H1–2009, influenza A/H3, influenza B, parainfluenza 1, parainfluenza 2, parainfluenza 3, parainfluenza 4, RSV) and several atypical bacteria (Bordetella pertussis, Chlamydia pneumoniae, and Mycoplasma pneumoniae).

Statistical Analysis
For purposes of our analysis serotypes not included in either PCV vaccine group were classified into 2 groups: nonvaccine serotype group and nontypeable group. Nonvaccine serotype group represented all serotypes not contained in PCV7 or PCV13. Nontypeable group included the bacterial isolates that were not typeable by either capsular swelling method or MLST. Descriptive analyses were performed using medians, ranges, frequency distributions, and percentages. Incidence of IPD was calculated for each year using the sum of all hospitalized and emergency center patients, identified by unique medical record number, as the denominator. The Z-test was used to compare these rates at 0.05 significance level. A χ² test and the Cochran–Armitage Trend test for trend analysis were used to test the change in serotype and antibiotic susceptibility patterns over time. The data were stratified into vaccine serotype and nonvaccine serotype invasive disease. Antimicrobial susceptibility patterns were determined for all strains. The association between serotypes, PCV7 serotypes and nonvaccine serotypes and the types of illnesses was determined by calculating odds ratios and 95% confidence intervals. SAS 9.3 software (SAS Institute Inc., Cary, North Carolina) was used for most of the statistical analyses.

Consent
This study was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center, Dallas and CMCD. Because this study did not require contact with patients or families, and because the identities of the patients were not revealed, informed consent was waived in accordance with the Institutional Review Board regulations.

RESULTS

Demographic Data
We identified 770 (52.3% male, 47.7% female) IPD cases at CMCD from January 1999 to June 2014 (Table 1). There were no cases of recurrent IPD during the study. Only 1 subject had invasive disease caused by 2 different serotypes: 14 and 19A. This patient was a 7-year-old female patient with Antley–Bixler syndrome and chronic lung disease who presented with bacteremia in 2014. Because both serotypes correspond to PCV13, they were included in the analysis as a single vaccine serotype. The median age of patients was 22 months (range, 0 months–18 years). The percentage of IPD cases in children younger than 24 months decreased consistently during the study time period (P = .008). In our study, 11% of all patients were 3 months of age or younger. The racial distribution showed the following: 260 (33.8%) children were Hispanic, 248 (32.2%) were White, 214 (27.8%) were Black, and 48 (6.2%) were classified as Other (Table 1).

Frequency of Disease
Analysis of the overall incidence of disease showed a significant decrease in IPD incidence rates (P < .0001) (Figure 1). When analysis was performed by study period, the difference in disease incidence between the PCV7 era and PCV13 era did not reach statistical significance (P < .11). Regarding the distribution of serotypes, PCV7 serotypes and nonvaccine serotypes continued to be an important cause of IPD in the PCV13 era (Figure 1).

Site of Infection
The principal illnesses observed during the study were as follows: isolated bacteremia 339 (44%), pneumonia 217 (28.2%), and meningitis 125 (16.2%). Analysis of IPD by site of infection during the 3 study periods did not show significant variation except for pneumonia and isolated bacteremia. Cases of

| Table 1. Demographics and Clinical Features of the Study Population by Study Period |
|---------------------------------------------------------------|
| Variable                | Pre-PCV Era 1999–2000 | PCV7 Era 2001–2010 | PCV13 Era 2011–2014* | Total   |
|-------------------------|------------------------|-------------------|----------------------|---------|
| (n)                     | (150)                  | (502)             | (118)                | (770)   |
| Age (months)            |                        |                   |                      |         |
| <24 Months              | 98 [65.3]              | 265 [52.6]        | 46 [38.9]            | 403 [52.3]|
| Gender (n [%])          |                        |                   |                      |         |
| Male                    | 65 [43.3]              | 279 [55.6]        | 59 [50]              | 403 [52.3]|
| Race (n [%])            |                        |                   |                      |         |
| Black                   | 60 [40]                | 125 [24.9]        | 29 [24.6]            | 214 [27.8]|
| Hispanic                | 37 [24.7]              | 174 [34.6]        | 49 [41.5]            | 260 [33.8]|
| White                   | 49 [32.6]              | 170 [33.8]        | 29 [25.6]            | 248 [32.2]|
| Other                   | 4 [2.7]                | 33 [6.7]          | 11 [9.3]             | 48 [6.2] |
| Diagnosis (n [%])       |                        |                   |                      |         |
| Isolated bacteremia     | 74 [49.3]              | 200 [39.8]        | 65 [55.1]            | 339 [44] |
| Pneumonia               | 38 [25.3]              | 159 [31.7]        | 20 [17]              | 217 [28.2]|
| Meningitis              | 22 [14.7]              | 83 [16.5]         | 20 [17]              | 125 [16.2]|
| Osteomuscular           | 9 [6]                  | 16 [3.2]          | 6 [5]                | 31 [4]  |
| Sinus/mastoid/ear       | 3 [2]                  | 32 [6.6]          | 5 [4.2]              | 40 [5.2] |
| Peritonitis             | 3 [2]                  | 10 [1.9]          | 2 [1.7]              | 15 [2]  |
| Cardiovascular          | 1 [0.7]                | 2 [0.3]           | 0 [0]                | 3 [0.4] |
| Comorbidity (n [%])     |                        |                   |                      |         |
| Yes                     | 49 [32.7]              | 212 [42.2]        | 68 [57.6]            | 329 [42.7]|
| Mortality (n [%])       | 6 [4]                  | 23 [4.6]          | 6 [5.1]              | 35 [4.5] |
| Vaccine serotype (n [%])|                        |                   |                      |         |
| PCV7                    | 71 [47.4]              | 84 [16.7]         | 29 [24.4]            | 184 [23.9]|
| PCV13                   | 17 [11.3]              | 239 [47.6]        | 47 [39.4]            | 303 [39.3]|
| Nonvaccine serotype     | 15 [10]                | 148 [29.5]        | 41 [34.5]            | 204 [26.5]|
| Nontypeable             | 0 [0]                  | 17 [3.4]          | 2 [1.7]              | 19 [2.4] |
| Nonavailable            | 47 [31.3]              | 14 [2.8]          | 0 [0]                | 61 [7.9] |

Abbreviations: IPD, invasive pneumococcal disease; PCV, pneumococcal conjugate vaccine.
*Until June 2014. In addition, in 2014, 2 different serotypes caused IPD in 1 patient.
pneumonia during PCV7 era were 31.7% compared with 17% during PCV13 era. \( P < .002 \). The opposite effect was observed for cases of isolated bacteremia with an increase from 39.8% to 55.1% between PCV7 and PCV13 eras \( P < .004 \).

**Changes in Serotype Distribution**

Pneumococcal isolates from 690 (89.6%) of the 770 patients included in the study were available for serotyping. Forty-seven (58.8%) of the missing samples were from 1999 and 2000 when the isolates were retrospectively collected from CMCD microbiology laboratory freezers. Twenty-nine serotypes were characterized by MLST between 2000 and 2010.

Analysis of each vaccine group by study time period showed that PCV7 serotypes decreased during PCV 7 era but continued to cause IPD in the PCV13 era. During the PCV13 era, 29 cases of IPD were caused by PCV7 isolates. Serotype 19F (38%) was the most common isolate in this group, followed by serotype 14 (31%), 6B (13.8%), 23 F (13.8%), and 9 V (3.4%). Patients with comorbidities represented 58.6% of these cases. Of the 41.4% of patients without known underlying medical conditions, 50% were patients younger than 6 months of age at the time of presentation.

Seroype 19A was the most common vaccine serotype isolated after PCV7 introduction. However, analysis of individual frequencies of vaccine serotypes in the PCV13 era showed that the most significant change noted in serotype distribution was the progressive reduction in the frequency of 19A isolates. The most common non-PCV13 serotypes identified were 23B \( n = 13, 30.2\% \), 6C \( n = 11, 25.5\% \), 23A \( n = 10, 23.3\% \), 9N/9L \( n = 3, 7\% \), and 12 \( n = 2, 4.7\% \).

**Antibiotic Susceptibility of Pneumococcal Isolates**

The resistance patterns of isolates to penicillin and cefotaxime by study time period are shown in Table 2. Two different breakpoints were included: isolate location breakpoint and meningitis breakpoint. Penicillin resistance interpreted by meningitis breakpoint has significantly decreased from the pre-PCV era (53.9%) to the PCV13 era (35.3%) \( P < .015 \). These results contrast with the pattern of resistance described by isolate location breakpoint. Resistance to penicillin according to that breakpoint increased significantly from pre-PCV era to PCV13 era from 0% to 8.2% \( P < .008 \). Cefotaxime susceptibility did not show a significant change between pre-PCV era and PCV13 era for either breakpoint.

**Comorbidities**

Three hundred twenty (41.6%) study patients had a documented comorbidity. The most common group of comorbidities included the following: malignancy (17.5%), history of prematurity (12.5%), genetic disorders (10.9%), renal disease...
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Table 2. Antibiotic Susceptibility to Penicillin and Cefotaxime by Study Time Period

| Antibiotic | Time Period | Susceptibility (%) |
|------------|-------------|-------------------|
| Penicillin | Pre-PCV Era | S | I | R |
|            |             | 96.7 | 3.3 | 0 |
| Meningitis breakpoint | 46.1 | 53.9 |
| PCV7 Era | 90.8 | 8.6 | 0.6 |
| Meningitis breakpoint | 51.1 | 48.9 |
| PCV13 Era | 88.2 | 3.4 | 8.2 |
| Meningitis breakpoint | 64.7 | 35.3 |
| Cefotaxime | Pre-PCV Era | 91.3 | 8 | 0.7 |
| Meningitis breakpoint | 72.1 | 18.6 | 9.3 |
| PCV7 Era | 91 | 6.8 | 2 |
| Meningitis breakpoint | 80.4 | 10.2 | 9.4 |
| PCV13 Era | 98.3 | 1.7 | 0 |
| Meningitis breakpoint | 90.5 | 7.8 | 1.7 |

Abbreviation: PCV, pneumococcal conjugate vaccine.

(9.1%), and asthma (7.5%). When information was analyzed by study period, no statistically significant variations were noted within the above-mentioned groups. The number of patients with sickle cell anemia who developed IPD during the study decreased from 7 (14.3%) in the pre-PCV era to 3 (4.5%) in the PCV13 era (P < .09). The majority of the patients who had comorbidity developed infection caused by a serotype included in PCV13 (36.6%) compared with PCV7 era (24%). It is interesting to note that the phenomenon of serotype replacement is more evident in the subgroup of patients with comorbidities, with nonvaccine serotypes causing 33.1% of the cases of IPD compared with 26.5% in the rest of the cohort (P < .05).

Viral Coinfection

Widespread use of viral respiratory panel was introduced in our hospital in 2009. Of the 56.1% of patients tested in 2009 and 2010 for viral coinfection, 46.8% had a positive result. For the PCV13 era, 43.7% had viral testing, with 60% of those results returning positive. The overall percentage of patients with IPD diagnosis and a viral coinfection was 21%. The most common respiratory viruses isolated were rhinovirus/enterovirus (26.2%), RSV (23%), and rhinovirus (18%).

DISCUSSION

Our study provides an epidemiologic description of IPD in Dallas, Texas, using continuous epidemiologic surveillance in infants and children hospitalized at CMCD before, during, and after the implementation of both PCV vaccines. Data on the immunogenicity of PCV13 vaccine were obtained from several noninferiority studies using PCV7 for comparison [18]. It has been described in the literature that even a single dose of PCV7 is able to elicit antibody response [18]. This response is presumed to be higher once PCV13 is given because of immunologic priming. Study of the kinetics on immune responses to PCV vaccines have shown comparable antibody persistence for the 7 common serotypes in cases of combined immunization. In addition, children with previous PCV13 immunization had higher antibody responses for the 6 additional serotypes compared with patients vaccinated with PCV7 alone [19].

It is interesting to note that in vitro cross-reactivity between S pneumoniae serotypes does not necessarily indicate clinical protection [13]. For example, antibodies produced by PCV7 immunization against 19F have produced limited cross-protective immunity against serotype 19A [18]. Functional analysis of antibody production regarding PCV13 showed that this vaccine could provide enhanced protection against serotypes included in the vaccine and certain serotypes such as 6C when compared with PCV7 [18]. Our data provide information about the persistence of PCV7 serotypes in the PCV13 era. The most frequent PCV7 serotypes in our population were 19F (38%) and 14 (31%). These findings contrast with recently reported IPD surveillance data [3, 7, 11, 20, 21]. However, our data are similar to findings of Loughlin et al [14] regarding PCV serotypes causing colonization. They describe that PCV7 serotypes persisted at low prevalence in their population in the following order of frequency: 19F, 23F, 18C, 14, and 6B.

Comparison of trends on IPD after the introduction of each vaccine must be done cautiously. Vaccine uptake for PCV7 and PCV13 was different because PCV7 was a new vaccine at introduction, and it took several years to reach vaccine uptake rates above 80% [4]. In addition, soon after PCV7 was licensed, several publications raised attention to the phenomenon of serotype replacement [5–7, 13]. There are data suggesting that the distribution of S pneumoniae serotypes in the community can evolve independently of the vaccine. This points to the importance of natural variations in serotype distribution unrelated to the vaccine and the ability of certain serotypes to fill the new ecologic niche. Natural trends in pneumococcal serotype colonization and the effect of antibiotic use should also be considered [22]. Observational studies have provided most of the available data regarding the serotype replacement. It is important to consider possible methodology issues such as ecologic and sampling bias that could affect the surveillance systems used by those studies. At the same time, changes in laboratory methods used in clinical practice or epidemiologic reporting tools can potentially influence observed changes in IPD incidence [22].

After PCV7 introduction, the nonvaccine serotypes that were the most commonly isolated in pneumococcal pneumonia and empyema in children in France were as follows: 1, 3, 7F, and 19A [11]. Serotype 19A has been the most common
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The PCV13 era already shows progressive reduction in the frequency of this isolate. Zuccotti et al [13] reported 6C as the most frequent serotype causing IPD in Italy after PCV13 introduction. Serotype 6C has also been reported as a cause of IPD in the United States [8]. In our study, frequency of 6C isolates reached 25.5% of nonvaccine serotypes in the PCV13 era. Epidemiologic data from France suggests a strong impact of PCV13 on pediatric community-acquired pneumonia cases among bacterial isolates in the Boston area [26]. On the other hand, Olarte et al [12] found that penicillin and ceftriaxone minimum inhibitory concentrations were similar between the pre- and post-PCV eras.

Several studies reporting the effectiveness of PCV13 continue to document IPD cases in children with underlying medical conditions [5, 21]. Our data shows that the most common comorbidities listed included the following: malignancy, history of prematurity (chronic lung disease), and asthma. It is important to consider that although the difference of IPD incidence between study periods did not reach statistically significance, cases of IPD in the PCV13 era were more common in patients with comorbidities. We consider that phenomena as an indirect indicator of vaccine effectiveness. Likewise, cases of IPD in patients with sickle cell anemia trended down in our cohort. This is most likely a consequence of PCV vaccination enforced in this population.

Conclusion: The present study shows some limitations. Approximately 10% of pneumococcal isolates were not available for typing or were found to be nontypeable. Most of those samples belong to the pre-PCV era (1999–2000). We began prospective collection of samples from the CMCD microbiology laboratory late in 2000. In addition, MLST was not available starting 2011. However, only 2 isolates were classified as nontypeable during the PCV13 period. Our study was based on results from a single tertiary referral center, and hence, our findings may not be generalizable to other areas of the country. Children’s Medical Center Dallas is the major referral center in the area, covering most of the pediatric population of the Dallas area. However, there are other medical centers, which were not included in the study, and the incidence of IPD could be overestimated or underestimated. Finally, although we could not associate vaccination status of individuals to development of IPD, we can point out that average PCV vaccination rates were slightly lower in the Dallas area compared with other areas of surveillance in the country. These findings could potentially contribute to some of the differences contained in this study in comparison with other reports.

CONCLUSIONS

Our findings show an overall reduction of IPD after introduction of PCV13 in the Dallas area. Penicillin resistance among bacterial isolates and occurrence of IPD caused by serotype 19A are decreasing as well. Interestingly, we documented persistence of PCV7 serotypes in the PCV13 era. The group with the most significant reduction of IPD corresponded to
children younger than 24 months with the highest impact by clinical syndrome seen in the pneumonia group. Patients with comorbidities represented a significant percentage of patients with IPD. Additionally, concerns for geographic variation in serotype replacement phenomena arise from the present study. Continuing careful monitoring through national and international population-based IPD surveillance is necessary to determine the long-term impact of PCV13. Groups of emerging serotypes (including persistence of PCV7 serotypes), changes in antimicrobial susceptibility, and possible geographic differences in serotype distribution should be addressed in future studies.

Notes
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