Epidemiology and Evolution of Invasive Pneumococcal Disease Caused by Multidrug Resistant Serotypes of 19A in the 8 Years After Implementation of Pneumococcal Conjugate Vaccine Immunization in Dallas, Texas

Chonnamet Techasaensiri, MD, Allison F. Messina, MD,*‡ Kathy Katz, BS,* Naveed Ahmad, MD, MPH,† Rong Huang, MS,† and George H. McCracken, Jr., MD**

Background: The heptavalent pneumococcal conjugate vaccine (PCV7) has significantly reduced vaccine-type invasive pneumococcal disease (IPD) in children. An increasing percentage of IPD cases are now caused by nonvaccine serotypes. The purpose of our observational study was to define the epidemiology of pneumococcal disease in Dallas, TX children for 8 years after implementation of PCV7 immunization.

Methods: *Streptococcus pneumoniae* isolates from normally sterile sites were collected at Children's Medical Center of Dallas from January 1, 1999 to December 31, 2008. 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 and cefotaxime susceptibilities were determined. Serotype 19A isolates were further characterized by multilocus sequence typing.

Results: Compared with the prevaccine period of 1999–2000, there was a significant reduction in the incidence of IPD from 2002 to 2008 ($P < 0.05$), although a significant increase in IPD incidence was observed from 2006 to 2008 ($P = 0.038$). The number of IPD cases caused by serotype 19A increased from 1999 to 2008 ($P < 0.001$). There were significant increases in penicillin and cefotaxime nonsusceptibile 19A isolates during this 10-year period ($P < 0.001$ and $P = 0.004$, respectively). The most common sequence type (ST) of the 19A isolates was ST-199 (42.7%). Clonal complex (cc-156) and cc-320 emerged in the period of 2005–2008 as penicillin and cefotaxime resistant 19A strains.

Conclusions: In Dallas, PCV7 immunization reduced significantly the incidence of IPD caused by vaccine-type strains. A significant increase in IPD caused by serotype 19A was observed. The penicillin and cefotaxime nonsusceptible STs, not previously identified in Dallas, have recently become an important cause of IPD.

Key Words: pneumococcus, serotype, invasive pneumococcal disease, 10-year experience after pneumococcal vaccine

(*Pediatr Infect Dis J* 2010;29: 294–300)

Streptococcus pneumoniae (SP) is a common cause of invasive infections worldwide especially in infants and children. In early 2000, the heptavalent pneumococcal conjugate vaccine (PCV7; Prevnar, Wyeth Lederle Vaccines) was licensed for use in infants and young children in the United States.[1] At the time of its introduction, the 7 serotypes included in PCV7 (4, 6B, 9V, 14, 18C, 19F, and 23F) accounted for approximately 80% of invasive pneumococcal disease (IPD) in children less than 4 years of age in the United States.[2,3] The introduction of PCV7 resulted in a dramatic decrease in the overall rate of IPD.[4–8] Recent studies, however, have shown an increased percentage of IPD cases caused by nonvaccine serotypes (NVTs).[6,8–15] In the United States, the incidence of IPD caused by 19A isolates has increased among vaccinated children, and these isolates can be resistant to commonly used antimicrobials.[6,11–15] In addition, the emergence of multidrug-resistant clones of SP serotype 19A has been reported.[16–18] We used multilocus sequence typing (MLST) to characterize invasive 19A strains isolated from children admitted to Children's Medical Center of Dallas (CMCD) to determine the association between antibiotic susceptibility pattern and sequence types.

Since our initial report that covered the period of 1999 to 2005,[6] we have collected data on IPD for 3 additional years at CMCD. These data have allowed us to assess the incidence, serotype distribution, and antibiotic resistance of isolates causing IPD in Dallas and to determine potential serotype coverage of investigational PCVs that comprise a greater number of serotypes.

METHODS

Identification of IPD

SP isolates from January 2006 to December 2008 from normally sterile sites were prospectively collected from the CMCD microbiology laboratory by the same method as mentioned in the previous report.[6]

Specimen Processing/Serotyping/Antimicrobial Susceptibility Testing

Pneumococcal isolates were serotyped by the capsular swelling method using commercially available rabbit antipneumococcal antisera (Statens Serum Institut, Copenhagen, Denmark). The pneumococcal strains that did not react with all pooled antisera were classified as nontypeable (NT). Thirty-two of 33 NT isolates from 2006 to 2008 were determined by MLST, and then were serotyped by using the antipneumococcal antisera based on the serotypes in the MLST database. One NT isolate in 2007 was not viable for MLST. 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 breakpoints for parenteral penicillin therapy for nonmeningitis.[19] To maintain consistency among all the isolates, the penicillin and cefotaxime breakpoints used in the analysis of this study are the "parenteral nonmeningeal" breakpoints, regardless of the site of isolation. From 1999 to

Accepted for publication September 22, 2009.
From the *Department of Pediatrics, Division of Infectious Diseases, University of Texas Southwestern Medical Center, Dallas, TX; †Children's Medical Center, Dallas, TX; and, currently, ‡All Children's Hospital, Saint Petersburg, FL.

Address for correspondence: Chonnamet Techasaensiri, MD, Ramathibodi Hospital, Mahidol University, 270 Rama VI Rd, Rajathevi, Bangkok 10400, Thailand. E-mail: chonmet@yahoo.com.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

ISSN: 0891-3668/10/2904-0294
DOI: 10.1097/INF.0b013e3181c2a229

2002 susceptibilities to penicillin and cefotaxime were performed by the epsilometric test (E-test). From 2003 to 2008 susceptibilities were determined by MICroSTREP microtiter method (Dade MicroScan Inc, West Sacramento, CA), a microbroth dilution method using Mueller-Hinton media with 3% lysed horse blood.[20]

Review of Medical Records

Medical records of patients with culture-confirmed IPD at CMCD were reviewed for demographic and clinical data. All patient information was obtained in accordance with the Health Information Protection and Portability Act guidelines.

Serotype Classification

For purposes of our analyses, NVTs represented all serotypes not contained in the PCV7 vaccine, including vaccine-related serotypes (6A, 6C, 9A, 9L, 9N, 18A, 18B, 18F, 19A, 19B, 19C, 23A, and 23B).

Genetic Analysis of 19A Isolates

Genetic relatedness of 19A serotypes was determined by multilocus sequence typing. MLST sequencing analysis was carried out according to previously published methods.[21] 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 19A SP isolates. The amplified PCR gene products were purified using the Qiagen QIAquick PCR purification kit (Qiagen Inc, Valencia, CA) and then sequenced in both the 5′ and 3′ directions using the ABI Big Dye Terminator 3.1 Sequencer (Applied Biosystems Inc, Foster City, CA) and analyzed on ABI capillary instruments by the University of Texas Southwestern Medical School's McDermott Center Sequencing Core Facility.

Allele and sequence type (ST) assignments were made using the Multi Locus Sequence Typing Web site (available at: http://spneumoniae.mlst.net). All alleles not already present in the pneumococcal MLST database were verified by resequencing the gene fragment on both strands. Assignment to clonal complexes was performed using the eBURST v3 software program (available at: http://eburst.mlst.net).[22] The eBURST program compared our SP19A MLST allelic profiles with those from the MLST SP reference database and assigned a primary founder ST to the member of the groups which contained the most alleles present within that group. The primary founder STs were defined as being the ST that has the greatest number of single-loci variants. The eBURST analysis shows single-, double-, and triple-locus variants of the founder STs and determines the relatedness of strains within a clonal group. Isolates in the group defined by eBURST were considered to belong to a single clonal complex (CC) when sharing of alleles at $\geq$5 of the 7 loci.

Further analysis of relationships between our SP19A pneumococcal isolates was examined by concatamerizing the DNA sequences of the 7 alleles by using the software (available at: www.mlst.net) and aligning the resulting sequences using the ClustalW analysis in the MEGA 3.1 software.[23] A phylogenetic neighbor-joining tree was prepared from the sequence alignment data using the MEGA 3.1 software program to show clonal relationship and evolutionary analysis.[23]

Statistical Analysis

Descriptive analyses were performed by using medians, ranges, frequency distributions, and percentages. Incidence rates of IPD were calculated for each year using the sum of all hospitalized and emergency center patients, identified by unique medical record number, as the denominator. Z-test was used to compare these rates at 95% confidence level. χ^2 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 VT and NVT disease, and antimicrobial susceptibility patterns determined for all strains. The association between serotypes and the types of illnesses were determined by calculating odds ratios and 95% confidence intervals. SAS 9.1 software (SAS Institute Inc, Cary, NC) 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, consent was waived in accordance with the Institutional Review Board regulations.

RESULTS

Demographics

We identified 538 (52.4% male, 47.6% female) cases of IPD from January 1999 to December 2008 (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A291). The median age was 23 months (range, 1 month to 18 years). The percentage of IPD cases in children younger than 24 months decreased significantly from 63.3% in 1999–2000 to 41.4% in 2008 ($P = 0.007$). Of them, 184 children (34.2%) were Hispanic, 164 children (30.5%) were white, and 164 (30.5%) were black. Distribution by race or ethnicity revealed over-representation of black and white children with IPD compared with the general hospital population (24.7% and 19.2%, respectively), and an under-representation of IPD in Hispanic children compared with the general population (48.7%) ($P < 0.001$).

Frequency of Disease

Because implementation of PCV7 immunization began at the end of 2000 in Dallas, we used data from 1999 and 2000 to represent the prevaccination period for our calculations. Compared with 1999–2000, there was a significant reduction in the incidence of IPD from 2002 to 2008 (Fig. 1). By contrast, there was a significant increase in incidence of IPD from 2006 (44 cases/100,000 CMCD patients) to 2008 (68.4 cases/100,000 CMCD patients) ($P = 0.038$).

Distribution of Serotypes

Pneumococcal isolates from 478 (89%) of the 538 patients identified with IPD were available for serotyping. Seventy-eight percent of the missing samples were from 1999 and 2000 when the isolates were retrospectively collected from CMCD microbiology laboratory freezers.

Disease caused by VT decreased significantly whereas that caused by NVT increased significantly from 1999 to 2008 ($P < 0.001$) (Figs. 2, 3). For serotype 19A isolates, the incidence rose significantly from 3.8 cases of 100,000 patients in 1999–2000 to 22.4 cases of 100,000 patients in 2008, an increase of 83.5% ($P < 0.001$) (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A292). Disease caused by serogroup 7 increased significantly from 1999 to 2008 ($P = 0.003$).

Types of Illnesses

The principal illnesses observed during the study were bacteremia, pneumonia, and meningitis. There was no significant difference in the percentages of these 3 illnesses by year.

From 1999 to 2008 pneumonia and meningitis caused by 19A increased significantly ($P < 0.001$ and 0.004, respectively). Additionally, bacteremia caused by 19A, 23A, and 7 increased significantly ($P = 0.002$, $P = 0.004$, $P < 0.001$, respectively) from 1999 to 2008.

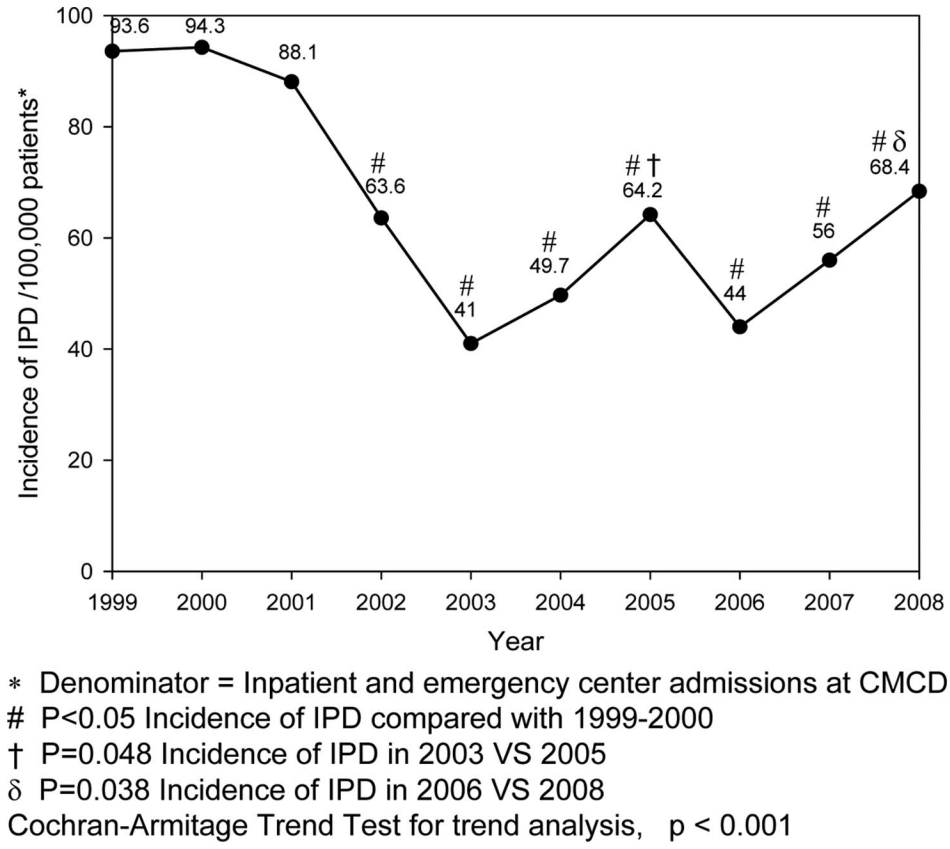


FIGURE 1. Annual incidence of invasive pneumococcal disease at Children's Medical Center Dallas, TX from 1999 to 2008.

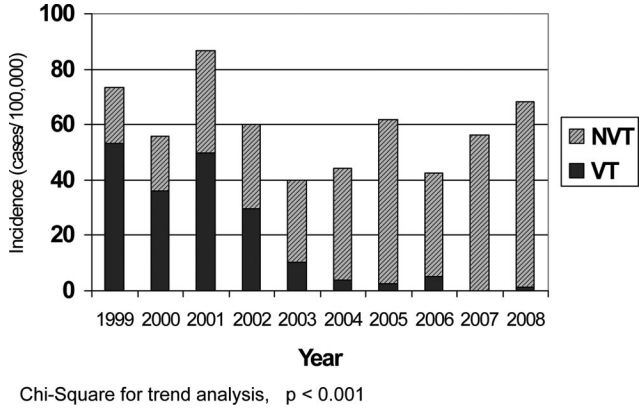


FIGURE 2. Incidence of invasive pneumococcal disease caused by vaccine (VT) and nonvaccine (NVT) serotypes at Children's Medical Center Dallas, Texas, from 1999 to 2008. Incidence based on cases per 100,000 emergency room and direct hospital admissions by year.

Antimicrobial Resistance of the Pneumococcal Isolates

The percentage of pneumococcal isolates that were penicillin nonsusceptible increased significantly from 1999 to 2008 ($P < 0.001$). Of the 141 isolates tested in 1999–2000, 5 (3.5%) were penicillin nonsusceptible compared with 8 of the 50 isolates (13.8%) in 2008 ($P = 0.019$). Cefotaxime susceptibility did not change significantly during 10-year study period. The antimicrobial susceptibility profile for 96 19A isolates recovered from 1999 to 2008 showed that penicillin and cefotaxime susceptibilities decreased significantly ($P < 0.001$ and $P = 0.005$, respectively).

Molecular Analysis of 19A Isolates

Of the 96 19A isolates collected, 89 (92.7%) were available for molecular analysis. The most common sequence type by MLST was 199, representing 38 of 89 (42.7%) of the strains. Between 1999 and 2008, the number of ST-199 isolates increased significantly but the proportion of ST-199 isolates among all strains decreased significantly ($P < 0.001$). Additionally, 23 sequence types not found during 1999–2003 were identified between 2004 and 2008. Of these, 8 (35%) were resistant to penicillin and cefotaxime (penicillin MIC $\geq$4, cefotaxime MIC $\geq$2) including ST-156 (n = 1), ST-320 (n = 3), ST-1451 (n = 2), ST-2432 (n = 2), ST-2514 (n = 2), ST-3425 (n = 2), ST-4153 (n = 1), and ST-4154 (n = 1). These 8 STs are closely related as demonstrated on the dendrogram (Fig. 4). Fourteen 19A isolates recovered between 1999 and 2008 were new to the MLST database. eBURST analysis of the 8 sequence types that were resistant to penicillin and cefotaxime belonged to 2 CCs-156 and 320 (Fig., Supplemental Digital Content 3, http://links.lww.com/INF/A293). ST-4153 is a double locus variant of ST-320 but could not be demonstrated by eBURST because there were no intermediate single locus variants descendent from ST-320 to ST-4153 identified in the MLST database.

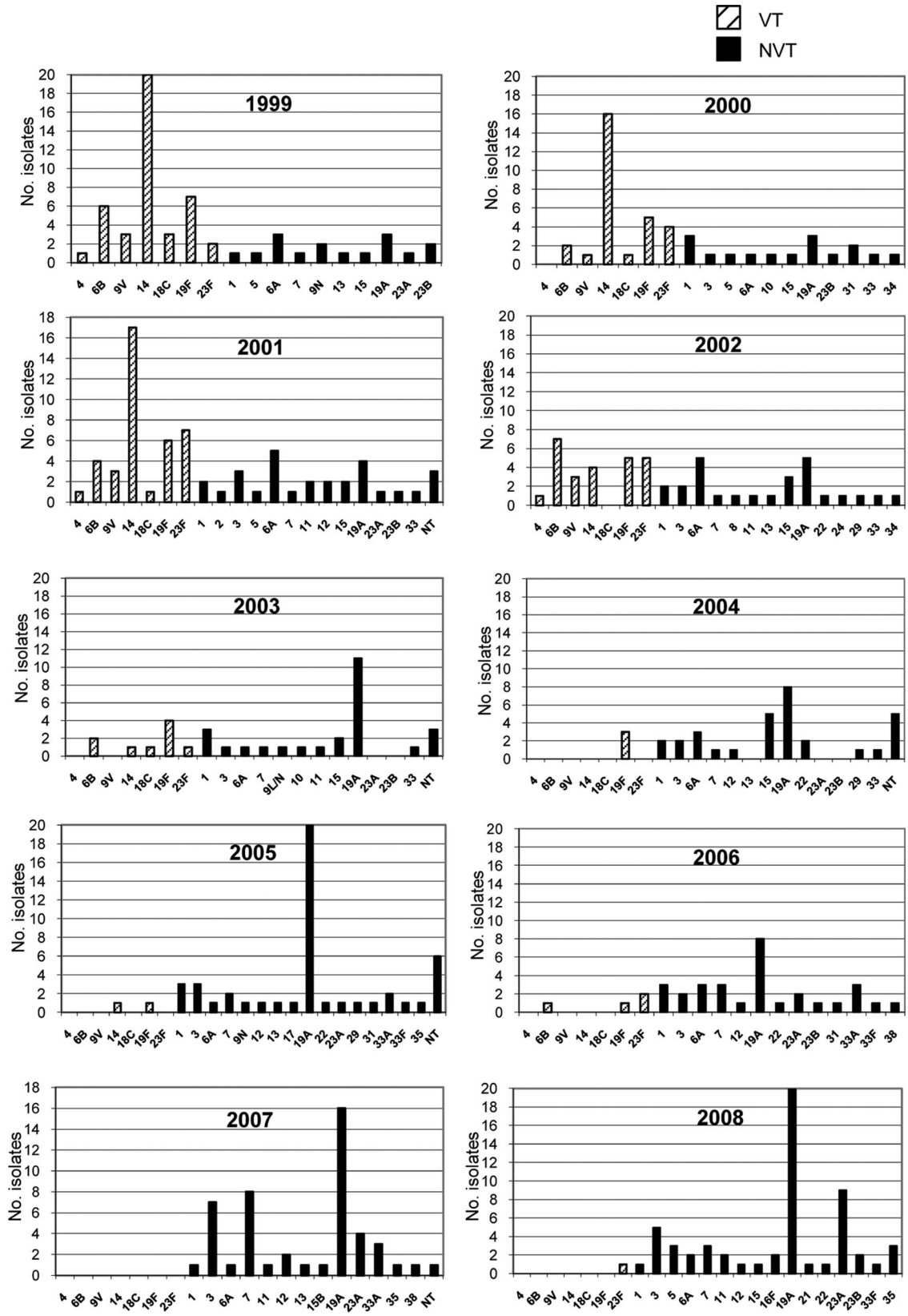
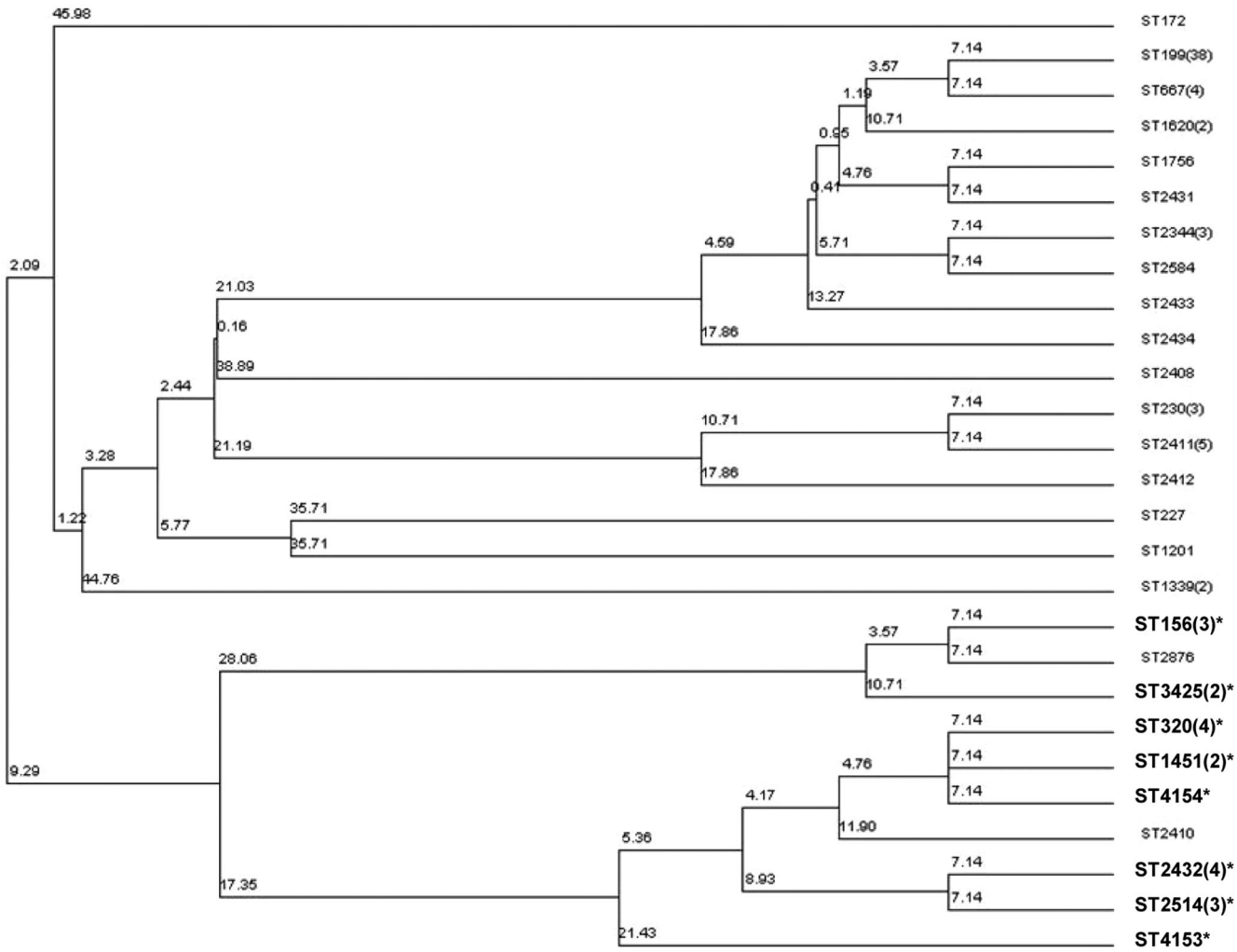


FIGURE 3. Number of patients with invasive pneumococcal disease caused by vaccine (VT) and nonvaccine (NVT) serotypes at Children's Medical Center Dallas, Texas, from 1999 to 2008.



* Penicillin and cefotaxime non-susceptible strains

FIGURE 4. Phylogenetic tree of MLST allele sequences of 19A isolates 1999–2008.

DISCUSSION

The pneumococcal conjugate vaccine has significantly reduced the incidence of IPD among Dallas children, especially that caused by the 7 serotypes contained in the vaccine. These data expand on our earlier observations[6] of a significant increase in NVTs causing disease, particularly 19A strains and are consistent with other reports.[7,12–14,16] The incidence of IPD in Dallas in 2006–2008 increased and the overall incidence of IPD in 2008 was significantly greater than that in 2006 and was associated with NVTs, principally 19A. Of the 19A isolates, both penicillin and cefotaxime nonsusceptibility increased significantly from 1999 to 2008. Others have reported a similar increase in both penicillin and cefotaxime nonsusceptibility among 19A strains after the introduction of PCV7.[11,16,24] Although cross-immunity is thought to extend to other related serotypes within the same serogroup except for serogroup for 19,[25] we found an increase in the incidence of pneumococcal bacteremia caused by serotype 23A in 2006–2008 suggesting that PCV7 offered limited cross-immunity between serotypes 23F and 23A.

Since the introduction of PCV7, most penicillin and cefotaxime resistant pneumococci are 19A strains.[11,14–18] In Dallas, penicillin-, and cefotaxime-resistant pneumococci were non ST-199 and limited to CC-156 (ST-156 and ST-3425) and CC-320 (ST-320, ST-1451, ST-2432, ST-2514, ST-4153, ST-4154). We observed that the ST-199 isolates persisted with penicillin MICs between 0.1 and 1.0 μg/mL, a finding that is consistent with a previous report.[16] In addition ST-156 and ST-320 have been previously reported to be associated with multidrug resistant 19A isolates.[6,16] By comparison with the MLST database, ST-156 has been reported to express different capsular serotypes, 9V, 11A, 14, and 19F whereas ST-320 has been reported to express serotypes 19F and 19A only. The increase in resistance among the CC-156 could be associated with capsule switching whereas the increase in resistance among the CC-320 could have resulted from clonal expansion, acquired resistance, or capsule switching.[11,26,27]

It is possible that the incidence of IPD could be further reduced by including additional serotypes in the vaccine. For example, a 10-valent PCV conjugated to *Haemophilus influenzae* glycoprotein-D (PCV10), adding serotypes 1, 5, and 7F to those contained in PCV7 has been licensed in several countries and would theoretically cover 19% and 14% of our isolates in 2007 and 2008, respectively. The CRM_{197} 13-valent PCV (PCV13), which

adds serotypes 3, 6A, and 19A to those contained in PCV10[28,29] would cover 68% and 60% of our isolates recovered in 2007 and 2008, respectively.

Our study has several limitations. First, the denominator we used to determine the incidence of disease was derived from the inpatient and emergency room populations at CMCD. The incidence rates calculated from our Dallas population are not comparable to those from studies in which the denominator is the general population. We were unable to use the latter as our denominator because there are 2 other hospitals in the Dallas area to which pediatric patients from the same catchment area as CMCD seek treatment. We could not assume, therefore, that all patients in the area with IPD were managed at our hospital. Most of our patients with IPD managed at CMCD were either directly admitted to the hospital or were treated in our emergency department. Accordingly, we believe that the using the total number of patients admitted to the emergency department and to the hospital directly represents a denominator that was inclusive of most patients in our area requiring medical attention for IPD at CMCD. Although the incidence rates in this study are unique to our CMCD population only, the changes in the rates during this 10-year study period in Dallas are similar to those derived from the general population.[2,4,6] Second, we did not begin prospectively collecting SP isolates from the CMCD laboratory until late 2000. Although we were able to identify all IPD cases and susceptibility information on the SP isolates from 1999 to 2000, we were unable to determine the serotype from 47 of the 150 patients from those 2 years. A third limitation of our study is that because the vaccination records were often incomplete, we were unable to report the vaccination status of the patients in the study. Finally, we used the capsular swelling method for pneumococcal serotyping. We may not be able to identify the new serotype 6C because the serotype 6C is reportedly indistinguishable from serotype 6A by this method.[30]

Based on our prospective observational study, the epidemiology of pneumococcal disease in Dallas, Texas has substantially changed in the past 10 years as a result of several factors, including natural variability of pneumococcal serotype ecology,[31] continued widespread use of antibiotics, and universal immunization with PCV7. These data do not allow us to determine which of these factors had the greatest impact on our population. Continued surveillance of IPD in areas where the conjugate vaccine is widely used will be important to identify changes in the serotypes and susceptibilities of pneumococci causing disease.

ACKNOWLEDGMENTS

The authors thank Shari Young for her assistance in procuring the pneumococcal isolates.

REFERENCES

1. Advisory Committee on Immunization Practices. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2000;49(RR-9):1–35.
2. Black S, Shinefield H, Fireman B, et al; Northern California Kaiser Permanente Vaccine Study Center Group. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J*. 2000;19:187–195.
3. Hausdorff WP, Bryant J, Paradiso PR, et al. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, Part I. *Clin Infect Dis*. 2000;30:100–121.
4. Whitney CG, Farley MM, Hadler J, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N Engl J Med*. 2003;348:1737–1746.
5. Kyaw MH, Lynfield R, Schaffner W, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med*. 2006;354:1455–1463.
6. Messina AF, Katz-Gaynor K, Barton T, et al. Impact of the pneumococcal conjugate vaccine on serotype distribution and antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates in Dallas, TX, children from 1999 through 2005. *Pediatr Infect Dis J*. 2007;26:461–467.
7. Centers for Disease Control and Prevention. Invasive pneumococcal disease in children 5 years after conjugate vaccine introduction–eight states, 1998–2005. *Morb Mortal Wkly Rep*. 2008;57:144–148.
8. Talbot TR, Poehling KA, Hartert TV, et al. Reduction in high rates of antibiotic-nonsusceptible invasive pneumococcal disease in tennessee after introduction of the pneumococcal conjugate vaccine. *Clin Infect Dis*. 2004;39:641–648.
9. Kaplan SL, Mason EO Jr, Wald ER, et al. Decrease of invasive pneumococcal infections in children among 8 children's hospitals in the United States after the introduction of the 7-valent pneumococcal conjugate vaccine. *Pediatrics*. 2004;113(3 pt 1):443–449.
10. Byington CL, Samore MH, Stoddard GJ, et al. Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the intermountain west: emergence of nonvaccine serogroups. *Clin Infect Dis*. 2005;41:21–29.
11. Pai R, Moore MR, Pilishvili T, et al. Postvaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *J Infect Dis*. 2005;192:1988–1995.
12. Beall B, McEllistrem MC, Gertz RE Jr, et al. Pre-and postvaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. *J Clin Microbiol*. 2006;44:999–1017.
13. Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med*. 2009;360:244–256.
14. Hicks LA, Harrison LH, Flannery B, et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. *J Infect Dis*. 2007;196:1346–1354.
15. Emergence of antimicrobial-resistant serotype 19A *Streptococcus pneumoniae*–Massachusetts, 2001–2006. *Morb Mortal Wkly Rep*. 2007;56:1077–1080.
16. Pelton SI, Huot H, Finkelstein JA, et al. Emergence of 19A as virulent and multidrug resistant pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. *Pediatr Infect Dis J*. 2007;26:468–472.
17. Pichichero ME, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *JAMA*. 2007;298:1772–1778.
18. Xu Q, Pichichero ME, Casey JR, et al. Novel type of *Streptococcus pneumoniae* causing multidrug-resistant acute otitis media in children. *Emerg Infect Dis*. 2009;15:547–551.
19. CLSI. Performance standards for antimicrobial susceptibility testing: 18th informational supplement M100-S18. Wayne, PA: CLSI; 2008.
20. Jorgensen JH, McElmeel ML, Crawford SA. Evaluation of the Dade MicroScan MICroSTREP antimicrobial susceptibility testing panel with selected *Streptococcus pneumoniae* challenge strains and recent clinical isolates. *J Clin Microbiol*. 1998;36:788–791.
21. Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiology*. 1998;144(pt 11):3049–3060.
22. Feil EJ, Li BC, Aanensen DM, et al. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol*. 2004;186:1518–1530.
23. Kumar S, Tamura K, Nei M. MEGA3: integrated software for Molecular Evolutionary Genetics analysis and sequence alignment. *Brief Bioinform*. 2004;5:150–163.
24. Mera R, Miller LA, Fritsche TR, et al. Serotype replacement and multiple resistance in *Streptococcus pneumoniae* after the introduction of the conjugate pneumococcal vaccine. *Microb Drug Resist*. 2008;14:101–107.
25. Lee H, Nahm MH, Burton R, et al. Immune response in infants to the heptavalent pneumococcal conjugate vaccine against vaccine-related serotypes 6A and 19A. *Clin Vaccine Immunol*. 2009;16:376–381.
26. Porat N, Arguedas A, Spratt BG, et al. Emergence of penicillin-nonsusceptible *Streptococcus pneumoniae* clones expressing serotypes not present in the antipneumococcal conjugate vaccine. *J Infect Dis*. 2004;190:2154–2161.

27. Hanage WP, Huang SS, Lipsitch M, et al. Diversity and antibiotic resistance among nonvaccine serotypes of *Streptococcus pneumoniae* carriage isolates in the post-heptavalent conjugate vaccine era. *J Infect Dis*. 2007;195:347–352.
28. Peters TR, Poehling KA. Invasive pneumococcal disease: the target is moving. *JAMA*. 2007;297:1825–1826.
29. Shouval DS, Greenberg D, Givon-Lavi N, et al. Serotype coverage of invasive and mucosal pneumococcal disease in Israeli children younger than 3 years by various pneumococcal conjugate vaccines. *Pediatr Infect Dis J*. 2009;28:277–282.
30. Park IH, Pritchard DG, Cartee R, et al. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. *J Clin Microbiol*. 2007;45:1225–1233.
31. Black S. The volatile nature of pneumococcal serotype epidemiology: potential for misinterpretation. *Pediatr Infect Dis*. 2009;28.