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Emergence of 19A as Virulent and Multidrug Resistant *Pneumococcus* in Massachusetts Following Universal Immunization of Infants With Pneumococcal Conjugate Vaccine

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Background: The long-term effects of selective pressure from conjugate pneumococcal vaccine on the serotype distribution and antimicrobial resistance of carriage and invasive isolates of *Streptococcus pneumoniae* are unknown. Early changes demonstrate a reduction in vaccine serotypes and an increase in nonvaccine serotypes (NVT) among both carriage and invasive isolates. Ongoing surveillance is necessary to identify emerging invasive serotypes and antimicrobial susceptibilities.

Methods: Enhanced surveillance of invasive pneumococcal disease in Massachusetts began in October 2001 and remains ongoing. Isolates from children less than 5 are sent to the Massachusetts Department of Public Health and subsequently to the Maxwell Finland laboratory for serotyping and determination of antimicrobial susceptibility. Annual incidence rates for vaccine serotype and NVT disease are calculated using 2000 census data.

Results: NVT caused 72%–91% of invasive pneumococcal disease annually in children less than 5 years of age between 2002 and 2005. Serotype 19A has emerged as the most frequent cause of IPD in Massachusetts. A multidrug-resistant clone (ceftriaxone, amoxicillin, azithromycin and trimethoprim-sulfamethoxazole) (MLST 320) was first identified in Massachusetts in 2005.

Conclusions: Three years after the introduction of pneumococcal conjugate vaccine for universal administration to children less than 2 in Massachusetts, a significant increase in invasive disease due to serotype 19A was observed. Although MLST 199 remains the most frequent sequence type among invasive isolates (of 19A), a multidrug-resistant sequence type, not previously identified in Massachusetts, has become an important cause of invasive disease. Further surveillance of the changing ecology of *S. pneumoniae* is necessary

as a 4-year time period is not sufficient to fully evaluate the impact of PCV of pneumococcal infections.

Key Words: immune pneumococcal disease, antimicrobial resistance, serotype 19A

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Universal immunization of infants with pneumococcal conjugate vaccine (PCV7) has markedly decreased the incidence of invasive pneumococcal disease (IPD) in children and adults as well as reduced the proportion of invasive isolates with decreased susceptibility to penicillin.^{1–3} Immunization has also had a dramatic impact on nasopharyngeal carriage of *Streptococcus pneumoniae* (SP) with a decline in carriage of vaccine serotypes and replacement with nonvaccine serotypes (NVT).^{4–7} Nevertheless, the long-term effects of selective pressure from antimicrobial use and conjugate pneumococcal vaccine on the serotype distribution and antimicrobial resistance of carriage and invasive isolates of SP are unknown. Replacement with NVT in the nasopharynx of asymptomatic individuals and capsular switching among vaccine serotypes to nonvaccine capsular serotypes have both been documented and raise concerns about the long-term success of capsular vaccine strategies for prevention of both invasive and local disease.^{4–7}

Success of the conjugate vaccine depends, in part, upon the relative inability of NVT to cause disease. Among vaccine serotypes, some, such as 14, have a high potential to cause invasive disease,^{8,9} whereas others, such as 23F, do not. Similarly, some NVT have begun to emerge as the cause of invasive disease occurring primarily in selected populations such as HIV infected adults and children under 5 years of age.^{10–14} These studies have reported the emergence of serotype 19A SP with reduced susceptibility to penicillin as an increasingly common cause of invasive disease.¹⁰ Although 19A is related to serotype 19F, which is included in the vaccine, cross protection appears very limited. Because the majority of NVT have been uncommon in the prevaccine population, additional information on the ability of these serotypes to cause invasive disease is needed. In contrast, there is good evidence that in local disease syndromes such as otitis media,

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differences among serotypes in their ability to cause disease are relatively small¹⁴ and hence replacement in the nasopharynx with NVT is likely to offset to some extent the decline in disease due to the decrease in vaccine serotypes.¹⁵ These observations emphasize the importance of ongoing surveillance of NVT.

Surveillance studies which focus on IPD isolates are insufficient to observe changes in the ecology of the nasopharynx, and fail to distinguish between strains that are extensively carried but have low potential for causing invasive disease from those carried on a more limited basis but with high capacity for invasion. Rates of greater than 20 cases of IPD per 100,000 acquisitions with serotypes 4, 14, 7F, 9V and 18C compared with 1 or fewer cases of IPD with serotypes 15A, B, and C, 11A, 33F, and 22F have been reported.¹⁶ Statewide surveillance for IPD in children less than 5 years old in Massachusetts enabled us to identify serotype 19A as a rapidly increasing cause of invasive disease. In addition we used multilocus sequence typing (MLST) to characterize the population of invasive 19A strains in Massachusetts to determine whether disease and/or antibiotic susceptibility was associated with the 19A capsular type, in general, or limited to one or several clones.

METHODS

Pneumococcal Isolates. Enhanced statewide surveillance for IPD was initiated in October 2001.¹⁷ All clinical microbiology laboratories in Massachusetts were requested to provide all SP isolates recovered from sterile sites from children less than 18 years of age to the Massachusetts Department of Public Health. Serotyping was performed on available isolates at the Maxwell Finland Laboratory for Infectious Diseases at Boston Medical Center, using the quellung reaction with pneumococcal antisera (Danish Statens Serum Institute, Copenhagen, Denmark). Serotypes 4, 6B, 9V, 14, 18C, 19F and 23C were classified as vaccine serotypes (VST) and all other serotypes as NVT. Antimicrobial susceptibility was determined by E test. Standard Clinical and Laboratory Standards Institute (CLSI) susceptibility cutoffs were used to classify organisms as susceptible, intermediate, or resistant to penicillin (sensitive: ≤ 0.06 ; intermediate: 0.12–1.0; resistant: ≥ 2.0), ceftriaxone (sensitive: ≤ 0.5 ; intermediate: 1.0; resistant: ≥ 2.0), azithromycin (sensitive: ≤ 0.5 ; intermediate: 1.0; resistant: ≥ 2.0), and trimethoprim/sulfamethoxazole (sensitive: $\leq 0.5/9.5$; intermediate: 1/19–2/38; resistant: $\geq 4/76$).

Multilocus Sequence Typing. MLST was performed as previously described.¹⁸ Allele assignments were made using the MLST website (www.spneumoniae.mlst.net). All alleles not already present in the pneumococcal MLST database were verified by resequencing the gene fragment from both strands. For serotypes in which a novel sequence type (ST)/serotype combination was observed when compared with published datasets, or where the ST/serotype combination was not previously reported in the MLST database, the strain was reserotyped and, if necessary, STs of the isolates were verified by resequencing of the MLST loci.

Statistical Analysis. Incidence rates for vaccine and NVT were derived using IPD case numbers as numerator values

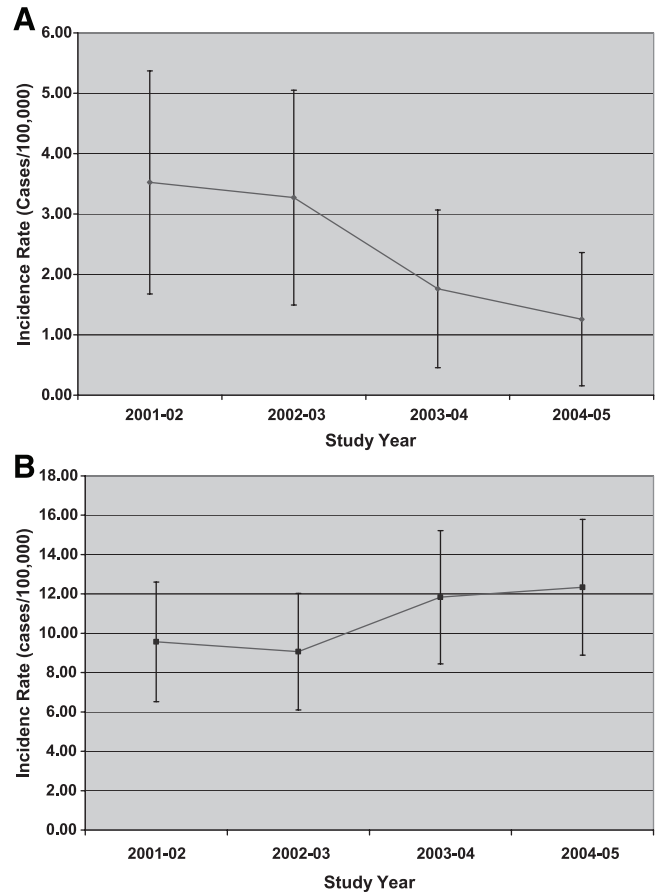


FIGURE 1. Annual incidence rate of invasive pneumococcal disease due to vaccine serotypes (A) and nonvaccine serotypes (B) in children less than 5 years in Massachusetts (annual incidence rate and 95% confidence intervals).

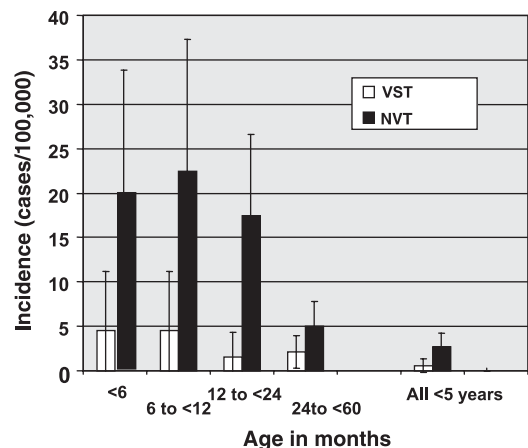


FIGURE 2. Mean incidence (cases per 100,000 population) of invasive pneumococcal disease by age group in Massachusetts: 2002–2005 (mean and 95% confidence interval).

and Census 2000 estimates of the Massachusetts population as denominators. Statistical analyses were conducted using Microsoft Access 2000 and SAS, version 9.1. Ninety-five

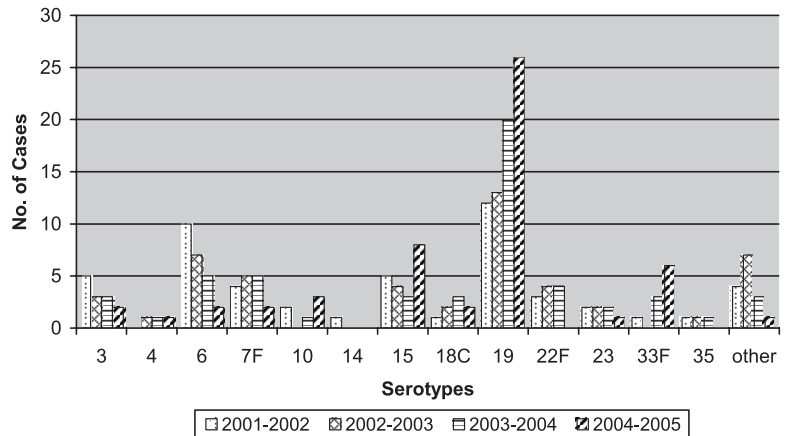


FIGURE 3. Serogroup/serotype specific cases of IPD in children less than 5 years of age in Massachusetts 2002–2005.

percent confidence intervals (CIs) were calculated, and two-sided *P* values that were less than 0.05 were considered statistically significant. Mantel–Haenszel χ^2 test for trend used to identify significant changes in serotype over time.

RESULTS

Figure 1A describes the incidence of IPD in children less than 5 years of age by vaccine serotypes and Figure 1B describes the incidence of IPD in children less than 5 years of age by NVT (IPD due to NVT increased from 73.7% of cases to 90.6% between October 2001 and September 2005). The average 2002–2005 incidence rate of NVT disease was greater than VST disease among all age cohorts (Fig. 2). Figure 3 details the serotype distribution of the 208 IPD cases in children less than 5 years of age in Massachusetts by year from October 2001 through October 2005. Serotype 19A was identified in 27% of cases of IPD among children less than 5 years of age between 2002 and 2005. The number and proportion of cases due to serotype 19A significantly increased over time, reaching 24 (44%) cases in 2005 (*P* < 0.001, Table 1), whereas 19F disease remained stable.

MLST analysis of IPD isolates of serotype 19A from 2001 to 2002 demonstrated limited diversity of invasive strains as only 5 MLST were present and the dominant MLST was 199 (58%). In 2004–2005, the diversity of serotype 19A had expanded to 11 sequence types. Although 199 still represented the most frequent sequence type (45.5%), it was no longer a majority of the 19A isolates. These data suggest broad expansion of the 19A population, not limited to a specific clone, concurrent with introduction of PCV7.

In 2002 through 2004 the majority of isolates of serotype 19A demonstrated intermediate susceptibility to penicillin and full susceptibility to ceftriaxone and amoxicillin (Table 3). A single multidrug resistance (MDR) 19A isolate was observed before 2005. Four 19A isolates (24%) were identified with MIC equal or greater than 2 $\mu\text{g/mL}$ to ceftriaxone that were also resistant to penicillin, amoxicillin, azithromycin and trimethoprim/sulfamethoxazole. These MDR SP were not present among the most prevalent sequence types (ST199, ST 63) but were limited to sequence type (320) (Table 1) not previously identified in the community.

DISCUSSION

Consistent with reports from the Centers for Disease Control and Prevention, we observed the emergence of 19A as a major cause of invasive disease in children in Massachusetts.⁸ Serotype 19A was identified from nearly 30% of all episodes of IPD from children less than 5 years of age in Massachusetts between October 2001 and September 2005 and 44% of cases in 2005. In Massachusetts, the pneumococcal population colonizing healthy children has undergone substantial shifts subsequent to PCV7 introduction, with expansion of 19A also seen among colonizing strains.⁷ Three possible explanations for the increasing prevalence of 19A as a cause of IPD can be considered: the expansion of sequence types existing before vaccine introduction (ST199, ST63), the introduction of sequence types that were not present in the

TABLE 1. Proportion of Invasive Pneumococcal Disease in Massachusetts Due to Serotype 19A and 19F in Children Less Than 5 Years of Age

| Cases | 2002* No. (%) | 2003* No. (%) | 2004* No. (%) | 2005* No. (%) | 2002–2005 No (%) | <i>P</i> |
|--------|------------------|------------------|------------------|------------------|---------------------|----------|
| 19A | 6 (11.5) | 8 (16) | 19 (36) | 24 (44) | 57 (27) | <0.001 |
| 19F | 6 (11.5) | 5 (10) | 1 (2) | 2 (4) | 14 (6.7) | NS |
| Total† | 52 | 49 | 53 | 54 | 208† | |

*From October 1 of the previous year through September 30 of designated year.

†Number of cases from which *S. pneumoniae* was isolated from sterile site and isolate available for serotyping.

TABLE 2. MLST Distribution of Serotype 19A Isolates of *S. pneumoniae* in Massachusetts by Year*

| Serotype/Serogroup | 2002* | 2003* | 2004* | 2005* |
|--------------------|-------------------------------------|---------------------------------------|--|--|
| 19A | 63 199 (×4) 667 | 199 (×3) 667 2081 | 199 (×8) 230 415 667 (×3) 695 1673 2081 | 199 (×7) 320 (×5) 695 1201 2081 2082 2083 |

ST present in multiple years are in bold type.

*From October 1 of the previous year through September 30 of designated year.

TABLE 3. Antimicrobial Susceptibility Among Invasive Isolates of Serotype 19A: October 2001–September 2005*

| 19A | Penicillin | | Ceftriaxone | | Azithromycin | | Amoxicillin | |
|---------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | MIC ₅₀ | MIC ₉₀ | MIC ₅₀ | MIC ₉₀ | MIC ₅₀ | MIC ₉₀ | MIC ₅₀ | MIC ₉₀ |
| 2002 (N = 6) | 0.125 | 0.25 | 0.047 | 0.125 | 0.38 | >256 | 0.094 | 0.125 |
| 2003 (N = 7) | 0.25 | 0.6 | 0.125 | 0.8 | 0.625 | >256 | 0.19 | 0.8 |
| 2004 (N = 19) | 0.19 | 0.75 | 0.125 | 0.38 | 0.38 | 0.75 | 0.19 | 0.38 |
| 2005 (N = 24) | 0.125 | 6 | 0.094 | 2 | 0.25 | >256 | 0.125 | 6 |

*MIC₅₀ and MIC₉₀ in $\mu\text{g/mL}$.

earliest samples (since these have been previously reported within the MLST database, we assume they are migrants from neighboring communities or were very rare prior to PCV7⁷), and capsule switching. Before 2000, only vaccine serotypes had been identified within ST230 and ST695 (one example of ST230 and 2 isolates of ST 695 were found with capsule type 19A in our collection). All 3 events, expansion of existing clones, introduction of new clones, and capsule switching have contributed to the observed trend.

A given serotype of SP may be a frequent cause of IPD either as a result of widespread colonization with a low invasive potential, or modest colonization with a high invasive potential. Little is known about the invasive potential of 19A and the current study design does not enable us to define the invasive potential for 19A. Hausdorff identifies 19A as common among nasopharyngeal isolates from children and among antibiotic resistant serotypes, but not among isolates commonly recovered from children with pneumococcal meningitis.¹⁸ Hanage reported that serotypes 19A SP had greater invasive potential greater than serotype 19F isolates.⁹ In the Finish OM study, increased cases of pneumococcal otitis due to serotypes 33, 35 and 38, but not 19A were observed in the vaccine cohort.¹⁸ Our data suggest that replacement disease may not be observed until several years after the introduction of vaccine; therefore, the absence of 19A as a replacement serotype in otitis media may reflect the time period for follow-up and not the capacity of 19A to cause replacement otitis media. A similar delay in emergence of replacement invasive disease has been reported among Alaskan natives (Singleton, R, personal communication).

We observed that isolates of ST199 persisted with intermediate resistance to penicillin, whereas ST 320 emerged as a MDR invasive isolate. Among 19A isolates with ST320, the MIC₅₀ to ceftriaxone was 3 $\mu\text{g/mL}$ (range 2–8 $\mu\text{g/mL}$) and the MIC₉₀ was 8 $\mu\text{g/mL}$. Four isolates were observed in children less than 5 years old during 2005 and 2 additional isolates in children 5 to 18 years old during the same year. As the breakpoint (CLSI defined MIC at or above which isolates are considered resistant) for ceftriaxone for noncentral nervous system infection is 4.0 $\mu\text{g/mL}$, these isolates may be a potential challenge to conventional therapy for the entire spectrum of pneumococcal disease including pneumonia. It will be critical to continue to monitor the frequency of disease because of this specific ST, as well as the effectiveness of current treatment paradigms for disease due to multidrug-resistant SP. Examples of 19A MDR ST 320 from other

locations are present within the MLST database [(www.spneumoniae.mlst.net; a website that contains all reported MLST/serotype combinations] suggesting that such isolates were migrants to the community or were previously very rare. Whether the MDR phenotype has facilitated emergence of 19A in the vaccinated communities studied here remains to be determined. Continuing studies of the changing ecology of SP are necessary as the 4-year time period of data collection reported here is not yet sufficient to determine whether the pace of diversification among SP isolates has been altered subsequent to the introduction of PCV7. Further studies are likely to reveal new nuances about virulence of SP, mechanisms for acquisition of resistance, and identification of serotypes or sequence types that require specific prevention strategies.

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