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Emergence of *Streptococcus pneumoniae* Serogroups 15 and 35 in Nasopharyngeal Cultures from Young Children with Acute Otitis Media

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Abstract

Background—Surveillance of children with acute otitis media (AOM) for nasopharyngeal colonization with *Streptococcus pneumoniae* before, during, and after the introduction of 7-valent pneumococcal conjugate vaccine (PCV7) indicated the near-complete elimination of PCV7 strains and the emergence of pneumococcal serotype 19A.

Methods—To determine effects of the introduction of 13-valent pneumococcal conjugate vaccine (PCV13) on pneumococcal nasopharyngeal colonization, we obtained nasopharyngeal cultures from 228 children 6 through 23 of age months presenting with a new episode of AOM during 2012 and 2013 and enrolled in an ongoing clinical trial of antimicrobial efficacy. All children had received at least 2 doses of PCV13. The *S. pneumoniae* isolates were subjected to serotyping and testing for antimicrobial susceptibility. We compared the findings with results obtained in three earlier studies.

Results—We found nasopharyngeal colonization with *S. pneumoniae* in 113 (50%) of the children with AOM. PCV7 and PCV13 serotypes accounted for 2% and 12%, respectively of the pneumococcal isolates. Of the 14 PCV13 isolates, 8 were serotype 19A. Nonvaccine serotypes accounted for 69% of the isolates. Most frequently occurring were subtypes of serotype 15 (23%) and serotype 35B (9%). Overall, 33% of the isolates were penicillin-nonsusceptible, a proportion not significantly different from proportions found in our three earlier studies (26%, 36%, and 37%, respectively). Serotypes 15 and 35B accounted for 51% of penicillin-nonsusceptible isolates.

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Conclusion—Expansion of contents of pneumococcal vaccine administered to children is followed by not-fully-predictable changes in nasopharyngeal pneumococcal colonization. Continued surveillance is required to help inform future vaccine development.

Keywords

AOM; Streptococcus pneumoniae; serotypes; vaccine; nasopharyngeal colonization

Introduction

Streptococcus pneumoniae remains a major cause of acute otitis media (AOM) and the major cause of invasive infection (e.g. bacteremia and meningitis) in young children. Acquisition of nasopharyngeal (NP) colonization with *S. pneumoniae* constitutes the first step in the pathogenesis of both superficial and invasive pneumococcal disease (IPD), and continuing NP carriage of *S. pneumoniae* serves as a reservoir for its spread. Of particular concern regarding *S. pneumoniae* has been an increase in the levels of resistance to penicillin among isolates of the organism. By the late 1990s, more than 30% of isolates were penicillin-nonsusceptible, with more than 20% of these isolates considered highly resistant.[1]

The introduction of PCV7 led to a dramatic decrease in the incidence of IPD in the pediatric population,[2,3] and also to a modest reduction in the incidence of AOM.[4,5] Routine use of PCV7 was also followed by a decrease in IPD among non-vaccinated children and adults, presumably the result of herd immunity.[6] In parallel with these changes was a shift in the serotypes of *S. pneumoniae* found to cause both IPD and AOM, with PCV7 serotypes having been almost completely eliminated and replaced by other serotypes. Notable among the changes was a sharp increase in the proportion of all isolates that were serotype 19A.[7-10]

It was hoped that the introduction of PCV7 would also result in reduced rates of pneumococcal resistance to penicillin by reducing the colonization with resistant serotypes. However, rates of penicillin resistance among non-PCV7 serotypes generally have not differed significantly from the relatively high rates observed among the PCV7 serotypes. [7-9, 11]

These developments were largely responsible for the recommendation in 2010 for routine use of an expanded, 13-valent conjugate vaccine (PCV13).[12] PCV13 includes all of the PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) along with serotypes 1, 3, 5, 6A, 7F and 19A, which were selected to provide protection against the “replacement” serotypes found to be responsible for most cases of IPD and of pneumococcal AOM after the approval of PCV7. With the introduction of PCV13, it was anticipated that the additional serotypes included would target both the replacement serotypes and the penicillin-nonsusceptible isolates that had been increasingly recovered from children with pneumococcal illness. To date, however, information concerning such effects of the routine use of PCV13 has been limited. [13-15]

In the present report we describe findings concerning serotypes and levels of penicillin resistance in NP isolates of *S. pneumoniae* in a group of children with newly diagnosed AOM whom we studied between January 2012 and March 2013, early after use of PCV13

became routine. We relate these findings to findings we had reported earlier concerning three similar groups of children who were enrolled in studies during three separate periods, respectively: Group 1, prior to routine PCV7 vaccination (1999-2000);[16] Group 2, early after the introduction of PCV7 (2003-2005);[7] and Group 3, late after use of PCV7 became routine (2006-2009).[17]

Three sets of findings among those children seemed particularly noteworthy. First, in the NP cultures obtained at the time of the children's study entry episode of AOM, the proportions of pneumococcal isolates that were PCV7 or PCV7-related serotypes declined progressively from 61% (52/85) in Group 1 to 15% (21/140) in Group 3. Second, increases occurred in the proportion of pneumococcal isolates that were Serotype 19A, from 5% (4/85) in Group 1, to 0% in Group 2, to 25% (35/140) in Group 3, and in the proportion of 19A isolates that were penicillin-nonsusceptible (0% and 69% in Groups 1 and 3, respectively). And third, serotypes that later would be incorporated in PCV13 accounted for 20% of pneumococcal isolates in Group 1, 21% in Group 2, and 40% in Group 3, while the proportion of serotypes not represented in either PCV7 or PCV13 increased progressively, accounting for 19%, 36%, and 45% of pneumococcal isolates in Groups 1, 2, and 3, respectively.[7,16,17]

Methods

Data for the present report were derived from children 6 to 23 months of age with AOM who were enrolled in an ongoing randomized clinical trial comparing the efficacy of 5 vs. 10 days of treatment with amoxicillin-clavulanate. The children were studied between January 1, 2012 and March 31, 2012 and October 1, 2012 and March 31, 2013, and are referred to in the present report as Group 4. We obtained written, informed consent from the children's parent(s) before undertaking any research procedures, and the study was approved by the University of Pittsburgh and Western Institutional Review Boards. We recruited children from the Primary Care Center of the Children's Hospital of Pittsburgh of UPMC, its affiliated Pediatric PittNet Practice-Based Research Network; and a pediatric practice in rural Kentucky. We excluded children with Down syndrome, a craniofacial abnormality, known immunodeficiency, or indwelling tympanostomy tube(s). All children were required to have received at least 2 doses of PCV13.

Procedures for examining children, diagnosing AOM, obtaining and processing NP specimens, and statistical analysis have been described in detail previously.[7] In brief, examinations were conducted by validated otoscopists using pneumatic otoscopy. The diagnosis of AOM was based on the presence of middle-ear effusion accompanied by one or more of the following: ear pain, marked redness of the tympanic membrane, and substantial bulging of the tympanic membrane. NP specimens were obtained in standard fashion at the time of initial diagnosis of AOM--i.e., at enrollment in the trial-- and were processed using standard techniques by the same laboratory personnel as for Groups 1, 2, and 3, under the direction of one of the authors (MG).

Pneumococcal isolates resistant to oxacillin (1μg) by disk diffusion were further tested against penicillin and other antimicrobials by microdilutional susceptibility testing methods according to the most current Clinical Laboratory Standards Institute (CLSI) guidelines[18]

(other susceptibility data not reported here). Strains with penicillin MIC ≥0.1 μg/mL were defined as penicillin-nonsusceptible. Of such strains, those with >0.1 μg /mL and MIC ≤1.0 μg/mL were considered intermediate and those with MIC >1.0 μg/mL were considered resistant. (These are the 2008 revised CLSI breakpoints for non-meningitis illnesses being treated orally, chosen because children in the parent study are treated with oral antibiotics. The same breakpoints had been used to compare results from study Groups 1, 2 and 3.) Pneumococcal isolates were typed by the Quellung reaction using serotype-specific antisera (Statens Serum Institute, Copenhagen, Denmark). Some isolates whose serotypes would have placed them into a designated serogroup containing ≤5 isolates were not further typed and were characterized only by their group identity. An isolate of each identified serotype/serogroup was sent to a reference laboratory for confirmation of the results.

Statistical Analyses

We used chi-square tests to test for differences between the Groups in selected demographic characteristics, and a logistic regression model to compare the Groups regarding the distribution of *S. pneumoniae* serotypes and penicillin susceptibility. We conducted tests for trend using the Cochran-Armitage test for trend in binomial proportions. We performed all analyses using two-tailed tests, with statistical significance set at $P<0.05$. For all analyses we used SAS software (Version 9.3) (SAS/STAT® 9.3 User's Guide. Cary, NC: SAS Institute Inc.).

Results

Demographics

Group 4 comprised 228 children. Of the total, 48% were aged 6 through 11 months, 29% were aged 12 through 17 months, and 23% were aged 18 through 23 months; 54% were male; 52% were Caucasian and 39% were African-American; 20% had a history of recurrent AOM; 68% were in households with one or more other children; 23% were exposed to household cigarette smoke; 54% were in day care; 65% were enrolled in a public health insurance program; and the mothers of 87% were high school or college graduates. There were no statistically significant differences in any demographic characteristic between the children in Group 4 and the children in Group 3. All of the children in Group 4 had received at least 2 doses of PCV13, and 90% had received 3 or more doses.

Pneumococcal serotypes

S. pneumoniae was found in 50% (113/228) of the NP cultures in Group 4 children. Table 1 shows the distribution of the pneumococcal isolates according to serotype, vaccine relationship, and penicillin susceptibility. PCV7 and PCV13 serotypes accounted for 2% (2/113) and 12% (14/113), respectively of the pneumococcal isolates. Of the 14 PCV13 isolates, 8 were serotype 19A. Nonvaccine serotypes accounted for 69% (78/113). Predominant were serotype 15 (all subtypes) and serotype 35B, which accounted for 33% (26/78) and 13% (10/78), respectively, of the nonvaccine isolates and for 23% (26/113) and 9% (10/113), respectively, of all the pneumococcal isolates.

Penicillin susceptibility

Overall, of the 113 pneumococcal isolates, 37 (32.7%) were penicillin-nonsusceptible; 23 (20.3%) were penicillin-intermediate and 14 (12.4%) were penicillin-resistant. Here also, serogroup 15 and 35B predominated, accounting for 18 (49%) of the penicillin-nonsusceptible isolates. Details concerning penicillin susceptibility of the various subtypes of serotypes 15 and 35 are shown in Table 2. Most (70%) of the 35B isolates had a penicillin MIC of 2 μg/mL and a ceftriaxone MIC of 1 μg/mL. In addition, six isolates of serotype 19A had a penicillin MIC of ≥4 μg/mL.

Comparison of the four Groups

Salient features of the comparison between the Groups are summarized in Table 3. The key findings were: in each Group a similar percentage of children demonstrated *S. pneumoniae* by NP culture at the time of diagnosis of AOM and the overall percentage of isolates that were nonsusceptible did not differ over time, however, the proportions that were PCV7 serotypes declined progressively.

Discussion

The findings reported here extend our previous observations of pneumococcal NP colonization in young children with AOM to encompass a 14-year period before and since the introduction and widespread use of PCV7, and subsequently, of PCV13. Over that period, we found no significant change in the prevalence of pneumococcal colonization, but significant changes in pneumococcal serotype distribution. Noteworthy among these were an increase in the prevalence of serotype 19A isolates, followed by a decrease, and the near elimination, after the introduction of PCV13, of all PCV7 serotypes, as noted in Group 4. In keeping with our findings, Pichichero and Casey (2007), Huang et al (2009), and Wroe et al (2012) noted an increase in the prevalence of serotype 19A after the introduction and use of PCV7, [8,1920] while Cohen et al (2012) noted a decline in its prevalence after the introduction and use of PCV13.[13] Similarly, Kaplan et al. (2013) noted a sharp decline, following general use of PCV13, in the prevalence of serotype 19A among children hospitalized with IPD.[14]

Of particular interest in our Group 4 children was the prevalence of nonvaccine serotypes that previously had been noted relatively infrequently--particularly serotypes 11A, 15A/B/C, and 35B. The clinical significance of this is not yet known. Kaplan et al. (2013) had found these serotypes in small numbers of children with IPD. [14] Recently, an increase in the prevalence of those serotypes was reported among children in France aged 6 to 24 months with AOM who were “adequately PCV13 vaccinated”-- i.e., had received two doses of PCV13 before age 1 year or at least one dose after age 1 year. In those children, Cohen et al. found that serotypes 11A, 15A/B/C, and 35B together accounted for 21% (100/486) of all pneumococcal isolates recovered from NP cultures at the time of diagnosis of AOM.[13] By comparison, in our Group 4 children those same serotypes together accounted for 39% (44/113) of all pneumococcal isolates ($P < 0.01$). For each of those serotypes, the prevalence we found was, to our knowledge, greater than any that have been reported previously.

Notwithstanding the changes that have occurred in the relative proportions of specific serotypes among the pneumococcal isolates recovered from these children over the 14-year period of the present report, the overall proportion of NP isolates from the first study episode of AOM that have been penicillin-nonsusceptible has remained relatively unchanged, with a range of 26% to 37% ($P = 0.38$). Other investigators in other regions have reported generally similar values and results. Finkelstein et al. found that, among Massachusetts children less than 7 years old examined at well-child or illness visits in 2001, 33% of 166 pneumococcal isolates were penicillin-nonsusceptible.[21] Proportions observed subsequently in similar populations were 34- 38% in three time periods: 2003-2004, 2006-2007, and 2008-2009.[21] Pelton et al.reported that among Boston children less than 2 years of age examined at well-child or AOM visits, 29% of 278 pneumococcal isolates were penicillin-nonsusceptible, and that during the 3-year period 2000-2003, no significant change was noted among such isolates in resistance to amoxicillin.[22] Moore et al. found that, among a demographically diverse group of children 3 to 59 months of age in Anchorage, Alaska, introduction of PCV7 into the routine immunization schedule had no significant impact over a 3-year period on the overall carriage of nonsusceptible *S. pneumoniae.*[23] And Cohen et al (2012) found the proportion of pneumococcal isolates that were penicillin-nonsusceptible to be 30% (85/285) in children who had received PCV7 only, compared with 23% (111/486) in “adequately PCV13-vaccinated” children ($P = 0.04$).[13]

A potential limitation of this study is the fact that serotyping by the Quellung method, as performed here, can mistakenly categorize isolates as serotype 6A that are actually serotype 6C. Accurate determination requires the use of polymerase chain reaction.[13] In addition, whereas the gold standard for determining the bacterial etiology of AOM would be recovery of the pathogen from middle-ear fluid, we did not perform tympanocentesis in any of these studies. Other studies, however, have shown that pathogens causing AOM are almost invariably recoverable at the same time from the nasopharynx, and that the pattern of pathogens recovered from children's nasopharynx at times of diagnosis of AOM is more likely to be reflective of the seroepidemiology of otopathogens than is the pattern of pathogens recovered from the nasopharynx of healthy children.[11,24]

It seems concerning that in our Group 4 children, the newly emergent serogroups 15 and 35B not only predominated among the nonvaccine serotypes recovered, but also accounted for 49% of the pneumococcal isolates that were penicillin-nonsusceptible. Among these nonvaccine serotypes, particularly concerning were the serotype 35B isolates, all of which were penicillin-nonsusceptible. On the other hand, the overall proportion of pneumococcal isolates that were penicillin-nonsusceptible appears not to have increased over time. Other studies suggest that these serotypes may be less likely to cause invasive disease,[15] however, it is unknown whether these resistant nonvaccine serotypes will continue to increase in prevalence, and whether they will be joined by other resistant serotypes. Continued surveillance will be required to monitor the effect of PCV13 on the burden of pneumococcal disease, and to monitor NP colonization as a potential marker of AOM and IPD pathogens and as a potential guide in planning vaccine modifications.

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Table 1

Distribution according to Serotype, Vaccine Relationship, and Penicillin Susceptibility of *Streptococcus pneumoniae* Isolates in Nasopharyngeal Cultures Obtained from Children with the Study Entry Episode of Acute Otitis Media during 2012-2013.

Serotype or Serogroup*	Total Number of Isolates	Susceptibility of Isolate to Penicillin	
		Susceptible†	Nonsusceptible
		Number of isolates (%)	
PCV7			
19F	1	1 (100)	---
23F	1	---	1 (100)
PCV7-related			
6, other than A and B	2	1 (50)	1 (50)
9, other than V	1	1 (100)	---
19 B	1	---	1 (100)
23, other than F	15	9 (60)	6 (40)
PCV13 (other than PCV7)			
3	3	3 (100)	---
6A	3	1 (33)	2 (67)
19A	8	2 (25)	6 (75)
Nonvaccine			
10A	3	3 (100)	---
11A	9	9 (100)	---
15‡	26	18 (69)	8 (31)
16F	3	3 (100)	---
17F	3	3 (100)	---
20	1	1 (100)	---
22	3	3 (100)	---
25A	1	1 (100)	---
33	3	3 (100)	---
34	1	1 (100)	---
35‡	13	2 (15)	11 (85)
37	1	1 (100)	---
40	4	4 (100)	---
44	1	---	1 (100)
group H (13, 28)	1	1 (100)	---
group E (21, 39)	5	5 (100)	---
TOTAL	**113**	**76 (67)**	**37 (33)**

PCV7 denotes 7-valent pneumococcal conjugate vaccine. PCV13 denotes 13-valent pneumococcal conjugate vaccine.

Numbers in parentheses refer to individual serotypes that constitute the respective Groups in their entirety. Isolates whose serotypes would have placed them into one of these Groups were characterized only by their Group identity; specific, individual serotype testing of these isolates was not performed.

*Other serotypes (vaccine-included, vaccine-related, and nonvaccine) that were not found are not listed.

[†] Penicillin-susceptible: Minimum inhibitory concentration (MIC) <0.1 μg/mL. Penicillin-nonsusceptible: MIC ≥0.1 μg/mL.

[‡] Includes multiple subtypes.

Table 2

Distribution according to Subtype and Penicillin Susceptibility of *Streptococcus pneumoniae* Serotype 15 and Serotype 35 Isolates from Nasopharyngeal Cultures Obtained from Children with the Study Entry Episode of Acute Otitis Media during 2012-2013.

Serotype	Serotype Subtype	Total Number of Isolates	Susceptibility of Isolate to Penicillin		
			Susceptible	Intermediate	Resistant
			number of isolates (%)		
15	A	5	2 (40)	3 (60)	---
	B	12	10 (83)	2 (17)	---
	C	8	6 (75)	2 (25)	---
	subtype not identified	1		1 (100)	
35	B	10		3 (30)	7 (70)
	F	3	2 (67)	1 (33)	---
Total, 15 + 35	N/A	39	20 (51)	12 (31)	7 (18)

Penicillin-susceptible: minimum inhibitory concentration (MIC) <0.1 ug/mL. Penicillin-intermediate: MIC ≥0.1 ug /mL and ≤ 1.0 μg/mL. Penicillin-resistant: MIC >1.0 μg/mL. N/A denotes not applicable.

Table 3

Selected Characteristics of Children Who Were Subjects of the Present Analysis, and Studies in which They Were Enrolled

	Group 1	Group 2	Group 3	Group 4
Study Topic	Effectiveness of influenza vaccine in preventing AOM[16]	Pneumococcal NP colonization before and after introduction of PCV7[7]	Antibiotic versus placebo for AOM[17]	5 days versus 10 days of antibiotic for aOm (currently ongoing)
Period included for present analysis	1999-2000	2003-2005	2006-2009	2012-2013
Number of children enrolled in parent study	417	326	282	228
Number of children from parent study with NP culture obtained at time of AOM diagnosis	175	87	282	228
Number (%) of children with NP colonization with *S. pneumoniae* at the time of AOM diagnosis	85 (49%)	33 (38%)	140 (50%)	113 (50%)
Pneumococcal serotype found: number (%) of children				
PCV 7	49 (58%)	14 (42%)	7 (5%)	2 (2%)
PCV7-related	3 (4%)	0	14 (10%)	19 (17%)
PCV13	17 (20%)	7 (21%)	56 (40%)	14 (12%)
Nonvaccine	16 (19%)	12 (36%)	63 (45%)	78 (69%)

NP denotes nasopharyngeal AOM denotes acute otitis media PCV7 denotes 7-valent pneumococcal conjugate vaccine PCV13 denotes 13-valent pneumococcal conjugate vaccine