

# Multinational study of pneumococcal serotypes causing acute otitis media in children

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**Background.** *Streptococcus pneumoniae* is a major cause of acute otitis media (AOM) in young children. More than 90 immunologically distinct pneumococcal serotypes have been identified, but limited information is available regarding their relative importance in AOM.

**Methods.** We analyzed nine existing datasets comprising pneumococcal isolates from middle ear fluid samples collected from 1994 through 2000 from 3232 children with AOM from Finland, France, Greece, Israel, several East European countries, the US and Argentina. We examined the distribution of pneumococcal serotypes in relation to several demographic and epidemiologic variables, including gender, age, antibiotic resistance and source of culture material.

**Results.** The major serotypes identified included 19F and 23F, each comprising 13 to 25% of pneumococcal middle ear fluid isolates in most datasets; 14 and 6B, comprising 6 to 18%; whereas 6A, 19A and 9V each comprised 5 to 10%. Despite differences in location, study design and antibiotic susceptibility, each major serotype was prominent in most age groups of each dataset. Serotypes represented in the 7-valent pneumococcal conjugate vaccine (PCV-7, 4, 6B, 9V, 14, 18C, 19F, 23F) accounted for 60 to 70% of all pneumococcal isolates in the 6- to 59-month age range, but only 40 to 50% of isolates in children

<6 or ≥60 months old. Serotype 3 and, in certain datasets, serotypes 1 and 5, were more important in the <6- and ≥60-month age groups. In each age group vaccine-related serotypes (mainly 6A and 19A) comprised an additional 10 to 15% of all pneumococcal isolates. Four serotypes (23F, 19F, 14 and 6B) accounted for 83% of all penicillin-resistant observations.

**Conclusions.** This analysis of several geographically diverse datasets indicates that a limited number of serotypes, largely represented in PCV-7, accounted for the majority of episodes of pneumococcal AOM in children between 6 and 59 months of age. Certain serotypes appeared to be relatively more significant in children <6 months or >59 months of age.

## INTRODUCTION

Acute otitis media (AOM) is by far the most common manifestation of disease caused by *Streptococcus pneumoniae*, with ~7 000 000 episodes of pneumococcal AOM estimated to occur annually in the US alone.<sup>1</sup> In addition to this considerable disease burden and the occasional long term or suppurative sequelae, aggressive antibiotic treatment of AOM is also believed to be a major factor in the rise of antimicrobial resistance in many countries.<sup>2</sup>

There are >90 immunologically distinct serotypes of pneumococci. These differ in the chemical composition of the polysaccharide capsules that constitute the major virulence factors for pneumococcal disease and also serve as the antigenic targets of current vaccination strategies. In contrast to invasive pneumococcal disease,<sup>3</sup> relatively few recent studies have characterized the specific serotypes most often responsible for AOM,<sup>4–10</sup> in part because diagnosis and treatment of AOM generally does not involve middle ear fluid (MEF) sampling.

Better understanding of the epidemiologic importance of individual serotypes could affect preventive and therapeutic strategies for AOM. For example a small subset of serotypes is responsible for most antibiotic resistance.<sup>7</sup> In addition a licensed 7-valent pneu-

Accepted for publication June 25, 2002.

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Key words: Pneumococcus, acute otitis media, serotypes.

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DOI: 10.1097/01.inf.0000035588.98856.05

mococcal conjugate vaccine has shown serotype-specific AOM efficacies that range considerably from 25% for 19F [95% confidence interval (95% CI), 14 to 51%] to 84% for 6B (95% CI 62 to 93%), with an overall efficacy against vaccine serotype AOM of 57% (95% CI 44 to 67%).<sup>11</sup> A similar variability in serotype-specific efficacies was also reported for a second vaccine.<sup>12</sup> In this study we analyzed the pneumococcal serotype distributions of several existing MEF datasets, all from the past 10 years.

## METHODS

**Abbreviations.** Tympanocentesis (T); myringotomy (M); otorrhea from AOM through spontaneously ruptured membranes (O); otorrhea through tympanostomy tubes (OT); invasive pneumococcal disease (IPD); 7-valent pneumococcal conjugate vaccine formulation, containing serotypes 4, 6B, 9V, 14, 18C, 19F, 23F (PCV-7); 9-valent pneumococcal conjugate vaccine formulation, containing PCV-7 serotypes plus 1 and 5 (PCV-9); 11-valent pneumococcal conjugate vaccine formulation, containing PCV-9 serotypes plus 3 and 7F (PCV-11).

**Datasets.** Nine datasets containing information on pneumococcal MEF isolates were available for analysis (Table 1). Each dataset had been assembled since 1994 in the course of routine clinical care, as part of an epidemiologic study or as part of an antibiotic or vaccine trial. For this reason specific inclusion/exclusion criteria differed by dataset (see below), although all except dataset US2 explicitly included only AOM patients. In several cases publications describing other analyses of these individual datasets have recently appeared.<sup>5-7, 13-16</sup> Other prerequisites for inclusion into this study included the existence of precise age information linked to each isolate and that the serotypes of most isolates were or could be determined by factor typing. A questionnaire was developed and investigators provided information on each isolate, where available, regarding geographic location, precise age of the patient, gender, antimicrobial susceptibility, copathogens and method of isolation.

**Inclusion and exclusion criteria specific to individual datasets.** Individual datasets were labeled with three character abbreviations in capital letters, as follows.

**ARG (Argentina).** Children with AOM were enrolled provided they had no known immunodeficiency, tympanostomy tube, spontaneous perforation or seriously underlying disease and who had not been given systemic antibiotics <48 h before isolation.

**FIN (Finland).** Dataset represents the control group from the Finnish Otitis Media Vaccine Trial, a Phase III vaccine trial in which children were followed in a study clinic setting from 2 to 24 months of age.<sup>11,14</sup> Children enrolled had no history of IPD or any known or suspected immunologic impairment. Myringotomy

(M) with aspiration was performed whenever AOM was diagnosed during the follow-up. Because in FIN myringotomies instead of tympanocentesis (T) were performed for routine sampling of MEF, samples coded as myringotomy isolates in this dataset were considered tympanocentesis isolates for purposes of analysis by method of isolation.

**FRA (France).** Children with nonresponsive AOM were enrolled, 26 of whom had otorrhea for <48 h. Nonresponsive AOM was defined as persistence or the recurrence of symptoms, i.e. fever, otalgia, irritability that was associated with otoscopic signs of AOM and occurred after at least 2 full days of antibiotic or if therapy had been discontinued within 3 days.

**GRE (Greece).** Children with AOM were enrolled and tympanocenteses were performed if they were seriously ill, did not respond to previous antibiotic therapy and/or had persistent bulging of the tympanic membrane. Only the first organism isolated from each sample was recorded.

**ISR (Israel).** This dataset comprised isolates obtained both from routine clinical care as well as those obtained as part of antibiotic studies of AOM. Precise diagnostic criteria differed by study.

**MUL (multicountry).** Children with AOM >3 months of age in US and >9 months of age in Eastern and Central Europe were enrolled.<sup>13</sup> Number of isolates in MUL from different study sites: Bulgaria, 33; Czech Republic, 24; Hungary, 10; Israel, 20; Romania, 81; Slovakia, 13; US 70.<sup>13</sup>

**US1 (USA).** This dataset comprised isolates obtained from routine clinical care as well as those obtained as part of antibiotic studies of AOM and included both inpatients and outpatients.<sup>5</sup> Precise diagnostic criteria varied by investigator.

**US2 (USA).** MEF isolates were obtained from routine clinical specimens submitted to commercial laboratories and chosen to be geographically representative of the US. However, the precise method of isolation and clinical presentation were unknown.<sup>6</sup>

**US3 (USA).** Exclusively enrolled children with AOM with patent tympanocentesis tubes who had mucopurulent or purulent otorrhea for <3 weeks.<sup>15, 16</sup>

**Isolates vs. observations vs. episodes.** Isolation and serotyping of a pneumococcus from MEF of a child was a prerequisite for inclusion in this analysis. The basic unit of analysis was observation, which was defined as an isolate from one individual that started a new episode. In the case of multiple isolates, a new episode was considered to have started if at least 30 days had elapsed since the beginning of a previous episode in that individual due to the same serotype, or if any interval had elapsed since the beginning of an episode caused by a different serotype. In three datasets (FRA, ISR and FIN), pneumococci were frequently isolated from both ears at the same time. If these were

TABLE 1. Description of each dataset

Dataset	Location	Years	No. of Observations	Age Range (mo)	Inclusion Criteria	Rationale for Study		Investigators and References
						Method of isolation	Penicillin nonsusceptibility (%)	
Studies with generally "open" enrollment, not weighted toward clinical failures								
MUL	US, Central Europe, Israel	1994–1995	251 (251)*	3.0–144 (36.0)†	AOM with effusion	Samples collected before antibiotic clinical study, 100% T	29.9%	Jacobs et al. <sup>1,3</sup>
FIN	Finland	1995–1999	489 (297)	1.9–24.2 (13.2)	AOM (routine myringotomy)	Control group of vaccine trial, 91% T, 3% O, 7% OT	5.6	Kilpi <sup>11</sup>
ARG	Argentina	1996–2000	86 (86)	1.5–132 (9.0)	AOM	Epi study of etiology and antibiotic susceptibility	80.2	Lopez‡
US1	US	1994–1997	619 (619)	1.0–165 (17.0)	AOM	Mixture of antibiotic studies or clinical care, 38% T, 54% O, 6% M, 2% U	43.9	Kaplan and Mason <sup>5</sup>
Studies including a large proportion of clinical failures								
FRA	France	1994–1998	155 (155)	3.9–51 (11.5)	Antibiotic-nonresponsive AOM	Epi study to examine etiology, antibiotic resistance 84% T, 15% O, 1% U	86.4	Cohen and Levy§
ISR	Israel	1998–1999	1250 (1127)	1.5–30 (9.0)	AOM	Mixture of antibiotic studies or clinical care, 82% T, 17% O, 1% OT	67.2	Dagan <sup>7</sup>
US2	US	1996–1999	425 (425)	1.2–168 (12.0)	MEF from commercial clinical laboratories	Study of antimicrobial susceptibility in US, 9% T, 91% U	58.4	Jacobs <sup>6</sup>
GRE	Greece	1999	79 (79)	1.5–132 (15.5)	Antibiotic-nonresponsive AOM	Epi study to examine etiology, antibiotic resistance, 25% T, 75% O	31.7	Syriopoulou¶
Study comprised solely of known chronic otitis patients								
US3	US	1995–1997	193 (193)	9.0–132 (21.0)	AOM in children with tympanostomy tubes	Epi study to examine etiology, 100% OT	Unknown	Pelton

\* Numbers in parentheses, number of individuals.

† Numbers in parentheses, median.

‡ EL Lopez, unpublished observations.

§ R Cohen and C Levy, unpublished observations.

¶ V Syriopoulou, unpublished observations.

|| SI Pelton, unpublished observations.

U, unknown; Epi, epidemiologic.

of the same serotype, they were considered to represent a single observation. If two serotypes were recovered from the same individual on the same date, either from the same ear or from opposite ears, each of them was considered to comprise a new episode and thus were regarded as two distinct observations. Only for analyses of vaccine coverage were two serotypes isolated on the same date (from either one ear or separate ears) considered a single observation, and this observation was deemed to be covered by the vaccine only if both isolates were represented in the vaccine formulation. We defined prevalence of a serotype as the proportion of observations occurring in a specific age range.

**Pneumococcal serotyping.** This was performed with the quellung reaction with antisera from Statens Seruminstitut, Copenhagen, Denmark. In one study (FIN) serotyping was performed by counterimmunoelectrophoresis or, for neutral serotypes/groups 7 and 14, by latex agglutination and confirmed by the quellung reaction when necessary.<sup>17</sup>

**Penicillin susceptibility.** Penicillin MICs were assessed by the agar dilution method, microdilution method or E-test. Isolates with MICs of  $\leq 0.06$   $\mu\text{g/ml}$  were considered penicillin-susceptible, those with MICs of 0.12 to 1.0  $\mu\text{g/ml}$  were considered penicillin-intermediate resistant and those with MICs of  $>1.0$   $\mu\text{g/ml}$  were considered resistant. Penicillin-intermediate and penicillin-resistant organisms were considered together as penicillin-nonsusceptible.

**Statistical analyses.** The nine studies that contributed data to the analyses reported here differed in important respects, including their rationales, inclusion and exclusion criteria and subject demographics. For this reason the data analysis methods used are largely descriptive and should be regarded as exploratory. Emphasis has been given to patterns that appeared to be reasonably consistent over the nine datasets.

Formal statistical tests were based on within-study comparisons. Test statistics were based on stratified (or matched) comparisons, where strata were defined by the individual datasets together with potentially relevant covariates (generally age and gender of the patient, method used to acquire the isolate and the penicillin resistance status of the isolate). For example an overall test of association between 7-valent vaccine serotype coverage and patient gender was based on the Mantel-Haenszel method, with strata jointly defined by datasets, age groupings (0 to 5 months, 6 to 23 months, 24+ months), method of isolation (T, O, other/unknown) and resistance to penicillin (susceptible, nonsusceptible, not tested). Therefore direct comparisons of serotype coverage between boys and girls were restricted to observations made within the same dataset, among subjects of similar ages, with isolates obtained by identical methods and having identical resis-

tance to penicillin. Aggregate statistics, such as the overall odds ratio, may be viewed as weighted averages over all strata.

Tests for the homogeneity of comparisons between strata were conducted with the Breslow-Day test, and logistic regression was used for more extensive modeling of factor interactions. Logistic regression was also used in some exploratory analyses in which covariate effects were modeled by linear functions or by unspecified smooth functions with the use of generalized additive models with logistic link functions.

## RESULTS

**Description of datasets.** A total of 3232 children provided 3559 pneumococcal MEF isolates, and for purposes of analysis these were considered 3520 distinct observations (see Methods for definition). Datasets were obtained from 11 countries in Europe, the Middle East, North America and South America (Table 1), including 1307 observations (37%) from the US, 1270 (36%) from Israel and 857 (24%) from Europe. A total of 3297 or 94% of the observations had linked penicillin susceptibility information, and datasets ranged in penicillin nonsusceptibility from 6% (Finland) to 86% (France), probably reflecting geographic variations in antimicrobial resistance levels as well as in inclusion criteria for the particular study populations examined.

Consistent with the age incidence of AOM,<sup>17</sup> the median age for eight of the nine datasets ranged from 9 to 21 months (Table 1). Overall the ages of the children ranged from 1 month to 14 years; 507 (14%) observations were from children  $<6$  months of age, 2301 (65%) from 6 to 23 month olds and 712 (20%) from children  $>23$  months of age.

For 3272 or 93% of the observations (essentially all but US3) gender of the patient was recorded, and boys accounted for 59% of the observations. Datasets did not differ in the proportion of boys comprising the study population (range, 53 to 63%;  $P = 0.1$ ), nor was there a difference in age distribution by gender. The method of isolation did not appear to be associated with the gender of the patient ( $P = 0.08$ ).

For 3120 or 89% of the observations (essentially all but US2), the method of obtaining the MEF specimen ("method of isolation") was recorded, and in most datasets more than one method was utilized (Table 1). Of those observations with a recorded method, 70% were derived from tympanocentesis (T), 21% from samples from otorrhea during AOM (O), 8% from otorrhea samples obtained from pressure equalization tubes (OT) (81% of which were from US3) and 1% from myringotomies. Children providing T isolates were generally younger than those providing O isolates (median ages, 9.6 and 16 months, respectively) ( $P < 0.0001$ ). Penicillin nonsusceptibility was more common



in children <2 years of age than in older children, after controlling for dataset, method of isolation and gender ( $P < 0.0001$ ).

**Nonpneumococcal bacterial copathogens.** In the four datasets with this information copathogens were isolated alongside pneumococci in 15 to 48.5% of all observations ( $n = 1943$ ). In three datasets (FRA, ISR, ARG) the most frequently isolated copathogen was *Haemophilus influenzae*, while in the fourth (FIN) *Moraxella catarrhalis* was isolated most frequently, in each case accounting for 70% or more of the observations with a copathogen. In the FRA dataset *M. catarrhalis* was the next most commonly isolated copathogen, in the FIN dataset it was *H. influenzae*, and in the ISR dataset it was *Pseudomonas aeruginosa*, though this was considered a likely contaminant (R Dagan, unpublished observations).

**Pneumococcal serotypes.** As depicted in Table 2, 19F and 23F each comprised 13 to 25% of pneumococcal isolates in most datasets usually followed by 14 and 6B, each representing 6 to 18% of the observations; whereas 6A, 19A and 9V each comprised 5 to 10%. Serotypes/serogroups consistently observed in most datasets at frequencies of 5% or less included serotypes 3, 4 and 18C and serogroups 7, 15 and 11. Serotypes 1 and 5, in many countries extremely prominent causes of invasive disease,<sup>3</sup> together represented 10% of all pneumococcal observations in the MUL dataset but no more than a few percent of pneumococcal isolates in any other dataset. Within the MUL dataset, which comprises isolates from the US (70), Eastern Europe (161) and Israel (20), serotypes 1 and 5 together constituted at least 5% of the pneumococcal observations in each geographic subset. For two relatively small datasets a single serotype appeared to predominate. In the French dataset (FRA) serogroup 23 represented 40.5% of the 155 isolates, whereas in the Argentine dataset (ARG) serotype 14 represented 53.5% of the 86 observations.

**Age dependence of serotype distribution.** For

the three age groups of 0 to 5, 6 to 23 and  $\geq 24$  months, serotypes 19F, 23F and 14 were among the most common four serotypes; in addition 6A, 6B, 19A and 9V were each represented among the most common eight serotypes. These seven serotypes represent 48, 79 and 65% of all observations in the 0- to 5-, 6- to 23- and  $\geq 24$ -month age groups, respectively, and overall 73% of all observations.

The relative importance of some serotypes appeared to vary by age group. A prominent example was serotype 3, which was the fifth and fourth most common serotype ( $\sim 6\%$  and  $\sim 10\%$  in the 0- to 5-month and  $\geq 24$ -month age ranges, respectively). However, in the age range where most otitis cases occur (6 to 23 months), serotype 3 was the ninth most common serotype, representing  $< 2\%$  of the observations. When each dataset was examined individually, serotype 3 consistently represented a higher percentage of all serotypes isolated from children  $> 18$  months of age than in children 6 to 17 months. In four (ISR, MUL, US1, ARG) of the six datasets with  $> 10$  isolates from children in the youngest age range, serotype 3 comprised a higher percentage of pneumococcal isolates in children  $< 6$  months of age than in children 6 to 17 months; in the other 2 datasets (GRE and FIN) the proportions of serotype 3 in the 2 age groups were similar.

The relative frequency of observation by age was examined more rigorously with the use of a smoothed fit analysis by age that controlled for gender, method of isolation, dataset and penicillin nonsusceptibility (Fig. 1). For serotypes 19F, 23F, 14 and 6B the relative rates of isolation peaked at  $\sim 10$  to 18 months and tapered off at older ages. The inverse pattern occurred with serotype 3, where the relative rate of isolation appeared lowest at 10 to 18 months. Finally for serotypes 9V and 6A the relative rate of isolation appeared to have little relationship to age. For 23F, 19F, 14, 6B and 3 the association of prevalence with age was highly significant ( $P < 0.01$ ); for 19A significance was at  $P < 0.05$ . Figure 1 depicts the relative rate of isolation of each

**TABLE 2.** Most frequently isolated pneumococcal serotypes from middle ear fluid in children, by dataset

Serotype	ARG	FIN	FRA	GRE	ISR	MUL	US1	US2	US3	All datasets
19F	3.5*	13.7	3.2†	12.7	13.9	12.8	23.3	24.5	17.6	16.1
23F	2.3	21.3	27.2†	15.2	14.0	9.2	15.4	11.1	16.1	14.9
14	53.5	6.3	18.4	8.9	13.4	12.0	10.2	16.0	11.9	13.1
6B	5.8	12.7	13.9	3.8	6.0	4.4	15.8	11.5	18.7	10.1
6A	1.2	9.8		10.1	5.8	8.8	7.0	10.4	10.9	7.3
19A	9.3	5.7	5.1†	11.4	8.9	2.0	6.0	4.9	2.1	6.6
9V	2.3	2.2	5.1	7.6	4.9	10.4	4.5	5.2		4.6
3	3.5	3.1		3.8	3.1	5.6	4.7	8.7	1.0	4.0
15	1.2	5.1		2.5	2.6	1.6	1.5	0.2	2.6	2.1
1	2.3			1.3	2.0	5.2	0.7			1.6
18C		3.7	0.6	3.8	1.8	3.2				1.4
11		5.1	0.6	1.3		0.8	1.6	0.5	2.6	1.3
5					1.6	4.8		0.5		1.0
7		0.6		3.8	1.6	2.4	0.8	0.5		0.8
4		0.9		1.3	0.9	1.6	1.0		1.0	0.7

\* Numbers represent each serotype as a percent of all serotypes isolated in that dataset.

† Not included are the contributions of unserotyped 23 and 19 to the FRA data set, 13.3 and 3.8%, respectively. See Table 1 for number of observations by dataset.

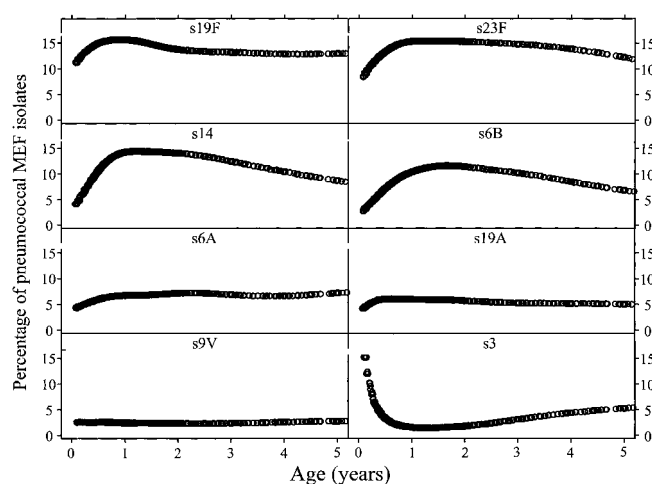


FIG. 1. Age dependence of serotype distribution-continuous fit. For each of the eight serotypes (s), the line represents that serotype as a percentage of all isolates at that age, controlling for dataset, gender, penicillin susceptibility and method of isolation. The density of circles within a given age interval reflects the number of data points in that interval.

serotype as compared with all other serotypes, not the absolute rate.

**Vaccine serotypes.** The seven serotypes in PCV-7 together represented 53 to 79% of all the pneumococcal AOM observations, depending on the dataset (Fig. 2, left). In addition serotypes immunologically related to those represented in the vaccine<sup>11, 18</sup> (i.e. all other members of serogroups 6, 9, 18, 19 and 23, but mainly 6A and 19A), comprised an additional 11 to 30% of all observations. These dataset-specific differences in vaccine serotype coverage remained highly significant ( $P < 0.00001$ ) even after controlling for differences in age distribution, gender, penicillin susceptibility and method of acquiring isolate.

Figure 2 (right) depicts the age dependence of vaccine serotype coverage and illustrates that overall

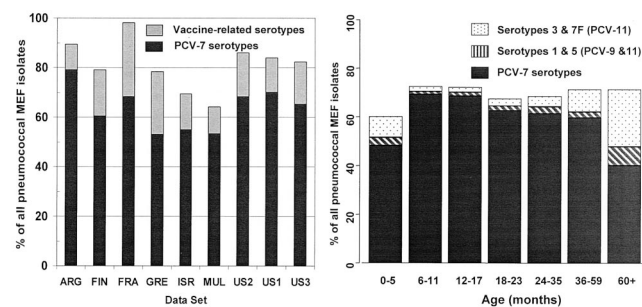


FIG. 2. Vaccine serotypes by dataset and by age. Left, percentage of pneumococcal MEF isolates in each dataset represented by serotypes 4, 6B, 9V, 14, 18C, 19F and 23F (PCV-7 serotypes) or by vaccine-related serotypes, such as 6A, 19A, 23B, etc.. Right, percentages of all pneumococcal MEF isolates in each age group with PCV-7 serotypes, incremental increase with serotypes 1 and 5 (included in PCV-9 and PCV-11) and incremental increase with serotypes 3 and 7F (included in PCV-11).

coverage remained fairly constant between 60 and 69% for the five age categories within the 6- to 59-month age range but tapered off markedly for children ages <6 months (48%) and >59 months (40%). The difference in vaccine serotype coverage between children 0 to 5 and 6 to 23 months of age was highly significant ( $P < 0.0001$ ), even after we controlled for dataset, gender, penicillin susceptibility and method of acquiring isolate.

Consistent with these observations, when we examined each dataset individually and restricted ourselves to penicillin-susceptible isolates only, serotypes represented in PCV-7 always represented a higher proportion of isolates in the 6- to 23-month-old age group than in the >24 month age group. For six of the eight datasets PCV-7 serotypes also comprised a higher proportion of isolates in the 6- to 23-month-olds than in the 0- to 5-month group (data not shown).

However, there were occasional deviations from this pattern. In particular in one dataset (FIN) PCV-7 coverage in children 0 to 5 months was appreciably higher than in older children. In each age category assessed, including the youngest and oldest, the contribution of vaccine-related serotypes (mainly 6A and 19A) remained in a narrow range of 14 to 18%.

Figure 2 (right) also illustrates the minimal incremental contribution that PCV-9 and PCV-11 formulations, still in clinical trials,<sup>19</sup> would make for children ages 6 to 35 months but also shows that for children in the younger and older age ranges PCV-11 serotype-specific coverage would rise to ~60 and 70%, respectively.

**Other variables affecting vaccine coverage.** After controlling for age, dataset, penicillin susceptibility and method of acquiring isolate, we observed only a marginal difference between boys and girls in PCV-7 serotype coverage (odds ratio, 1.18;  $P = 0.04$ ). When examined by specific age grouping, PCV-7 coverage for T isolates from children in the 6- to 23-month age range was slightly higher (66%) than for O isolates (62%;  $P < 0.001$ ), as assessed in the five datasets that allowed for direct comparison. However, there was insufficient power to determine whether a similar difference existed in the <6- and >23-month age groups as well.

**Penicillin susceptibility and vaccine coverage.** Of 3335 isolates for which penicillin susceptibility was assessed, 1646 (49%) were susceptible, 1005 (30%) showed intermediate susceptibility and 684 (20.5%) were resistant. Four serotypes (in decreasing rank order of prominence, 23F, 19F, 14 and 6B) comprised 83% of all penicillin-resistant observations, 7 serotypes (14, 19F, 19A, 23F, 6B, 6A, 9V) accounted for 83% of all observations with intermediate susceptibility to penicillin but at least 15 serotypes [19F, 23F, 3, 14, 6B, 6A, 19A, 9V, 18C, 15 (unserotyped), 11 (unserotyped), 1, 5, 4, 7F] were needed to account for 85% of penicillin-

susceptible observations. In general the most common penicillin-susceptible serotypes were also the most common penicillin-nonsusceptible serotypes, with one exception; serotype 3 was the third most common susceptible serotype, representing 8% of all penicillin-susceptible observations, but virtually never showed diminished susceptibility to penicillin.

Figure 3 illustrates the association of penicillin susceptibility in 6 to 23 month olds with vaccine serotype coverage for each of the three formulations. Vaccine coverage is substantially different when one separately examines isolates with differing penicillin susceptibilities, with resistant serotypes largely represented in the PCV-7 vaccine formulation. This finding was also reflected within each of the individual datasets, with 86 to 100% of resistant serotypes represented in PCV-7, as compared with 30 to 91% of the susceptible isolates. In fact, of all variables examined in this study, penicillin nonsusceptibility was the variable most strongly associated with PCV-7 serotype coverage. After controlling for age, gender, method and dataset, the odds ratio relating nonsusceptibility to PCV-7 coverage was 3.77 ( $P < 0.0001$ ), firmly establishing that the serotypes in the PCV-7 formulation are far more likely to be resistant to penicillin than the others.

## DISCUSSION

This retrospective analysis of nine datasets had two major purposes: (1) to identify the pneumococcal serotypes most responsible for AOM in children and relate those to specific vaccine formulations; and (2) to help define the relative importance of certain demographic

and methodologic variables as determinants of serotype distribution.

We found that despite significant variation in geographic location, dataset design and population and prevalence of antibiotic resistance, the most prominent serotypes in each dataset and in each age group tended to be the same, namely 23F, 19F and 14, followed by 6B, 6A, 19A and 9V. This finding is consistent with the prominence of serogroups 6, 19, 23 (and often 14) as previously reported in several pediatric studies of AOM in the US, Australia, China, Europe<sup>4,20</sup> and Brazil,<sup>21</sup> including two published 25 or more years ago.<sup>8,9</sup> It appears that the serotypes represented in PCV-11, plus 6A and 19A, comprise all major serotypes in each age group studied. Conversely the less common serotypes (usually 5% or less) were minor in each of the datasets. Next most prominent appear to be members of serogroup 15, but these totaled a maximum of only 2% of all pneumococcal MEF isolates in any age group.

This study and others<sup>7,22</sup> have shown the intimate relationship between age and antimicrobial susceptibility, with younger children having a higher proportion of penicillin-nonsusceptible isolates than older children. By looking for similar trends within several individual datasets while controlling several variables, we were able to confirm that age and penicillin susceptibility are each independently associated with variations in serotype distribution. However, other variables that we were not able to explore in this study, such as the extent and nature of pretreatment with antibiotics or the relative proportions of recurrent otitis cases, may also contribute to differences in serotype distribution by dataset.

We saw slight differences in serotype distribution when we compared isolates obtained by tympanocentesis *vs.* those obtained from spontaneously ruptured membranes, but differing indications for tympanocentesis in different datasets probably yielded a clinically heterogeneous group of patients that makes interpretation difficult. There were too few isolates to assess the serotype distribution of isolates obtained by other methods.

Each of the vaccine serotypes 19F, 23F, 14 and 6B represented a significant proportion of isolates in the 6- to 36-month age range and gradually waned in relative importance in older and younger children. 6A, 9V and 19A in contrast showed little or no age association. A significant age dependence was seen for serotype 3 (represented along with 7F in PCV-11). Within the peak AOM incidence age range (6 to 23 months) serotype 3 represented <2% of all isolates, but in children <6 months or ≥60 months of age type 3 represented ~5.8 and 21.5% of all isolates, respectively. This age dependence is consistent with observations made in the 1970s that showed that the relative importance of serotype 3 was much greater in the youngest and

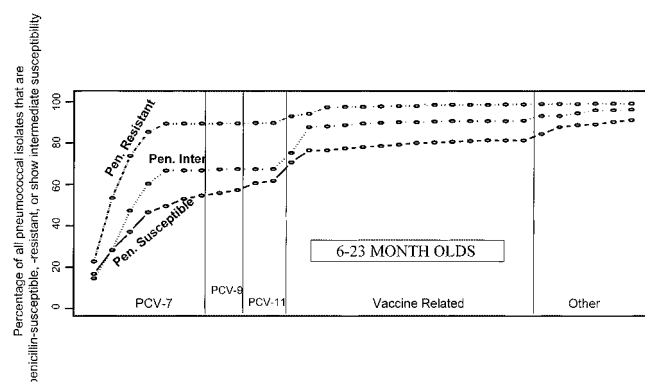


FIG. 3. Penicillin (*Pen.*) susceptibility and vaccine coverage. *Ovals*, incremental contribution of each serotype within the categories of "PCV-7" (4, 6B, 9V, 14, 18C, 19F, 23F), "PCV-9" (1, 5), "PCV-11" (3, 7F), "Vaccine Related" (other members of PCV-7, PCV-9 and PCV-11 serogroups) and "Other." Within each category serotypes are presented in order of decreasing prominence for all datasets as a whole. *Lines* represent the cumulative serotype coverage in each case. Data depicted comprise pneumococcal isolates from 6- to 23-month olds whose penicillin susceptibility has been assessed. A qualitatively similar pattern (highest coverage in penicillin-resistant isolates, lowest coverage in penicillin-susceptible isolates) was obtained in <6- and >23-month-old groups (not shown). *Inter.*, intermediate.



especially oldest age groups than within the age range of highest AOM incidence.<sup>8,9</sup> Similarly a Spanish study of MEF isolates in individuals >14 years of age reported serotype 3 as the most prominent serotype in that age group, comprising 21% of the 84 isolates.<sup>23</sup>

Serotypes 1, 5 and 7F are represented in the PCV-9 (1 and 5) and/or PCV-11 (1, 5 and 7F) conjugate vaccine formulations because of their prominence as causes of pediatric IPD in many countries<sup>3,24,25</sup> but nonetheless contributed little to overall pneumococcal AOM (<3% of all observations) and were not even represented in some datasets. Serotypes 1 and 5 comprised ~10% of the isolates in the MUL dataset, and this finding may reflect a higher proportion of older children in that set compared with the others. Indeed when age association was analyzed after controlling for other variables, including dataset, serotype 1 was relatively more prominent in children in the oldest age groups (>5 years; data not shown), a finding also reported by Austrian et al.<sup>8</sup> Two recent IPD studies in Denmark<sup>26</sup> and England and Wales<sup>27</sup> also showed a relatively high proportion of serotype 1 and 3 isolates in the youngest and oldest pediatric age groups.

To understand the differences in serotype prevalence by age, it is necessary to keep in mind that the overall incidence of AOM rises sharply in the first several months of life and that it wanes steadily after 2 to 3 years of age.<sup>17</sup> Thus the increasing prominence of serotypes 1 and 3, when expressed as percentages of all pneumococcal serotypes, in the youngest and especially oldest age groups may not reflect increases in the actual numbers of AOM cases caused by these serotypes. Rather it is possible that the absolute incidence of serotype 1 and 3 when is fairly constant throughout the first several years of life and that they are less susceptible than other serotypes to the age-associated factors that cause the overall rate of pneumococcal AOM to rise and then decrease with age.

These factors could include serotype-specific differences in the amount of antibody in breast milk or cord blood,<sup>28</sup> age-related variations in exposure to the pneumococcal serotypes carried by older children and adults inside and outside the home<sup>29</sup> and differential rates of development of protective immunologic responses to different serotypes.<sup>30,31</sup>

In this study we were not able to directly evaluate the clinical consequences of infection with any particular serotype. However, in light of evidence linking the age of the first episode of AOM to a higher frequency of recurrent disease later in childhood,<sup>17</sup> the relatively high prevalence of 19F and 23F AOM (and perhaps serotype 3) in the first 6 months of life might suggest that early disease caused by these serotypes could have important clinical consequences. These particular serotypes were also highlighted in a recent study of pneumococcal mastoiditis in 28 US children, where

serotypes 19F, 19A, 23F and 3 comprised 35.7, 14.3, 14.3 and 10.7% of cases, respectively, with an additional 7.1% cases caused by unserotyped serogroup 19 isolates.<sup>32</sup>

Based on these findings, the potential incremental benefit of the PCV-9 and PCV-11 conjugate vaccine formulations still in clinical development may be restricted to the youngest and oldest age groups. Depending on the local immunization schedule, vaccination may come too late to directly prevent disease in the first few months of life, unless vaccination of older children prevents sufficient transmission to provide indirect protection (herd immunity) for the youngest.

In our retrospective analysis of nine recent datasets, we have noted that relatively few serotypes account for most AOM in young children. In addition we documented strong associations between patient age and the relative frequency of specific serotypes and replicated earlier analyses showing that only a few serotypes are responsible for most penicillin resistance. These results support the proposal that each serotype be considered as a separate pathogen from an epidemiologic standpoint<sup>33</sup> and also allow a better appreciation of the epidemiologic usefulness of various conjugate vaccine formulations.

## ACKNOWLEDGMENTS

This study was supported by Wyeth-Lederle Vaccines. We thank Dr. George Siber for the concept and Drs. M. Fletcher, B. Fritzell, D. Laufer, P. Paradiso and B. Kohberger for helpful discussions. We also acknowledge the following collaborating investigators: Dr. Mindy Seidlin (US3); Professor Eugene Leibovitz and Anat Kaner (ISR); and Drs. M. Vazquez and A. Procopio (ARG).

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