MAJOR ARTICLE

Pneumococcal Conjugate Vaccine Serotypes of *Streptococcus pneumoniae* Isolates and the Antimicrobial Susceptibility of Such Isolates in Children with Otitis Media

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The ability of the recently licensed 7-valent pneumococcal conjugate vaccine to cover isolates that cause otitis media, especially drug-resistant ones, was assessed using 500 recently obtained US isolates. Of these isolates, 418 (84%) belonged to vaccine-related serogroups, whereas 82 (16%) belonged to non–vaccine-related serogroups. Serotype 3 accounted for 48 (59%) of the non–vaccine-related serogroups. In addition, 93% of the isolates from patients ≤3 years of age belonged to serotypes that were included in or related to the heptavalent vaccine, compared with 49% of the isolates from older patients ($P = .001$). Most of the isolates (98%–100%) that were resistant to the antimicrobial agents tested were covered by the heptavalent vaccine, including 95.1% of the isolates from patients <2 years of age. The 7-valent pneumococcal conjugate vaccine could therefore potentially provide protection against all but 1 (type 3) of the common otitis media–associated pneumococcal serogroups identified in this study as well as against 98% of antibiotic-resistant isolates.

In both developed and developing nations, acute otitis media (AOM) accounts for most bacterial respiratory tract infections in children [1, 2]. *Streptococcus pneumoniae* is responsible for 30%–50% of all cases of AOM worldwide [3], and, in the United States, *S. pneumoniae* causes 7 million episodes of AOM annually [4]. AOM caused by *S. pneumoniae* is associated with a low frequency of spontaneous cure and is more likely to result in complications if poorly managed [5, 6]. The emergence and worldwide spread of drug-resistant *S. pneumoniae* has complicated antimicrobial therapy for patients with AOM [7, 8], and there is an overwhelming need for an effective vaccine that can prevent pneumococcal disease.

Unlike the serotypes of *Haemophilus influenzae* (one [type b] of which is responsible for most invasive infections), 90 serotypes of *S. pneumoniae* are capable of causing invasive disease. A vaccine to cover all the *S. pneumoniae* serotypes is not yet available. In addition, the 23-valent purified polysaccharide pneumococcal vaccine, which is used in the United States and Europe, is ineffective in children <2 years of age, a group that experiences the greatest burden of *S. pneumoniae* infections [9–11]. Therefore, 7-, 9-, and 11-valent protein conjugate vaccines, which are immunogenic in children <2 years of age, have been developed, and a 7-valent vaccine (Prevnar; Wyeth-Lederle Vaccines) has recently

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been licensed in the United States [12–14]. The pneumococcal serotypes included in these conjugate vaccines were designed to cover strains that predominantly cause disease in children worldwide [15, 16], as well as to cover antibiotic-resistant strains [8, 17, 18]. Because of geographic variation in serotypes and drug-resistant strains, the vaccines may not be universally optimal. The new 7-valent pneumococcal conjugate vaccine has recently been shown to be effective in reducing the incidence of meningitis, bacteremia, pneumonia, and otitis media among infants, although the reduction in the incidence of otitis media is more modest than the reduction in incidence associated with other diseases [12]. In addition, the 7-valent vaccine has been shown to have similar efficacy against other serotypes within the serogroups included in the vaccine [14]. We evaluated the potential coverage of conjugate vaccines against serotypes of *S. pneumoniae* that cause otitis media in the United States, and we assessed their efficacy against antibiotic-resistant isolates.

PATIENTS AND METHODS

Study isolates. Isolates of *S. pneumoniae* that were recovered, from 1996 through 1999, from samples of middle ear fluid obtained from patients with otitis media throughout the United States and that were then received as part of AOM [19] and surveillance studies conducted by some of our investigators (P.C.A. and M.R.J.) [20–22] were chosen from our culture collection for inclusion in this study. Isolates were selected to provide geographic distribution throughout the continental United States. They were stored at −70°C in serum-glycerol freezing media.

Identification, serotyping, and antimicrobial susceptibility testing. Optochin susceptibility and bile solubility were used to confirm that the isolates were *S. pneumoniae* [19]. The capsular reaction test, which used group- and type-specific antiserum from Statens Seruminstitut (Copenhagen) [19], was used to determine the serogroups and serotypes of the isolates.

Antimicrobial susceptibility of the isolates was assessed by determination of MICs of penicillin, amoxicillin, azithromycin, clindamycin, and trimethoprim-sulfamethoxazole (TMP-SMZ). MICs were determined by broth microdilution, in accordance with recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [23], in dried 96-well microdilution trays (Sensititre Division, Trek Diagnostics). Inocula were prepared from colonies grown on trypticase soy agar plates supplemented with 5% whole sheep blood (Becton Dickson Microbiology Systems) incubated in 5%–10% CO_2 for 18–20 h. The colonies were directly suspended in tubes of Mueller-Hinton broth (Sensititre), and turbidity was adjusted to a density that was equivalent to 0.5 McFarland's standard. After preparation, 200 μL of the inoculum was transferred to a 10-mL tube of Mueller-Hinton broth (Sensititre) supplemented with 5% lysed horse blood (Cleveland Scientific). Mueller-Hinton lysed horse-blood broth was freshly prepared on the day of testing by adding 1 mL of lysed horse blood to 10 mL of Mueller-Hinton broth. Doseheads were fitted on the tubes, and 100-μL volumes were dispensed into each well of the Sensititre microdilution trays with an autoinoculator (Sensititre). All inocula were checked to ensure that they contained the required inocula of 3×10^5 to 7×10^5 cfu/mL. The trays were sealed and incubated for 22–24 h at 35°C aerobically. The lowest drug concentration with no visible growth was read as the MIC. MICs were interpreted according to NCCLS criteria. Isolates that were intermediate or resistant to penicillin are analyzed separately, whereas isolates that were intermediate to other agents are included in the resistant groups.

Quality control (QC). The following QC strains recommended by the NCCLS were used on each day of testing: *S. pneumoniae,* American Type Culture Collection (ATCC) 49619; *Haemophilus influenzae,* ATCC 49247 and 49766; *Enterococcus faecalis,* ATCC 29212; and *Escherichia coli,* ATCC 25922. Susceptibility results were accepted if the MICs obtained for the QC strains were within NCCLS-specified limits [23].

RESULTS

A total of 500 pneumococcal isolates recovered from samples of middle ear fluid obtained from patients with AOM were selected. All isolates originated from outpatients in 19 states. Isolates had been recovered from surveillance ($n = 361$) and otitis media ($n = 39$) studies. Patients from whom the isolates were recovered included 392 children and 35 adults; the ages of 73 patients were unknown. The age distribution of the children was as follows: <1 year, 116 children; 1 year, 131; 2–3 years, 73; 4–9 years, 64; and 10–18 years, 10. The age distribution of the adults was as follows: 19–30 years, 8 adults; 31–40 years, 7; 41–50 years, 5; 51–60 years, 4; 61–70 years, 3; and >70 years, 8.

Serotype distribution. Table 1 and figure 1 show the overall distribution of the serotypes of the isolates. Of the 500 isolates, 333 (66.7%) belonged to the serotypes included in the 7-valent vaccine (19F, 14, 6B, 23F, 9V, 18C, and 4). It is significant that 3 serotypes—19F, 14, and 6B—account for >50% of isolates and that, as serogroups, they account for almost two-thirds of isolates. When analyzed as serogroups, 418 isolates (83.6%) belonged to vaccine-related serogroups (148 isolates belonged to serogroup 19; 79, to serotype 14; 102, to serogroup 6; 54, to serogroup 23; 26, to serogroup 9; 7, to serogroup 18; and 2, to serotype 4). Eighty-two isolates (16%) belonged to non–vaccine-related serogroups; 48 belonged to serotype 3, 26 belonged to 14 other serotypes, and 8 were untypeable. The 9-valent vaccine, which contains serotypes 1 and 5 in addition to the serotypes included in the heptavalent vaccine, would

Table 1. Distribution of 500 otitis media isolates, by serotype and antimicrobial susceptibility, according to serotypes included in protein conjugate vaccines.

Vaccine, serotype	No. of isolates	Antimicrobial susceptibility					
		Penicillin intermediate	Penicillin resistant	Amoxicillin resistant	Azithromycin resistant	Clindamycin resistant	TMP-SMZ resistant
7-valent							
19F	119	14	60	20	72	33	81
14	79	7	44	30	47	10	55
6B	54	12	35	12	40	21	46
23F	50	7	26	11	13	8	34
9V	26	3	16	3	8	1	19
18C	3	0	0	0	0	0	0
4	2	0	0	0	1	0	0
Subtotal, no.	**333**	**43**	**181**	**76**	**181**	**73**	**235**
7-valent related							
6A	48	7	23	5	25	4	32
19A	27	18	6	4	9	2	19
18A/B	4	0	0	0	0	0	0
19B/C	2	1	1	0	0	0	2
23A	2	1	0	0	0	0	1
23B	2	0	0	0	0	0	0
Subtotal, no.	**85**	**27**	**30**	**9**	**34**	**6**	**54**
9-valent							
5	2	0	0	0	0	0	0
1	0	0	0	0	0	0	0
Subtotal, no.	**2**	**0**	**0**	**0**	**0**	**0**	**0**
11-valent							
3	48	0	0	0	0	0	0
7	2	0	0	0	0	0	0
Subtotal, no.	**50**	**0**	**0**	**0**	**0**	**0**	**0**
Nonvaccine							
NT	8	1	1	1	0	0	2
29	4	0	3	1	0	0	1
16	1	0	0	0	1	0	0
Other[a]	17	0	0	0	0	0	1
Subtotal, no.	**30**	**1**	**4**	**2**	**1**	**0**	**4**
Total, no. (% total isolates)	**500**	**71** (14.2)	**215** (42.6)	**87** (17.4)	**216** (43.2)	**79** (15.8)	**293** (58.6)

NOTE. NT, not typeable; intermediate, intermediately resistant; TMP-SMZ, trimethoprim-sulfamethoxazole.

[a] Serotype 22 ($n = 4$); serotypes 8, 12, 15, 25, and 28 ($n = 1$ each); serotypes 11 and 31 ($n = 2$ each); and 4 other non–vaccine-related types.

cover virtually the same proportion of isolates as the 7-valent vaccine (420 vs. 418). The 11-valent vaccine, which has the further addition of serotypes 3 and 7, would cover 470 (94%) of the isolates, with most of the improvement caused by the 48 serotype 3 isolates.

Distribution of vaccine-related and non–vaccine-related serogroups by patient age. Table 2 shows the age-specific coverage of the 7-valent vaccine for the 429 study isolates for which patient age was known. Analysis of vaccine coverage among different age groups showed that coverage by the 7-valent vaccine was 93.1% (298 of 320) among children <4 years of age, compared with 48.6% (53 of 109) among older children and adults ($P < .001$; Fisher's exact test). The majority of the 45 isolates of the predominant nonvaccine serotype, type 3, were found among older children or adults.

Serotypes found in adults included serotype 3 (10 adults), serotype 19F (8), serotype 14 (5), and 12 other isolates belonging to 8 serotypes. Fifteen (71.4%) of 21 vaccine-related isolates from adults were from patients <50 years of age, compared with 5 (35.7%) of 14 non–vaccine-related isolates ($P = .04$; by Fisher's exact test).

Antimicrobial susceptibility. Overall, 42.6% of isolates

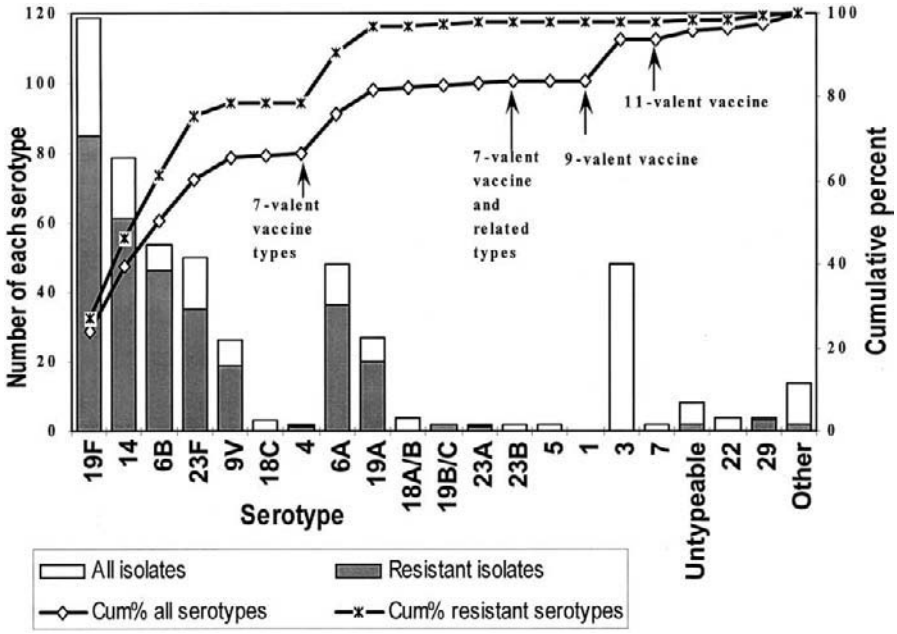

Figure 1. Serotypes included in 7-, 9-, and 11-valent vaccines, shown as the number and the cumulative percentage of each serotype ($n = 500$). Also shown are the number and the cumulative percentage of each serotype for isolates resistant ($n = 313$) to $\geqslant 1$ of the following: penicillin, azithromycin, and trimethoprim-sulfamethoxaole; for this analysis, isolates that were immediately resistant to penicillin are not included in the resistant group. Types 4, 6B, 9V, 14, 18C, 19F, and 23F are included in the 7-valent vaccine. Types 6A, 18A, 18B, 19B, 19C, 23A, and 23B are 7-valent vaccine related. The 9-valent vaccine includes serotypes included in the 7-valent vaccine plus types 1 and 5, and the 11-valent vaccine additionally includes types 3 and 7.

were susceptible to penicillin, whereas 14.2% were intermediate to penicillin and 43.2% were penicillin resistant (table 1). Virtually all penicillin-nonsusceptible isolates were in the vaccine-related group (table 3). A notable exception was serotype 29, for which 3 of 4 isolates identified were penicillin resistant. These resistant isolates were recovered from 3 cultures performed for patients in Florida, Georgia, and Ohio (patient age, 77 years, 5 years, and 1 year, respectively).

The proportion of isolates resistant to several of the antimicrobial agents was remarkably similar for the 7 most common serotypes (table 1). This was particularly noticeable with regard to resistance of serotypes 19F, 14, 6B, 23F, 9V, and 6A to penicillin and TMP-SMZ. Resistance to azithromycin was more variable, with the prevalence of resistance being highest in serotypes 19F, 14, 6B, and 6A (52.1%–74.4%) and lowest in serotypes 23F, 9V, and 19A (26.0%–33.3%). However, approximately half of the azithromycin-resistant isolates that belonged to serotypes 19F, 6B, and 23F were clindamycin resistant, compared with fewer than a quarter of isolates that belonged to serotypes 14, 9V, 6A, and 19A. Resistance to amoxicillin also varied, with 38% of serotype 14 isolates being resistant, compared with 10.4% of serotype 6A isolates.

Multiple-drug resistance was present for 3 of the agents tested (penicillin, azithromycin, and TMP-SMZ) in 161 isolates (32.2%), all of which were in the vaccine-related group. Of these isolates, 65 were resistant to penicillin, azithromycin, TMP-SMZ, and amoxicillin; 61, to penicillin, azithromycin, TMP-SMZ, and clindamycin; and 19, to all 5 agents. Overall, amoxicillin and clindamycin were the most active agents (82.6% and 84.2% susceptible, respectively), followed by azithromycin (66.8% susceptible) and TMP-SMZ (41.4% susceptible).

Table 2. Correlation between age and vaccine-related and non–vaccine-related serotypes of 429 isolates for which patient age was known.

	No. (%) of serotypes		
Patient age, years	7-valent vaccine or 7-valent vaccine related ($n = 351$)	Not related to 7-valent vaccine ($n = 78$)	Serotype 3 ($n = 45$)[a]
<1	112 (96.6)	4 (3.4)	2 (1.7)
1	123 (93.9)	8 (6.1)	0 (0)
2–3	63 (86.3)	10 (13.7)	6 (8.2)
4–9	31 (48.4)	33 (51.6)	21 (32.8)
10–18	1 (10.0)	9 (90.0)	6 (60.0)
>18	21 (60.0)	14 (40.0)	10 (28.6)

NOTE. Percentages shown are based on the total number of patients in each age group (see paragraph 1 of the Results section).

[a] The 45 serotype 3 isolates are included in the group of 78 isolates not related to the 7-valent vaccine.

Table 3. Susceptibility of vaccine-related and non–vaccine-related serotypes.

Agent	Percentage of isolates susceptible			
	All (n = 500)	Vaccine related (n = 418)	Not vaccine related (n = 82)	P[a]
Penicillin	46.2	32.8	92.7	<.001
Amoxicillin	82.6	79.7	97.6	<.001
Azithromycin	56.8	48.6	98.7	<.001
Clindamycin	84.2	81.1	100	<.001
TMP-SMZ	41.2	30.9	93.9	<.001

NOTE. TMP-SMZ, trimethoprim-sulfamethoxazole.

[a] Comparing vaccine-related with non–vaccine-related groups.

Coverage of the drug-resistant strains by the 7-valent vaccine. Table 1 and figure 1 show the heptavalent vaccine's potential coverage of antibiotic-resistant isolates. Coverage of the resistant strains by the 7-valent vaccine, as well as by the candidate 9- and 11-valent vaccines, was >97%. The majority (93%–100%) of the nonvaccine serogroup isolates were susceptible to the antibiotics tested. There was no significant difference in rates of coverage for resistant isolates when the 7-, 9-, and 11-valent vaccines were compared. Five serotypes—19F, 14, 6B, 23F, and 9V—accounted for 328 (66%) of all the isolates and 224 (78%) of the penicillin-nonsusceptible strains; these 5 serotypes are included in all 3 conjugate vaccines. When other serotypes of each serogroup are considered to be covered by the vaccines, 409 (82%) of all the isolates and 280 (98%) of the penicillin-nonsusceptible isolates would be covered. The addition of serotype 3 to these 5 vaccine-related serotypes would improve overall coverage to 454 isolates (91%) but would result in no increase in coverage of penicillin-nonsusceptible isolates. However, most of the type 3 isolates were recovered from older children and adults.

When vaccine coverage of isolates resistant to penicillin (excluding those that were intermediate), azithromycin, or TMP-SMZ was considered, virtually all resistant isolates again belonged to serotypes included in the 7-valent vaccine or to related serotypes (figure 1). Therefore, although the 7-valent vaccine potentially covers 83.6% of vaccine and vaccine-related types, it potentially covers 97.7% of antibiotic-resistant types.

DISCUSSION

The recently licensed 7-valent conjugate vaccine has been shown to reduce mortality and morbidity due to pneumococcal disease [12, 14]. Evidence of such reductions occurring among patients with AOM was recently shown by a Finnish otitis media vaccine efficacy study [14]. This study showed that vaccine efficacy in patients with AOM was 57% for vaccine types and that, in addition, the vaccine's efficacy was 51% for vaccine-related types. Vaccine efficacy was highest for vaccine type 6B (84%), and efficacy was also statistically significant for vaccine-related type 6A (57%). However, vaccine efficacy was lowest for vaccine type 19F (25%), and there was a 34% increase in nonvaccine types; therefore, the overall reduction in pneumococcal AOM was 34%.

Although vaccination with conjugate vaccines reduces the rate of nasopharyngeal colonization with *S. pneumoniae* serotypes in the vaccine [24], nonvaccine serotypes have been found to colonize vaccinated individuals, replacing vaccine serotypes [25]. This substitution of vaccine serotypes with nonvaccine serotypes, as well as geographic- and age-associated variations in serotype distribution, may affect the coverage of the pneumococcal conjugate vaccines, which contain a limited number of serotypes. As such, continued surveillance of the pneumococcal serotype distribution is necessary to assess coverage and guide the choice of the serotypes to be included in future conjugate vaccines.

In this cross-sectional study, we found that most of the otitis media isolates studied would be covered by the conjugate vaccines. Because there is cross-reactivity among serotypes of the same serogroup [14], coverage of a particular serotype should be extended to other members of the serogroup. Coverage by the 7-valent and 9-valent vaccines was similar (at 83.6% and 84%, respectively), but it increased to 94% for the 11-valent vaccine. Serotype 3, which is included in the 11-valent vaccine but is *not* included in the 7- and 9-valent vaccines, was responsible for most of the difference in coverage. Two limitations of this study are that it is cross-sectional analysis of isolates obtained from patients in surveillance and otitis media studies and that information on the clinical presentation and antimicrobial history of patients was not available for most isolates. However, it would be difficult to obtain a large number of isolates from patients presenting with AOM, because tympanocentesis would have to be performed for >2000 patients. Nevertheless, this study provides information on available isolates and may provide more clinically relevant information than a planned study, because our study isolates are likely to have been recovered from patients with severe disease or treatment failure.

Inability to respond to treatment because of antibiotic-resistant pneumococci has been reported [7, 26]. Antibiotic pressure results in selection of resistant strains in the nasopharynx, and, from that location, they cause disease or spread to other individuals [27–29]. In our study, >97% of all isolates that were resistant to any drug tested would be covered by all 3 conjugate vaccines. Because the pneumococcal conjugate vaccines are able to prevent colonization with *S. pneumoniae* of the serotypes included in the vaccine, these vaccines are potentially able to markedly reduce AOM due to antibiotic-resistant serotypes [26]. However, it is possible that nonvaccine serotypes that replace vaccine serotypes in the nasopharynx will

develop resistance and spread in the populations, causing disease of a similar magnitude if virulence is comparable. However, even under these circumstances, these nonvaccine serotypes would initially have little antimicrobial resistance, and this would be a significant improvement over the current situation. However, detection of penicillin resistance in nonvaccine serotype 29 isolates is a particular concern because resistance has not been described previously in this serotype.

This study has documented the predominant serotypes of *S. pneumoniae* isolated from middle ear fluid samples from outpatients in the United States, the extensive antimicrobial resistance of the most common types, and the potential effect on AOM of the newly licensed 7-valent pneumococcal vaccine. Use of this vaccine, as well as judicious antimicrobial use, has the potential to greatly reduce the incidence of pneumococcal AOM in children and, hence, decrease the overall selective pressure of antimicrobials on bacterial pathogens and commensals.

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