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Antibiotic-Resistant *Streptococcus pneumoniae* in the Heptavalent Pneumococcal Conjugate Vaccine Era: Predictors of Carriage in a Multicommunity Sample

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ABSTRACT. *Objective.* **Despite immunization with heptavalent pneumococcal conjugate vaccine (PCV7), the rising prevalence of antibiotic resistance makes *Streptococcus pneumoniae* a continuing threat to child health. Data on carriage of resistant organisms by healthy children in communities in which immunization with PCV7 has been implemented will help to define and decrease these risks further.**

Methods. **Children who were <7 years old, resided in a study community, and presented for routine well care or a "sick" visit between March 13 and May 11, 2001, at 31 primary care practices in 16 geographically distinct Massachusetts communities were studied. Consenting parents provided demographic information and data on potential risk factors for carriage of *S pneumoniae* and of penicillin-nonsusceptible *S pneumoniae* (PNSP). *S pneumoniae* isolates from nasopharyngeal specimens were tested for resistance to commonly used antibiotics including penicillin, ceftriaxone, erythromycin, and trimethoprim/sulfamethoxazole. Isolates were serotyped and grouped into PCV7-included serotypes, potentially cross-reactive serotypes (ie, an organism of a serogroup included in the vaccine), or non-PCV7 serotypes. Diagnosis on the day of collection, history of recent antibiotic use, and history of PCV7 immunization were obtained by chart review. Separate bivariate and multivariate analyses were performed to identify correlates of colonization with *S pneumoniae* and colonization with PNSP, accounting for clustering within communities.**

Results. ***S pneumoniae* was isolated from the nasopharynx of 190 (26%) of the 742 children studied. Of the 166 tested, 33% were nonsusceptible to penicillin, with 14% showing intermediate susceptibility (minimum inhibitory concentration [MIC] 0.12–1.0) and 19% fully resistant (MIC ≥2). Nonsusceptibility to other antibiotics was common, including ceftriaxone (14%), erythromycin (22%), and trimethoprim/sulfa (31%); 20% of *S pneumoniae* isolates were not susceptible to ≥3 antibiotics. Thirty-six percent of isolates were of serotypes covered by PCV7; 30% were of PCV7 serogroups and potentially cross-reactive, but not 1 of the 7 included serotypes; and 34% were unrelated to PCV7 serogroups. Nonsusceptibility to penicillin was more common in PCV7-included strains (45%) and potentially cross-reactive strains (51%) than in non-PCV7 serotypes (8%). Risk factors for PNSP colonization included child care attendance (odds ratio [OR]: 3.9; 95% confidence interval [CI]: 2.3–6.5), current respiratory tract infection (OR: 4.7; 95% CI: 2.5–8.6), and recent antibiotic use (OR: 1.7; 95% CI: 1.0–2.8). PCV7 immunization was associated with decreased carriage of PCV7-included serotypes but not with an overall decrease in *S pneumoniae* colonization or with a decline in PNSP colonization.**

Conclusions. **In this multicommunity sample, pneumococcal antibiotic resistance was common and was most frequently found in PCV7-included and PCV7 serogroup strains. The long-term impact of PCV7 immunization will be partially determined by the protection that it affords against invasive infection with potentially cross-reactive serotypes, as well as the virulence and future resistance patterns of unrelated serotypes. *Pediatrics* 2003;112:862–869; *antibiotic resistance, penicillin resistance, Streptococcus pneumoniae, nasopharyngeal carriage.***

ABBREVIATIONS. PNSP, penicillin-nonsusceptible *Streptococcus pneumoniae*; MDR, multidrug resistance; PCV7, heptavalent pneumococcal conjugate vaccine; RTI, respiratory tract infection; OR, odds ratio; CI, confidence interval.

The rising prevalence of antibiotic-resistant bacterial pathogens in the community is an emerging threat to the health of US children.[1–3] Resistance among *Streptococcus pneumoniae* is of particular concern[4,5] because this organism is the leading cause of meningitis[6] and other serious bacterial infections[7] in children, as well as a major cause of more common localized infections such as otitis media.[8] Penicillin-nonsusceptible *S pneumoniae* (PNSP) have become increasingly common in the United States over the past decade. In 1992, PNSP accounted for 7% of *S pneumoniae* isolates in a sample of hospital laboratories.[4] A more recent national hospital surveillance study reported a PNSP rate of 24% overall and 32% among children younger than 5 years.[9] Multidrug resistance (MDR) to several classes of antibiotics is also increasingly prevalent.[9,10]

Despite broad consensus that pneumococcal resis-

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tance constitutes a significant threat, recent studies of nasopharyngeal carriage of resistant organisms among generally healthy children are limited. Community-based studies of colonization in the United States over the past decade have revealed varying rates of *S pneumoniae* colonization,[10–14] with even more extreme international variability reported.[15,16] Resistance profiles differ as well, with some regions reporting less resistance to penicillin but high rates of resistance to macrolides and other agents.[15] Recently published studies from Wisconsin and Utah showed substantial fractions of *S pneumoniae* with reduced susceptibility to penicillin, but in these rural communities, only 2% to 4% were fully resistant to penicillin (minimum inhibitory concentration [MIC] ≥2.0).[17,18] Many of these surveillance studies have been based in child care centers,[12–14] where particular strains may be circulating,[19] thus limiting the generalizability of findings to the entire community.

The distribution of resistance among *S pneumoniae* serotypes is of particular interest in the context of the recent introduction of heptavalent pneumococcal conjugate vaccine (PCV7). Universal immunization of all infants against the 7 most common invasive serotypes began in the spring/summer of 2000. Data from vaccine trials show a substantially reduced risk of invasive infection and a more modest decrease in otitis media from included (and perhaps cross-reactive) serotypes among vaccinees.[20,21] However, children remain at risk from serotypes not included in PCV7. Colonization with pneumococcal vaccine serotypes[16,22,23] has been shown to decrease shortly after immunization, but longer-term changes in colonization patterns in communities remain unclear. The possibility exists that serotypes with which children have traditionally been colonized may be replaced by nonvaccine serotypes as predominant colonizers in a community, possibly leading to increased risk of infection by these organisms.[24,25] To date, however, there has been no evidence of an increase in invasive disease caused by nonvaccine serotypes in PCV7 recipients.[26] A second, related concern is the possibility of genetic recombinational events in microenvironments such as child care centers that could transfer resistance and virulence factors to serotypes not covered by the current vaccine. The finding in such centers of multiple serotypes with the same DNA type support this possibility.[19]

Finally, rates of antibiotic prescribing to children, which peaked in the early-1990s, have reportedly decreased by 40%.[27] Although examples of correlation between resistance and antibiotic use rates have been reported, studies in 2 areas of the United States—Wisconsin and Alaska—showed no sustained decrease in pneumococcal resistance with decreasing antibiotic prescribing.[18,28] Effects of judicious prescribing on community resistance rates in other locales is unknown. In the context of these simultaneous changes, current information is needed on carriage of *S pneumoniae* among generally healthy children and the serotypes and susceptibilities of prevalent organisms. We undertook surveillance for nasopharyngeal carriage of *S pneumoniae* in children who live in Massachusetts communities, not grouped by child care arrangements. Our goals were 1) to determine the resistance profiles (including MDR) for *S pneumoniae* in generally healthy children who were younger than 7 years and seeking office-based medical care across 16 distinct communities; 2) to evaluate further the reported risk factors for carriage of PNSP among generally healthy children; and 3) to determine the predominant susceptible and resistant serotypes of *S pneumoniae* carried in these populations after addition of PCV7 to the schedule of recommended routine immunization.

METHODS

Setting

Nasopharyngeal specimens were collected in primary care physician offices in 16 Massachusetts communities between March 13 and May 11, 2001, as part of baseline microbiologic data collection for a randomized trial of community-level intervention to promote judicious antibiotic prescribing for children. The 16 communities studied were selected for participation in the trial on the basis of geographic separation and evidence that few individuals crossed community boundaries to seek medical care, as well as diversity of size and demographic characteristics, using available US Census data.[29] Fifty primary care practices that serve children were approached for participation, and 31 agreed to participate.

Data Collection

Children were eligible for inclusion when they were younger than 7 years, resided in a study community, and presented for either routine well care or a "sick visit" at 1 of the participating practices. This age group was chosen to include the period of highest risk of pneumococcal infection and to be large enough to accrue efficiently the required sample. Some practices, after training by study personnel, had their own staff approach parents, obtain written informed consent (and verbal assent from children), and collect the nasopharyngeal specimens. In other practices, study personnel recruited patients and collected specimens on designated days. In either case, consenting parents filled out a brief structured questionnaire that included demographic information, current symptoms, and data on potential risk factors for carriage of susceptible and resistant organisms such as current (or recent) antibiotic use, attendance at group child care, age of siblings, smoking by a family member, room sharing with 1 or more other children, and duration of breastfeeding. One physician (S.H.) performed a structured review of the medical record of each patient to confirm data on symptoms and diagnosis on the day of collection, antibiotic use in the previous 2 months, PCV7 vaccination status, and the presence of chronic illness. A summary variable, respiratory tract infection (RTI), was created to include viral upper respiratory tract infection, sinusitis, bronchitis, pharyngitis, and otitis media. Recent antibiotic use was defined as use in the previous 2 months, on the basis of either parent report or medical record documentation. Analyses regarding PCV7 immunization separated children who received at least 1 dose from those who received none. All study procedures were approved by the Harvard Pilgrim Health Care institutional review board.

Microbiologic Methods

Specimens were collected by inserting a calgiswab, bent at 30 degrees, into the nares of each child approximately the distance to the tragus. Swabs were placed directly into STGG transport medium, an oxoid tryptone soy broth containing skim milk, glycine, and glycerol.[30] Samples were delivered by overnight courier to the microbiology laboratory at Children's Hospital, Boston, where specimens were plated on blood agar, blood agar + gentamicin, and chocolate agar plates (Becton, Dickinson and Company, Franklin Lakes, NJ), and incubated at 36°C. Alpha hemolytic colonies were initially identified using an optichin disk, followed by definitive identification of *S pneumoniae*. *S pneumoniae* isolates were frozen and transported to the State Laboratory Institute of the Massachusetts Department of Public Health for resistance testing using microdilution titer plates meeting all applicable National Committee for Clinical Laboratory Standards[31] standards

(Microstrep panel, Dade Behring, West Sacramento, CA). Standard National Committee for Clinical Laboratory Standards susceptibility cutoffs were used to classify organisms as susceptible, intermediate, and resistant for concentrations (in μg/mL) of the following antibiotics: penicillin (sensitive: ≤0.06; intermediate: 0.12–1.0; resistant: ≥2.0), ceftriaxone (sensitive: ≤0.5; intermediate: 1.0; resistant: ≥2.0), erythromycin (sensitive: ≤0.25; intermediate: 0.5; resistant: ≥1.0), trimethoprim/sulfamethoxazole (sensitive: ≤0.5/9.5; intermediate: 1/19–2/38; resistant: ≥4/76). MDR was defined as resistance to at least 3 antibiotics. Serotype was determined for a single colony from a pure culture of each isolate by the Quellung reaction, using serotype-specific pneumococcal antisera (Serum Statens Institute, Copenhagen, Denmark). There are inconsistent data on which serotypes, within a serogroup, may have vaccine cross-reactivity.[16,32] Therefore, for some analyses, isolates were categorized as vaccine serotypes (the 7 included strains), vaccine serogroups (those for which another member of the serogroup is included in PCV7), and nonvaccine serotypes.

Data Analysis

Parent report, chart review, and microbiologic data were analyzed using SAS software (SAS Institute, Cary, NC). Resistance patterns were analyzed for the sample overall, by community, and by serotype. Variability among communities was assessed using likelihood ratio tests for the community variance parameter from generalized linear mixed models[33] both without covariates and controlling for the covariates in each final model. Potential risk factors were tested in separate bivariate analyses for their association with 1) nasopharyngeal carriage of *S pneumoniae* and 2) carriage of PNSP among all children tested (population percentage resistant), rather than only those colonized with *S pneumoniae.* Children were grouped by age (<5 months, 5–<24 months, 24–<36 months, and ≥36 months), and cutoffs for the remaining continuous predictor variables were selected to ensure a sufficient number of observations in each stratum.

Generalized estimating equations methods[34] were used to account for clustering of outcomes within each of the 16 communities. Separate multivariate models were constructed for carriage of *S pneumoniae* and for carriage of PNSP. Significant bivariate predictors ($P < .10$ by χ^2 statistic) of either outcome were tested for inclusion in the multivariate models of both. A model including all such predictors was constructed for each outcome, and nonsignificant variables were removed sequentially until only those significant at $P < .05$ remained. Variables of particular interest based on previous studies, such as patient age and recent antibiotic use, were included even when not statistically significant. In all cases, we report odds ratios (ORs) and 95% confidence intervals (CIs). Because members of sibling pairs were included in the primary analysis, a confirmatory analysis was performed with random selection of 1 sibling per family to confirm the direction and magnitude of effects. Analyses testing the impact of PCV immunization on colonization were limited to children ≥5 months of age at the time of specimen collection.

RESULTS

Specimens were received from 766 children in the 31 participating practices. Twenty-four (3%) specimens were excluded from analysis for the following reasons: patient age outside the specified range (5) and patient residence outside of study communities (19). The median number of specimens analyzed per practice was 24 (interquartile range: 5–40), and the median number of specimens per community was 41 (interquartile range: 35–52). Characteristics of children contributing specimens are summarized in Table 1. Siblings from 78 families contributed 165 (22%) specimens. Only 12 sets of siblings both carried *S pneumoniae*, and only 4 were concordant for serotype; therefore, isolates from siblings were analyzed as independent observations. Results were not significantly different when analyses were rerun including only 1 member of sibling pairs. Forty-six percent of patients were female, and 78% were of white race by self-report. A total of 195 (27%) presented to the practice for treatment of an RTI, including 72 (10%) with acute otitis media. According to parental report and medical record review, 70 (10%) were taking an antibiotic at the time of specimen collection, and 294 (40%) had taken an antibiotic within the previous 2 months. The 16 communities studied, according to 2000 US census data, ranged in population size from 28 445 to 139 025, in median income from $31 809 to $93 354, and in percentage nonwhite from 2% to 39% (Table 2).[29]

TABLE 1. Characteristics of 742* Massachusetts Children Providing Nasopharyngeal Specimens

	N (%)
Age (mo; *n* = 742)	
0 to <5	109 (15)
5 to <24	270 (36)
24 to <36	104 (14)
36+	259 (35)
Sex distribution (*n* = 741)	
Female	340 (46)
Race (*n* = 707)	
White	548 (78)
Black	50 (7)
Hispanic	53 (8)
Asian	20 (3)
Other	36 (5)
Current (RTI)† (*n* = 729)	195 (27)
Current antibiotic use (*n* = 735)	70 (10)
Antibiotic use within 2 mo (*n* = 742)	294 (40)
Vaccination (Prevnar‡; *n* = 729)	
0 doses	398 (55)
1 dose	113 (16)
>1 dose	218 (30)
Child care participant (*n* = 695)	303 (44)
No. of siblings <6 y old (*n* = 701)	
0	367 (52)
1	266 (38)
>1	68 (10)
Roommates <6 y old (*n* = 676)	140 (21)
Smoking in home (*n* = 700)	209 (30)
Breastfed >2 mo (*n* = 679)	274 (40)
Prematurity <36 wk (*n* = 729)	29 (4)

* *N*s for individual items vary because of age restrictions and missing data.
† Includes viral upper respiratory tract infection, otitis media, sinusitis, and cough illness.
‡ Doses must be completed 30 days before swab date.

A total of 190 (26%) children were colonized with *S pneumoniae*, with the percentage colonized varying among the 16 communities from 14% to 46% (Table 2). This degree of variation in colonization rates did not exceed what would be expected by chance alone, after controlling for potential confounders ($P = .11$). Factors associated with colonization with *S pneumoniae* in bivariate analyses are summarized in Table 3. Colonization was more frequent among children older than 5 months, those with a current RTI (including otitis media), those who attended group child care, or those who had siblings.

In the multivariate model predicting colonization with *S pneumoniae* (Table 4), independent predictors of colonization included age, child care participation (OR: 2.3; CI: 1.6–3.4), current respiratory tract infection (including otitis media; OR: 2.5; CI: 1.7–3.6), and having 1 (OR: 1.5; CI: 1.0–2.3) or more (OR: 2.5; CI: 1.5–4.2) siblings. A history of being breastfed was

TABLE 2. Prevalence of Carriage of All *S pneumoniae* and PNSP Among Children in 16 Massachusetts Communities

Community	Population Size (1000s)*	Median Family Income ($1000s)*	Percentage Nonwhite*	Specimens (*N*)	Carriage of *S pneumoniae* (*N* [%])	*S pneumoniae* Penicillin Resistant† (*N* [%])	*S pneumoniae* Penicillin Intermediate† (*N* [%])
1	46	36	7	33	8 (24)	1 (14)	2 (29)
2	67	52	7	41	13 (32)	2 (15)	1 (8)
3	52	76	5	41	12 (29)	4 (36)	1 (9)
4	94	40	39	78	21 (27)	4 (21)	2 (11)
5	102	32	8	49	19 (39)	3 (20)	4 (27)
6	34	56	2	35	5 (14)	1 (25)	1 (25)
7	30	59	3	44	20 (46)	3 (16)	1 (5)
8	30	50	7	94	23 (25)	6 (32)	3 (16)
9	40	45	5	60	12 (20)	3 (25)	4 (33)
10	80	41	15	55	9 (16)	0	1 (17)
11	37	93	10	28	6 (21)	0	0
12	139	47	25	35	8 (23)	3 (50)	0
13	115	33	19	48	9 (19)	0	1 (17)
14	28	67	3	35	11 (31)	1 (10)	1 (10)
15	52	56	5	30	8 (27)	0	1 (13)
16	38	48	3	36	6 (17)	0	1 (17)
Total	983	Avg. 52	Avg. 10	742	190/742 (26)	31/166 (19)	24/166 (14)

* Based on US Census data for 2000.
† Of 166 *S pneumoniae* isolates tested for penicillin susceptibility.

protective (OR: 0.6; CI: 0.4–0.9), and there was a trend toward a protective effect of antibiotic use in the previous 2 months (OR: 0.7; CI: 0.5–1.0). The impact of a history of prematurity (<36 weeks) was significant in bivariate analysis. However, because of the small number of premature patients, this variable was unstable in multivariate models and was excluded from the final models.

A total of 166 *S pneumoniae* isolates were tested for susceptibility to commonly used antibiotics (Fig 1); the remaining 24 isolates did not survive storage and transport. A total of 55 (33%) were not susceptible to penicillin; 24 (14%) showed intermediate susceptibility to penicillin, and 31 (19%) were resistant. The fraction nonsusceptible varied by community from 0% (0 of 5) to 58% (7 of 12). The variation observed by community, after controlling for potential confounders, did not exceed what would be expected by chance alone ($P = .25$). Although less common than penicillin resistance, substantial levels of third-generation cephalosporin resistance (14% nonsusceptible) were observed. Nonsusceptibility to erythromycin (22% nonsusceptible) and trimethoprim/sulfamethoxazole (31% nonsusceptible) was common. Only 4 isolates were resistant to clindamycin, and no vancomycin-resistant organisms were isolated in this community sample. MDR, defined as resistance to at least 3 antibiotics, was present in 20% of isolates.

Serotype was determined for the 143 *S pneumoniae* isolates that survived subculture and transport (Table 5). Fifty-one (36%) of the 143 isolates were of serotypes included in PCV7 (most commonly 19F, 23F, and 6B); 43 (30%) of 143 were contained within serogroups included in the vaccine but not vaccine serotypes (most commonly 6A, 19A, and 9A); and, 49 of 143 (34%) were nonvaccine serotypes (most commonly 15C, 11, and 22F). Of the organisms within the PCV7-covered group, 23 (45%) of 51 were not susceptible to penicillin; all of these organisms were also nonsusceptible to at least 1 drug in addition to penicillin. The 3 most prevalent PCV7 serotypes all showed substantial penicillin nonsusceptibility: 19F (69%), 23F (50%), and 6B (45%). Of those isolates belonging to PCV7 serogroups (but not vaccine serotypes), 22 (51%) of 43 isolates were not susceptible to penicillin. Among nonvaccine strains, penicillin resistance was concentrated in specific strains, with 6A, 19A, and 9A, which together accounted for 77% of the resistant organisms not included in PCV7. Finally, nonsusceptibility was relatively low (4 [8%] of 49) among those serotypes that were neither vaccine-included nor potentially cross-reactive strains. In summary, although we found a high proportion of antibiotic-resistant organisms among serotypes included in the PCV7 vaccine, the highest prevalence of resistance was found in those serotypes belonging to PCV7 serogroups but not specifically included in the heptavalent vaccine.

Our analyses of carriage of penicillin nonsusceptible (PNSP) strains are based on the denominator of all children tested (population percentage resistant), rather than only on those carrying *S pneumoniae*. In bivariate analyses (Table 3), PNSP carriage was most frequent among children between 5 and 24 months (12%; $P = .01$) compared with older and younger children and more frequent among children with an RTI (19% vs 4%; $P < .001$) or otitis media (33% vs 5%; $P < .001$) or who had received antibiotics in the previous 2 months (12% vs 5%; $P < .001$). Colonization with a nonsusceptible strain was also more common with child care participation of 4 hours or more each week (12% vs 4%; $P < .001$) and among those with a history of prematurity (gestational age <36 weeks; 19% vs 7%; $P = .03$). The prematurity effect was based on only 5 PNSP colonized children (of 27 with a history of prematurity) and therefore was excluded from multivariate models. In a multivariate model (Table 4), colonization with a penicillin-nonsusceptible strain was independently predicted by child care participation (OR: 3.9; CI: 2.3–6.5), current RTI (OR: 4.7; CI: 2.5–8.6), and antibiotic use in the previous 2 months (OR: 1.7; CI: 1.0–2.8).

TABLE 3. Bivariate Associations of Colonization With Penicillin-Susceptible *S pneumoniae* and PNSP Among 742 Children* From 16 Massachusetts Communities

Variable	Colonized With *S pneumoniae* (% [*N*])	*P* Value	Colonized With PNSP (% [*N*])	*P* Value
Age (mo)		<.001		.01
0 to <5	8 (9/109)		2 (2/107)	
5 to <24	29 (78/270)		12 (30/262)	
24 to <36	25 (26/104)		6 (6/102)	
36+	30 (77/259)		7 (17/247)	
Sex				
Male	26 (106/401)	NS	8 (32/389)	NS
Female	25 (84/340)		7 (23/328)	
Race				
White	26 (144/548)	NS	8 (42/532)	NS
Nonwhite	26 (41/159)		7 (11/151)	
Current RTI				
Yes	40 (78/195)	<.001	19 (35/187)	<.001
No	20 (109/534)		4 (20/519)	
Current otitis media				
Yes	54 (39/72)	<.001	33 (22/67)	<.001
No	23 (148/657)		5 (33/639)	
Current antibiotic use				
Yes	20 (14/70)	NS	10 (7/68)	NS
No	26 (175/665)		8 (48/643)	
Antibiotic use in past 2 mo				
Yes	25 (74/294)	NS	12 (34/286)	<.001
No	26 (116/448)		5 (21/432)	
Child care				
≥4 h/wk	35 (105/303)	<.001	12 (35/291)	<.001
<4 h/wk	20 (77/392)		4 (16/380)	
Siblings <6 y old				
0	23 (83/367)	.04	8 (29/361)	NS
1	29 (77/266)		8 (19/251)	
>1	35 (24/68)		8 (5/65)	
Share a room with child <6 y old				
Yes	37 (52/140)	<.01	8 (10/129)	NS
No	24 (128/536)		8 (40/523)	
Breastfed (mo)				
>2	23 (62/274)	.08	8 (22/269)	NS
≤2	29 (116/405)		8 (30/386)	
Smoking				
Yes	25 (53/209)	NS	7 (13/201)	NS
No	26 (128/491)		8 (38/476)	
Prematurity-gestational age (wk)				
<36	38 (11/29)	NS	19 (5/27)	.03
≥36	25 (176/700)		7 (50/679)	

NS indicates not significant.
* *N*s for individual items vary because of age restrictions and missing data. Analysis of colonization with *S pneumoniae* based on 742 isolates. Analysis of colonization with PNSP based on 718 isolates for which resistance data were available.

This surveillance was conducted approximately 9 months after introduction of PCV7. At the time of surveillance, 74% of children aged 5 months to <24 months had received ≥2 doses, and 51% of those 24 to 36 months of age had received at least 1 dose. In crude analyses of the sample limited to children older than 5 months, rates of *S pneumoniae* carriage were similar among immunized and nonimmunized children (28% vs 29%; $P = .84$), and immunized children had similar rates of PNSP carriage compared with unimmunized children (10% vs 7%; $P = .17$). After controlling for age and other variables associated with *S pneumoniae* carriage and clustering by community, however, PCV7 immunization was associated with lower rates of colonization with PCV7-included serotypes (OR: 0.46; CI: 0.27–0.78). In multivariate models predicting *S pneumoniae* colonization or PNSP colonization overall, PCV7 immunization status was not statistically significant and was removed from the final models reported.

DISCUSSION

Isolates from generally healthy children in 16 Massachusetts communities support the growing concern regarding antibiotic-resistant *S pneumoniae*.[3,4,35] Twenty-six percent of children carried *S pneumoniae*, and 8% carried a penicillin-nonsusceptible strain. Independent risk factors for carriage of PNSP included age, group child care attendance, current RTI, and recent antibiotic use. Finally, these specimens, collected shortly after introduction of PCV7, showed similar rates of *S pneumoniae* colonization overall among vaccine recipients and nonrecipients, although decreased colonization with vaccine-included strains.

The fractions of PNSP (33%) and multidrug-resis-

TABLE 4. Multivariate Predictors of Carriage of *S pneumoniae* and PNSP Among Children in Massachusetts Communities

	Predictors of Carriage of Any *S pneumoniae**		Predictors of PNSP†	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age (mo)				
<5	1		1	
5 to <24	4.5 (2.2–9.5)	<.001	3.7 (0.81–17.1)	.09
24 to <36	3.0 (1.4–6.2)	<.01	1.2 (0.20–7.5)	NS
36+	3.4 (1.6–7.3)	<.01	1.4 (0.24–8.4)	NS
Recent antibiotics	0.69 (0.45–1.0)	.08	1.7 (1.0–2.8)	.03
Child care	2.3 (1.6–3.4)	<.001	3.9 (2.3–6.5)	<.001
RTI/AOM	2.5 (1.7–3.6)	<.001	4.7 (2.5–8.6)	<.001
Siblings at home			NI‡	
0	1			
1	1.5 (1.0–2.3)	.04		
>1	2.5 (1.5–4.2)	.0007		
Breastfed	0.58 (0.38–0.89)	.013	NI‡	

AOM indicates acute otitis media; NS, not significant.
* Analysis includes 729 children with complete data on resistance and predictors included in final model.
† Analysis includes 706 children with complete data on resistance and predictors included in final model.
‡ Not significant and therefore not included in final model.

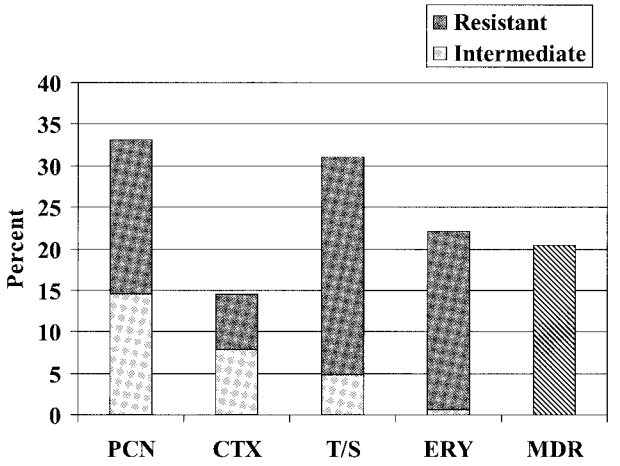

Fig 1. Percentage of *S pneumoniae* isolates with decreased susceptibility to commonly used antibiotics from children in 16 Massachusetts communities ($N = 166$). PCN, penicillin (sensitive: ≤0.06; intermediate: 0.12–1.0; resistant: ≥2.0); CTX, ceftriaxone (sensitive: ≤0.05; intermediate: 1.0; resistant: ≥2.0); T/S, trimethoprim/sulfamethoxazole (sensitive: ≤0.05/0.95; intermediate: 1/19–2/38; resistant: ≥4/76); ERY, erythromycin (sensitive: ≤0.25; intermediate: 0.05; resistant: ≥1.0); MDR, multidrug resistant, defined as nonsusceptibility to 3 or more antibiotics.

tant *S pneumoniae* are consistent with recent hospital-based reports.[9,36] Although the high prevalence of penicillin-resistant strains (MIC ≥2) is similar to that reported from some areas,[10,12] it far exceeds that found in most studies.[11,13,17] The variability in these reports is not surprising because most reflected a single geographic area. Specimens for this analysis were obtained from children in 16 noncontiguous communities, some of which are more densely populated than some regions previously studied.[13,17,18] The variability seen here by community in the fraction of *S pneumoniae* nonsusceptible to penicillin (0%–58%) partly reflects the small number of isolates from some communities but has also been seen in a study of French schools.[37] However, when we used multivariate techniques to examine the degree of variability observed, we found that it did not exceed what would be expected by chance. Sampling in primary care offices avoids the problem of clustering in settings in which children have close contact (eg, child care centers, schools) and therefore should better reflect the organisms carried in a community, as well as variation among communities.

The positive association with current RTI, reported previously,[38] may be a reflection of increased recovery of organisms from the nasopharynx of children with upper respiratory tract infections rather than of differences in colonization rates per se. We were surprised by the protective effect of breastfeeding on pneumococcal carriage, especially because the exposure was distant in time for most of these children. Although an immunologic explanation is possible, inadequate control for confounding factors (eg, socioeconomic status) may also be responsible for this finding, which requires additional study.

Contrary to many previous studies, we have distinguished the probability of carriage of a nonsusceptible organism among all children in the community (population percentage resistant) from the probability of a nonsusceptible organism among *S pneumoniae* carriers (typically reported). The analysis of this absolute risk among members of a community, dependent on both the fraction of resistant *S pneumoniae* and the rate of carriage, is more important in gauging the public health and clinical impacts of antibiotic resistance. Attendance at group child care has been a consistently identified risk factors for *S pneumoniae*[39] and PNSP colonization in previous studies[10] and is a strong predictor in our sample as well (OR: 3.9). Previous antibiotic use, especially of low dosage and long duration, has been associated with increased risk of carriage of resistant *S pneumoniae* as well.[10,11,37]

This report represents one of the first US community surveillance studies since implementation of universal immunization with PCV7 and highlights the critical need for ongoing evaluation of its impact in actual practice. Only 36% of the *S pneumoniae* recovered in this study belonged to 1 of the 7 covered serotypes, with another 30% accounted for by organisms of an included serogroup (but not a PCV7 serotype). Previous studies have documented reduced carriage of vaccine-included strains after immunization.[16,22,23] Our data confirm that PCV7 immunization substantially reduced carriage with PCV7 serotypes, but immunization was not associated with lower rates of *S pneumoniae* carriage overall. This is additional indirect evidence for serotype replacement among organisms colonizing the nasopharynx after immunization.[16,23,25] Previous surveillance in 2 Massachusetts communities showed a higher prevalence of serotype 6B and much lower prevalence of 6A than was found here (S.I.P., personal communication). However, we cannot rule out secular changes in serotype prevalence unrelated to PCV7 introduction.[40] Some clinical trials of PCVs have shown substantial decreases in 6A colonization after immunization,[16] but others have not.[23] The clinical

TABLE 5. Resistance (*N* [%]) to Common Antibiotics of 166 *S pneumoniae* Isolates, by Serotypes Grouped by Inclusion in PCV7

	N	Penicillin		Erythromycin*		Trimethoprim/Sulfa*		Ceftriaxone		MDR
		I	R	I	R	I	R	I	R	
PCV7-included										
4	2	0	0	0	0	0	0	0	0	0
6B	11	3 (27)	2 (18)	0	4 (36)	2 (18)	2 (18)	1 (9)	1 (9)	4 (36)
9V	0	-	-	-	-	-	-	-	-	-
14	6	0	1 (17)	0	1 (17)	0	1 (17)	1 (17)	0	1 (17)
18C	4	0	0	0	0	0	0	0	0	0
19F	16	2 (13)	9 (56)	0	9 (56)	1 (7)	8 (53)	3 (19)	3 (19)	10 (63)
23F	12	1 (8)	5 (42)	0	4 (36)	2 (17)	5 (42)	0	5 (42)	4 (33)
Total PCV7	51	6 (12)	17 (33)	0	18 (36)	5 (10)	16 (32)	5 (10)	9 (18)	19 (37)
PCV7 serogroups										
6A	23	6 (26)	3 (13)	0	7 (32)	0	13 (57)	0	0	3 (13)
9A	5	0	5 (100)	0	0	0	5 (100)	4 (80)	1 (20)	5 (100)
9L	2	0	1 (50)	0	1 (50)	0	1 (50)	1 (50)	0	1 (50)
9N	3	0	1 (33)	0	0	0	1 (33)	0	1 (33)	1 (33)
19A	6	6 (100)	0	0	0	0	2 (33)	0	0	0
23A	1	0	0	0	0	0	0	0	0	0
23B	3	0	0	0	0	0	0	0	0	0
Total PCV7 serogroups	43	12 (28)	10 (23)	0	8 (19)	0	22 (51)	5 (12)	2 (5)	10 (23)
Non-PCV7†										
15C	8	0	0	0	0	1 (13)	0	0	0	0
11	8	0	0	0	0	1 (13)	0	0	0	0
22F	5	0	0	0	0	0	0	0	0	0
Other	28	1 (4)	3 (11)	1 (4)	2 (7)	0	2 (7)	3 (11)	0	2 (7)
Total Non-PCV7	49	1 (2)	3 (6)	1 (2)	2 (4)	2 (4)	2 (4)	3 (6)	0	2 (4)
Total all serotyped‡	143	19 (13)	30 (21)	1 (0.7)	28 (20)	7 (5)	40 (28)	13 (9)	11 (8)	31 (22)
All specimens	166	24 (14)	31 (19)	1 (0.6)	35 (21)	8 (5)	43 (26)	13 (8)	11 (7)	34 (20)

* Sensitivity results are missing for erythromycin (3 isolates) and trimethoprim/sulfamethoxazole (2 isolates).
† Twenty serotypes total; 3 most frequent serotypes shown.
‡ 23 of 166 *S pneumoniae* isolates were not serotyped because isolates did not survive subculture and transport.

importance of serotype replacement will depend on 2 possible effects for which evidence is incomplete: 1) the capacity of PCV7 to protect against invasive disease with serotypes not included in PCV7 but within PCV7 serogroups (especially 6A and 19A) and 2) the virulence of nonvaccine strains in the absence of competition from PCV7 serotypes. Cross-protection from nonvaccine serotypes has been studied in the laboratory with mixed results. Measures of opsonophagocytic activity and antibody titers often conflict,[32] and correlation of humoral immunity with clinical effect may be poor.[22] More information clearly is needed on the capacity of non-PCV7 strains to cause serious illness in an era of universal PCV7 immunization.

Evidence regarding the virulence of non-PCV7 strains is also lacking. Although the PCV7 vaccine was developed to cover strains responsible for the majority of invasive infections, it is also true that this century has seen dramatic shifts in the most clinically significant serotypes.[40,41] A recent multicenter study of children who were hospitalized with pneumococcal pneumonia before PCV7 release showed non-PCV7 strains disproportionately responsible for complications.[42] Contrary to previous reports,[23] in this sample, PCV7 did not seem to offer protection, overall, from colonization with penicillin-nonsusceptible strains. In our sample, high rates of resistance were found in a number of non-PCV7 strains, including 6A, 9A, and 19A. Current efforts to expand the number of covered serotypes in future generations of PCV7 vaccine, to the extent possible, are likely to be important.

These results should be interpreted in the context of several limitations. Although we sampled children in 16 communities, we do not have large enough numbers of isolates to obtain precise community-specific estimates. The study cohort represents those seeking care, often for an acute illness, and only those who consented to participate. Although these communities were selected as discrete areas both geographically and demographically, we cannot rule out some contact among their inhabitants. Because the colonization rate was only 26% and only a minority of those were penicillin nonsusceptible, the analyses of predictors of colonization with nonsusceptible *S pneumoniae* are based on small numbers of isolates. Furthermore, because this surveillance was conducted relatively soon after introduction of PCV7, many of the younger children had not yet received the full PCV7 series, and most of the older children (>2 years) had not been immunized at all.

This study adds to our understanding of the prevalence of *S pneumoniae* serotypes in communities and their relationship to antibiotic resistance. There is continuing risk of infection with resistant strains, despite the benefits of universal immunization with PCV7. These data underscore the need for monitoring of both colonizing organisms and those responsible for invasive infections. For example, if the vaccine protects well against invasive pneumococcal disease with covered serotypes but does not as dramatically change colonization with these strains among young children, then we would expect limited "herd immunity" among unimmunized children and their adult (especially elderly) contacts. Over the next several years, there will be continuing value in assessing the complex interaction of PCV7 and anti-

biotic use among children on pneumococcal colonization, infection, and antimicrobial resistance.

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