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Epidemiological Markers for Interactions Among *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* in Upper Respiratory Tract Carriage

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Background. Cocolonization by *Streptococcus pneumoniae* and *Haemophilus influenzae* among children has been noted in numerous studies, as has an inverse relationship involving colonization with these species and *Staphylococcus aureus*. Interactions among these pathogens could mediate unanticipated outcomes of clinical interventions, including changes in *H. influenzae* and *S. aureus* disease incidence following pneumococcal vaccine introduction. However, it remains unclear whether cocolonization patterns represent true interspecies interactions or whether they result from confounding factors.

Methods. We investigated polymicrobial carriage using longitudinal data from 369 Bedouin children and 400 Jewish children in Israel who were enrolled in a 7-valent pneumococcal conjugate vaccine (PCV7) trial. Children were swabbed 10 times between 2 and 30 months of age.

Results. The pathogens followed distinct age and seasonal distributions, but polymicrobial carriage associations persisted after controlling for these and other confounding factors. Receipt of PCV7 resulted in pneumococcal serotype replacement but did not influence total carriage of *S. pneumoniae*, *H. influenzae*, or *S. aureus*.

Conclusions. The fact that *S. pneumoniae*, *H. influenzae*, and *S. aureus* polymicrobial carriage patterns do not result from confounding by age and season supports the idea of active interspecies interactions. However, pneumococcal serotype replacement may prevent changes in *H. influenzae* and *S. aureus* carriage among PCV7 recipients.

Keywords. *Streptococcus pneumoniae*; *Haemophilus influenzae*; *Staphylococcus aureus*; pneumococcal conjugate vaccine; serotype replacement.

Upper respiratory tract colonization is the source of transmission of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* and precedes disease. *S. pneumoniae* and *H. influenzae* are more likely to cocolonize with one another than independently, suggesting these species have a cooperative relationship; in contrast, colonization with these species is associated with lower prevalence of *S. aureus* [1]. Negative associations between carriage of *S. pneumoniae* and *S. aureus* led to concerns that pneumococcal conjugate vaccine (PCV) could have unintended consequences, such as increasing carriage or disease caused by *S. aureus* [1–3]. However, it remains unclear whether observed patterns in cocolonization result from true interspecies interactions or confounding epidemiological risk factors.

Differences in the ages when children typically carry these pathogens may contribute to confounding [4]. Colonization by *S. aureus* is most frequent among neonates and declines in

prevalence following the first weeks of life, increasing again as children approach school age [5–8]. In contrast, *S. pneumoniae* and *H. influenzae* colonization are most prevalent during later infancy [9, 10]. Another potential confounder, seasonality of carriage, has been minimally assessed in studies reporting cocolonization [1, 5, 11–14]. *S. pneumoniae* and *H. influenzae* are carried most often during the winter months, when the incidence of community-acquired *S. aureus* infections is typically lowest [9, 15]. Little is known regarding the seasonality of *S. aureus* colonization [16]. Last, most previous studies reporting associations involving *S. aureus* relied on nasopharyngeal isolates, whereas the anterior nares are the dominant anatomical niche for staphylococcal carriage [17].

Several epidemiological markers would indicate whether reported carriage associations result from interspecies interactions or confounding. First, polymicrobial carriage patterns should persist after adjustment for age, season, and other exposures expected to influence carriage. Earlier-life reductions in *S. aureus* carriage among populations acquiring *S. pneumoniae* and *H. influenzae* at younger ages would suggest that competitive interactions explain inverse associations between carriage of these pathogens and *S. aureus* [5, 18]. Additionally, differences in *H. influenzae* and *S. aureus* carriage among PCV recipients

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and nonrecipients would suggest that *S. pneumoniae* serotypes contained in PCV influence acquisition or clearance of the other species [1].

Comparative data from Jewish and Bedouin populations in southern Israel provide a unique opportunity to investigate these markers for interspecies interactions. Although they have access to the same healthcare services, Jewish and Bedouin children are socioeconomically distinct and have limited contact, living in predominantly urban areas and in scattered rural townships, respectively [19]. Geographic and climatic exposures are shared by the 2 groups and cannot account for differences in carriage rates or seasonality. While Bedouin children acquire *S. pneumoniae* earlier in life and carry pneumococci more frequently than nearby Jewish children [20], less is known about differences in *H. influenzae* and *S. aureus* carriage between the 2 populations. We used data from a randomized controlled trial of 7-valent PCV (PCV7) conducted among Jewish and Bedouin children to investigate *S. pneumoniae*, *H. influenzae*, and *S. aureus* cocolonization and risk factors, together with interspecies vaccine effects.

METHODS

Study Population and Design

The study design and pneumococcal and staphylococcal carriage data have been reported previously [21, 22]. The study received ethical approval from the Soroka University Medical Center, Maccabi Health Services, and the Israeli Ministry of Health. Participants were healthy Jewish and Bedouin children from southern Israel enrolled at public-sector mother-and-child primary healthcare centers. The Bedouin population is transitioning from a nomadic lifestyle to permanent settlements and tends to have lower socioeconomic status, larger family sizes, and higher rates of overcrowding relative to Jews in the region [19].

Jewish (n = 400) and Bedouin (n = 369) children were randomized to one of 4 study arms receiving PCV7 (containing serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) with differing dosing schedules (Supplementary Table 1). Of the total study population, 382 Jewish and 351 Bedouin children were recruited at 2 months of age and received either a 3-dose primary series (at ages 2, 4, and 6 months), with or without a booster dose at 12 months; a 2-dose primary series (at ages 4 and 6 months), with a booster at 12 months; or a booster-only series with doses at ages 12 and 18 months. The remaining 18 Jewish and 18 Bedouin children were recruited at 18 months of age and given a single booster dose. Nasopharyngeal, oropharyngeal, and nasal swabs were collected to detect bacterial carriage at 10 scheduled visits over the first 30 months of life (ages 2, 4, 6, 7, 12, 13, 18, 19, 24, and 30 months). Of 7474 scheduled visits, 6909 (92.4%) were completed, and swabs were analyzed for 6764 (90.5%). Enrollment continued from August 2005 to April 2007, with the last follow-up occurring in March 2009,

prior to the addition of PCV7 to the Israeli National Immunization Plan (July 2009). Children received all other routine immunizations according to standard schedules [21].

Laboratory Procedures

Procedures for swabbing, culturing, and pneumococcal serotyping have been reported previously [21, 22]. Carriage was defined by a positive oropharyngeal or nasopharyngeal swab for *S. pneumoniae* and *H. influenzae*, and by a positive nasal swab for *S. aureus*. Antimicrobial susceptibility for all 3 pathogens was assessed by broth dilution and disk diffusion according to US National Committee for Clinical Laboratory Standards protocols [23].

Statistical Analysis

We calculated the prevalence of *S. pneumoniae* (overall and for PCV7 and non-PCV7 serotypes separately), *S. aureus*, and *H. influenzae* carriage among children, stratifying by ethnicity (Jewish or Bedouin), study arm, vaccine doses received, age, and calendar week. We computed 95% confidence intervals (CIs) for prevalence estimates using a cluster bootstrap of individuals, thereby adjusting variance estimates to account for children's repeated sampling [24].

We constructed 3 logistic regression models, with each of *S. pneumoniae*, *H. influenzae*, or *S. aureus* carriage as the outcome, to test whether interspecies associations in carriage of each pathogen persisted after accounting for potential confounders. The models included carriage of each of the 2 other pathogens, vaccine receipt (described below), age (log transformed), sex, ethnicity, antibiotic receipt in the prior month, number of children at the home or day care where participants spent most days (log transformed), and seasonal pattern (described below). We accounted for distinct seasonal and age patterns in Jewish and Bedouin children using interaction terms. We constructed 2 additional models examining these factors as predictors for PCV7-serotype and non-vaccine-serotype *S. pneumoniae* carriage. We controlled for age as a continuous variable. This was necessary because there would be complete separation between discrete age categories and number of vaccine doses under the trial design. As with the descriptive analyses, we fitted models on cluster-bootstrapped replicates to account for serial observations of individuals when estimating 95% CIs surrounding adjusted odds ratios (aORs).

Seasonality was accounted for using sine and cosine transformations of the sampling date with periods of 4, 6, and 12 months, allowing for an asymmetrically shaped seasonal curve. We also considered whether harmonics with a 3-month period should be included in the seasonal curve, and whether age should be modeled with a linear, quadratic, or cubic transformation. We compared these alternatives using the Bayesian information criterion and found that they did not improve model fit for *S. pneumoniae*, *H. influenzae*, or *S. aureus*. We accounted for differences in the age and seasonal patterns of carriage for Jewish and Bedouin children using

interaction terms. We estimated vaccine effects as the aOR for carriage associated with having received any doses of PCV7. We also defined a second model for each pathogen, in which we estimated aORs associated with receiving 1, 2, or ≥ 3 doses (relative to 0 doses), controlling again for the previously listed confounding exposures. Analyses were implemented in R [25].

RESULTS

Population Characteristics

Study enrollment is summarized in Table 1 [21, 22]. Bedouin children inhabited households with an average (\pm SD) of 3.5 ± 1.3 persons per bedroom, compared with 2.7 ± 0.6 persons per bedroom in Jewish households (Table 1). While Bedouin children were less likely than Jewish children to attend day care (17% vs 79%), Bedouin children were on average (\pm SD) exposed to more children during the day (3.5 ± 2.3) than their Jewish counterparts (2.2 ± 1.1).

Prevalence and Characteristics of *S. pneumoniae*, *H. influenzae*, and *S. aureus* Carriage

Jewish children carried *S. pneumoniae* at 40% of visits (95% CI, 37%–42%), in contrast to 71% of visits (95% CI, 69%–73%) by Bedouin children (Table 1). *H. influenzae* carriage occurred at 42% (95% CI, 39%–44%) and 65% (95% CI, 62%–67%) of visits by Jewish and Bedouin children, respectively. In contrast, *S. aureus* was significantly more prevalent among Jewish children (18% [95% CI, 16%–20%]) than Bedouin children (14% [95% CI, 12%–16%]). PCV7 serotypes represented an equivalent proportion (31%) of overall *S. pneumoniae* carriage in the 2 populations (Table 1; serotype frequencies are in Supplementary Table 2). Overall, 60% of pneumococcal isolates exhibited reduced susceptibility to ≥ 1 antibiotic [26, 27]. Resistance was more prevalent among Bedouin children than among Jewish children and among PCV7 serotypes than among non-PCV7 serotypes. Detailed antibiotic resistance data for the pneumococcal isolates are presented in Supplementary Table 3.

Over 90% of *H. influenzae* isolates were nontypeable in both populations, and <1% of isolates were the vaccine-targeted serotype b; 17% were β -lactamase positive. Nine percent of staphylococcal isolates were methicillin resistant (Table 1).

Variation in Carriage by Age and Season

In addition to carrying these pathogens at higher overall rates, Bedouin children acquired *S. pneumoniae* and *H. influenzae* earlier than Jewish children. At 2 months of age, the prevalence of *S. pneumoniae* and *H. influenzae* was 64% (95% CI, 59%–69%) and 50% (95% CI, 44%–55%) among Bedouin children, respectively, in contrast to 21% (95% CI, 17%–26%) and 13% (95% CI, 10%–17%) among Jewish children (Figure 1). PCV7 receipt was not associated with a lower odds of carrying *S. pneumoniae* and did not alter the age distribution of carriage for this pathogen (data are presented below). Earlier *S. pneumoniae* and *H. influenzae* acquisition among Bedouin children

Table 1. Description of the Study Population

Characteristic	Bedouin (n = 369)	Jewish (n = 400)
Male sex	195 (53)	214 (54)
Day care attendance	61 (17)	316 (79)
Antibiotic use within 1 mo of any visit	245 (66)	211 (53)
Gestational age, wks	39.4 \pm 1.6	39.3 \pm 1.5
Persons per bedroom in household, no.	3.5 \pm 1.3	2.7 \pm 0.6
Children at place where child spends the day, no.	3.5 \pm 2.3	2.2 \pm 1.1
Visits completed, no.	3308	3601
Swabs analyzed/swabs collected (%) ^a	3217/3308 (97)	3547/3601 (99)
<i>S. pneumoniae</i> -positive swabs/swabs analyzed (%) ^{a,b,c}	2292/3217 (71)	1409/3547 (40)
PCV7 serotype-positive swabs	724 (32)	438 (31)
Oropharyngeal carriage	29 (1)	26 (2)
Polymicrobial carriage	1725 (75)	950 (67)
<i>H. influenzae</i> cocolonization	1628 (71)	847 (60)
<i>S. aureus</i> cocolonization	302 (13)	201 (14)
Dual cocolonization	205 (9)	98 (7)
<i>H. influenzae</i> -positive swabs/swabs analyzed (%) ^d	2081/3217 (65)	1485/3547 (42)
Beta-lactamase positive	386 (19)	225 (15)
Unencapsulated types	1933 (93)	1430 (96)
Type B	16 (1)	14 (1)
Oropharyngeal carriage only	277 (13)	287 (19)
Nasopharyngeal carriage only	67 (3)	49 (3)
Polymicrobial carriage	1687 (81)	928 (62)
<i>S. pneumoniae</i> cocolonization	1628 (78)	847 (57)
<i>S. aureus</i> cocolonization	264 (13)	179 (12)
Dual cocolonization	205 (10)	98 (7)
<i>S. aureus</i> -positive swabs/swabs analyzed (%) ^e	449/3308 (14)	642/3547 (18)
Methicillin resistant	87 (19)	10 (2)
Polymicrobial carriage	361 (80)	282 (44)
<i>S. pneumoniae</i> cocolonization	302 (67)	201 (31)
<i>H. influenzae</i> cocolonization	264 (59)	179 (28)
Dual cocolonization	205 (46)	98 (15)

Data are no. of participants (%) or mean \pm SD, unless otherwise indicated.

Abbreviations: *H. influenzae*, *Haemophilus influenzae*; PCV7, 7-valent pneumococcal conjugate vaccine; *S. aureus*, *Staphylococcus aureus*; *S. pneumoniae*, *Streptococcus pneumoniae*.

^a Further details about antimicrobial susceptibility are presented in Supplementary Table 3.

^b Serotype-specific frequencies are presented in Supplementary Table 2.

^c The numbers of *S. pneumoniae*-positive swabs in each group are used as the denominators for the remaining characteristics subsumed under this row.

^d The numbers of *H. influenzae*-positive swabs in each group are used as the denominators for the remaining characteristics subsumed under this row.

^e The numbers of *S. aureus*-positive swabs in each group are used as the denominators for the remaining characteristics subsumed under this row.

corresponded to earlier decreases in *S. aureus* carriage. Whereas an equal proportion of Jewish children (32% [95% CI, 28%–37%]) and Bedouin children (35% [95% CI, 30%–40%]) carried *S. aureus* at age 2 months, for subsequent increases in age (log scale), Bedouin children experienced 25% greater reductions (95% CI, 10%–38%) in the adjusted odds of carrying *S. aureus*, compared with Jewish children (Table 2). By 30 months of age, the prevalence of *S. aureus* carriage was 53% lower (95% CI,

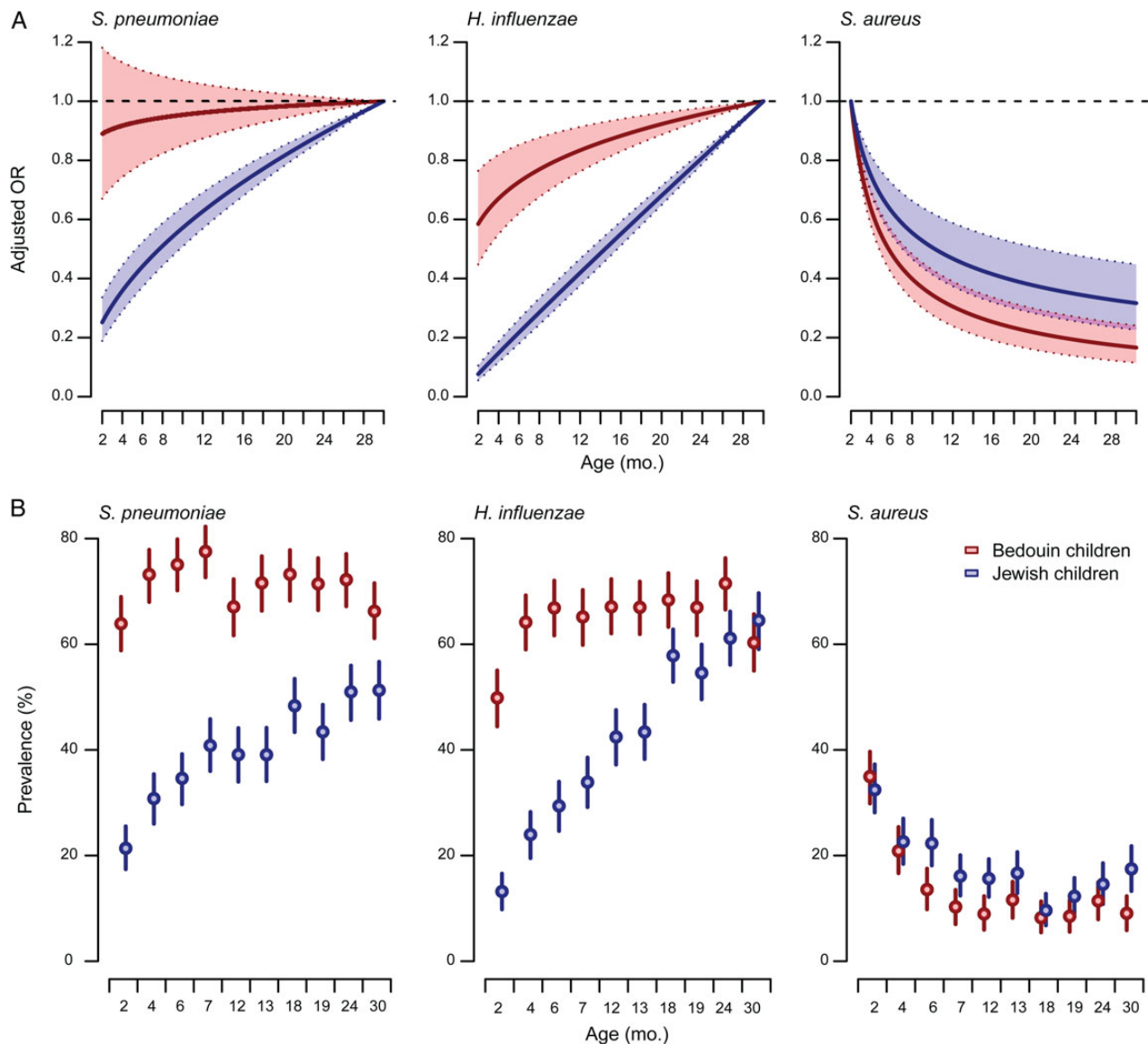


Figure 1. Variation in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* colonization, by age, among Jewish and Bedouin children. *Top*, Plots illustrate variation in carriage odds by age within each ethnic group, with age at which carriage odds were predicted to be highest as a reference (30 months, *S. pneumoniae* and *H. influenzae*; 2 months, *S. aureus*). We identified that a logarithmic trend in carriage odds across ages provided the optimal fit to data (in comparison to linear and quadratic and cubic polynomial trends) via the Bayesian information criterion. Odds ratios (ORs) are estimated via cluster-bootstrapped logistic regression adjusting for seasonal trend, antibiotic receipt, child contacts, and ethnicity. Shaded regions delineate 95% confidence intervals (CIs). *Bottom*, Plots illustrate prevalence of carriage for each pathogen by study visit age. Error bars delineate 95% CIs.

23%–72%) among Bedouin children, compared with Jewish children (aOR 0.55 [95% CI, 0.40, 0.77]).

Pathogen carriage rates varied throughout the year (Figure 2). *S. pneumoniae* carriage peaked biannually in November and June. Biannual patterns persisted after controlling for seasonally varying exposures, such as recent antibiotic use and number of daily child contacts at the time of swabbing, and were observed in both antibiotic-resistant and antibiotic-susceptible pneumococci (Supplementary Figure 1). *H. influenzae* carriage increased concurrently with *S. pneumoniae* carriage from September to November in the 2 populations, but *H. influenzae*

showed less of a biannual pattern (Figure 2). The late-summer nadir in *S. pneumoniae* and *H. influenzae* carriage coincided with a seasonal peak in *S. aureus* carriage within both populations (Figure 2). Lower *S. aureus* carriage was sustained during the autumn-winter peaks in *S. pneumoniae* and *H. influenzae* among Bedouin and Jewish children. In contrast to *S. pneumoniae*, no biannual pattern was noted in *S. aureus* carriage.

Polymicrobial Carriage

If host factors and epidemiological exposures accounted for differences in age and seasonal patterns of *S. pneumoniae*, *H. influenzae*, and *S. aureus* carriage, cocolonization associations

Table 2. Factors Associated With *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* Carriage

Factor	<i>S. pneumoniae</i>		<i>H. influenzae</i>		<i>S. aureus</i>	
	OR (95% CI)	aOR (95% CI) ^{a,b}	OR (95% CI)	aOR (95% CI) ^{a,b}	OR (95% CI)	aOR (95% CI) ^{a,b}
Ethnicity						
Bedouin (reference)
Jewish	0.27 (.23–.31)	0.14 (.10–.21)	0.39 (.34–.45)	0.11 (.07–.17)	1.36 (1.13–1.65)	0.64 (.41–.98)
Age (per log mo)^c						
Bedouin	1.02 (.92–1.12)	0.98 (.88–1.09)	1.22 (1.11–1.34)	1.21 (1.07–1.37)	0.52 (.45–.59)	0.56 (.48–.66)
Jewish	1.58 (1.43–1.76)	1.36 (1.17–1.53)	2.51 (2.24–2.81)	2.45 (2.14–2.80)	0.65 (.58–.74)	0.75 (.65–.88)
Sex						
Female (reference)
Male	1.07 (.91–1.27)	1.13 (.98–1.31)	1.04 (.89–1.21)	1.02 (.89–1.18)	1.26 (1.04–1.53)	1.26 (1.03–1.54)
Child contacts (per log no.)	1.99 (1.74–2.27)	1.49 (1.32–1.70)	1.93 (1.72–2.16)	1.55 (1.38–1.74)	0.83 (.72–.96)	0.94 (.79–1.10)
Recent antibiotic use	0.76 (.65–.88)	0.44 (.37–.52)	1.84 (1.56–2.15)	1.57 (1.31–1.87)	0.64 (.51–.80)	0.80 (.63–1.01)
PCV7 receipt (I)^b						
No doses (reference)
Any doses	...	1.05 (.85–1.30)	...	0.94 (.77–1.14)	...	0.83 (.67–1.04)
PCV7 receipt (II)^b						
0 doses (reference)
1 dose	...	1.10 (.89–1.37)	...	0.97 (.79–1.19)	...	0.84 (.67–1.05)
2 doses	...	1.07 (.84–1.37)	...	0.94 (.75–1.18)	...	0.84 (.65–1.09)
≥3 doses	...	0.95 (.73–1.23)	...	0.88 (.69–1.13)	...	0.81 (.59–1.10)
Bacterial cocolonization						
<i>S. pneumoniae</i>	3.67 (3.26–4.13)	2.82 (2.50–3.18)	0.67 (.57–.77)	0.84 (.71–.98)
<i>H. influenzae</i>	3.67 (3.26–4.13)	2.80 (2.48–3.15)	0.56 (.48–.65)	0.74 (.63–.87)
<i>S. aureus</i>	0.67 (.57–.77)	0.83 (.70–.97)	0.56 (.48–.65)	0.73 (.62–.86)

Three models were fitted, with carriage of *S. pneumoniae*, *H. influenzae*, or *S. aureus* as the outcomes.

Abbreviations: CI, confidence interval; *H. influenzae*, *Haemophilus influenzae*; PCV7, 7-valent pneumococcal conjugate vaccine; *S. aureus*, *Staphylococcus aureus*; *S. pneumoniae*, *Streptococcus pneumoniae*.

^a Multivariate analyses additionally control for sine and cosine transformations of calendar week with 4-, 6-, and 12-month periods, stratified by ethnicity (Figure 2).

^b Adjusted odds ratios (aORs), calculated by logistic regression, are presented with adjustment for having received any dose of PCV7 (I) and differ by <5% after adjustment for receipt of 1, 2, or ≥3 doses (II).

^c Unadjusted odds ratios (ORs), calculated by logistic regression, are estimated from substrata of the data from Bedouin and Jewish children only for this variable.

would not be expected to persist after adjustment for age, season, and other confounding risk factors. We found instead that interspecies bacterial carriage remained a significant predictor for carriage of each pathogen in multivariate models controlling for age, season, and other exposures (Table 2), suggesting that the patterns of carriage across ages and seasons do not explain characteristic interspecies carriage associations. *S. pneumoniae* and *H. influenzae* were each associated with 2.8-fold higher (95% CI, 2.5-fold–3.2-fold) adjusted odds of carrying the other pathogen and with an 0.8-fold lower (95% CI, 0.7-fold–1.0-fold) and 0.7-fold lower (95% CI, 0.6-fold–0.9-fold) adjusted odds of carrying *S. aureus*, respectively. Associations persisted in analyses stratified by vaccine- or non-vaccine-serotype pneumococcal carriage (Table 3). However, it is likely that our study was underpowered to provide statistically significant estimates for the inverse association with *S. aureus* in these strata.

Pneumococcal Vaccine Effects on *S. pneumoniae*, *H. influenzae*, and *S. aureus* Carriage

Children who had received PCV7 had lower odds of carrying vaccine-serotype pneumococci, compared with children who

had not received PCV7 (aOR, 0.8 [95% CI, 0.6–1.0]; Table 3 and Figure 3). In a separate model accounting for PCV7 doses received, incrementally lower odds of vaccine-serotype carriage were noted among children who received 2 doses (aOR, 0.7 [95% CI, 0.5–1.0]) and ≥3 doses (aOR 0.5 [95% CI, 0.4–0.7]), compared with unvaccinated children. Children who received PCV7 also tended to have higher odds of carrying nonvaccine pneumococcal serotypes, compared with children who did not receive PCV7 (aOR, 1.2 [95% CI, 1.0–1.5]). We noted incrementally higher odds of non-vaccine-serotype carriage after receiving 2 doses (aOR, 1.3 [95% CI, 1.0–1.7]) and ≥3 doses (aOR, 1.4 [95% CI, 1.1–1.8]). As a result of this replacement in carried pneumococcal serotypes, PCV7 receipt was not associated with any net change in children's odds for carrying *S. pneumoniae*, nor did we identify associations between vaccination and *H. influenzae* or *S. aureus* carriage (Table 3). Similarly, differences were seen primarily in vaccine-serotype and non-vaccine-serotype pneumococcal carriage when comparing the prevalence of each pathogen across the study arms within each age and ethnic group (Supplementary Table 4).

Table 3. Factors Associated With Vaccine-Serotype and Non-Vaccine-Serotype *Streptococcus pneumoniae* Carriage

Factor	PCV7 Serotypes		Non-PCV7 Serotypes	
	OR (95% CI)	aOR (95% CI) ^{a,b}	OR (95% CI)	aOR (95% CI) ^{a,b}
Ethnicity				
Bedouin (reference)
Jewish	0.49 (.41–.59)	0.27 (.16–.44)	0.40 (.34–.46)	0.27 (.17–.42)
Age (per log-month)^c				
Bedouin	0.82 (.73–.92)	1.01 (.85–1.19)	1.14 (1.03–1.25)	1.00 (.88–1.15)
Jewish	1.24 (1.07–1.43)	1.36 (1.13–1.64)	1.54 (1.37–1.74)	1.25 (1.07–1.46)
Sex				
Female (reference)
Male	1.22 (1.02–1.47)	1.20 (1.01–1.44)	0.96 (.82–1.12)	1.01 (.88–1.16)
Child contacts (per log no.)	1.31 (1.14–1.52)	1.05 (.91–1.21)	1.72 (1.53–1.94)	1.40 (1.24–1.57)
Recent antibiotic use ^d	1.27 (1.04–1.54)	1.04 (.86–1.26)	0.63 (.53–.74)	0.44 (.37–.53)
PCV7 receipt (I)^b				
No doses (reference)
Any doses	...	0.78 (.61–1.00)	...	1.23 (.99–1.53)
PCV7 receipt (II)^b				
0 doses (reference)
1 dose	...	1.01 (.79–1.28)	...	1.07 (.86–1.35)
2 doses	...	0.73 (.55–.98)	...	1.32 (1.03–1.70)
≥3 doses	...	0.54 (.40–.74)	...	1.41 (1.09–1.83)
Bacterial cocolonization				
<i>H. influenzae</i>	2.34 (2.01–2.73)	1.99 (1.69–2.35)	2.37 (2.11–2.66)	0.88 (.75–1.03)
<i>S. aureus</i>	0.80 (.65–.99)	0.90 (.73–1.12)	0.73 (.63–.84)	1.83 (1.63–2.06)

Three models were fitted, with carriage of *S. pneumoniae*, *H. influenzae*, or *S. aureus* as the outcomes.

Abbreviations: CI, confidence interval; *H. influenzae*, *Haemophilus influenzae*; PCV7, 7-valent pneumococcal conjugate vaccine; *S. aureus*, *Staphylococcus aureus*; *S. pneumoniae*, *Streptococcus pneumoniae*.

^a Multivariate analyses additionally control for sine and cosine transformations of calendar week, with 4-, 6-, and 12-month periods, stratified by ethnicity (Figure 2).

^b Adjusted odds ratios (aORs), calculated by logistic regression, control for 1, 2, and ≥3 doses of PCV7 received (I) and differ by <5% after controlling for receipt of any PCV7 dose (II).

^c Unadjusted odds ratios (ORs), calculated by logistic regression, are estimated from substrata of the data from Bedouin and Jewish children only for this variable.

^d Differences in antimicrobial susceptibility profiles of PCV7 serotypes and non-PCV7 serotypes are reported in [Supplementary Table 3](#).

DISCUSSION

Cross-sectional studies in diverse settings have identified positive correlations in carriage of *S. pneumoniae* and *H. influenzae* among children, in contrast to inverse correlations between these pathogens and *S. aureus* [1]. These findings raised concern that using PCVs to prevent certain *S. pneumoniae* serotypes from colonizing the nasopharynx could have unforeseen consequences. Comparative longitudinal data on bacterial carriage among Bedouin and Jewish children in southern Israel allowed us to investigate whether (1) polymicrobial carriage patterns are attributable to the confounding influences of age and season and (2) PCV7 causes interspecies replacement in bacterial carriage. We noted similarities in age and seasonal patterns of *S. pneumoniae* and *H. influenzae* carriage, contrasting starkly with those for *S. aureus* carriage. However, interspecies correlations persisted in our analysis after accounting for these covarying factors. Because nonvaccine serotypes offset reductions in PCV7 serotype carriage among vaccine recipients, immunization did not influence individuals' odds for carrying *S. pneumoniae*. Because *H. influenzae* and *S. aureus* carriage did not differ according to vaccine receipt, factors mediating *S. pneumoniae*

associations with these pathogens may not relate specifically to vaccine-targeted pneumococcal serotypes [3, 11].

Differences in age distributions of *S. pneumoniae*, *H. influenzae*, and *S. aureus* between Bedouin and Jewish populations indicate that epidemiological exposures, rather than host factors alone, contribute to variation in carriage risk by age. The higher prevalence of *S. pneumoniae* and *H. influenzae* in Bedouin communities is of particular importance to transmission dynamics and age of acquisition [28]. In this regard, several observations from our study and others comport with the hypothesis that competition involving *S. pneumoniae* and *H. influenzae* influences the population distribution of *S. aureus* carriage. We found higher *S. aureus* carriage among Jewish children, in contrast to higher *S. pneumoniae* and *H. influenzae* carriage among Bedouin children. Similarly, in the United States, *S. aureus* colonization is more prevalent among white, non-Hispanic children and adults in comparison to their black or Hispanic counterparts, among whom *S. pneumoniae* and *H. influenzae* carriage is more prevalent [10, 29, 30]. We also observed reductions in *S. aureus* carriage at younger ages among Bedouin children, who carried *S. pneumoniae* and *H. influenzae* earlier and

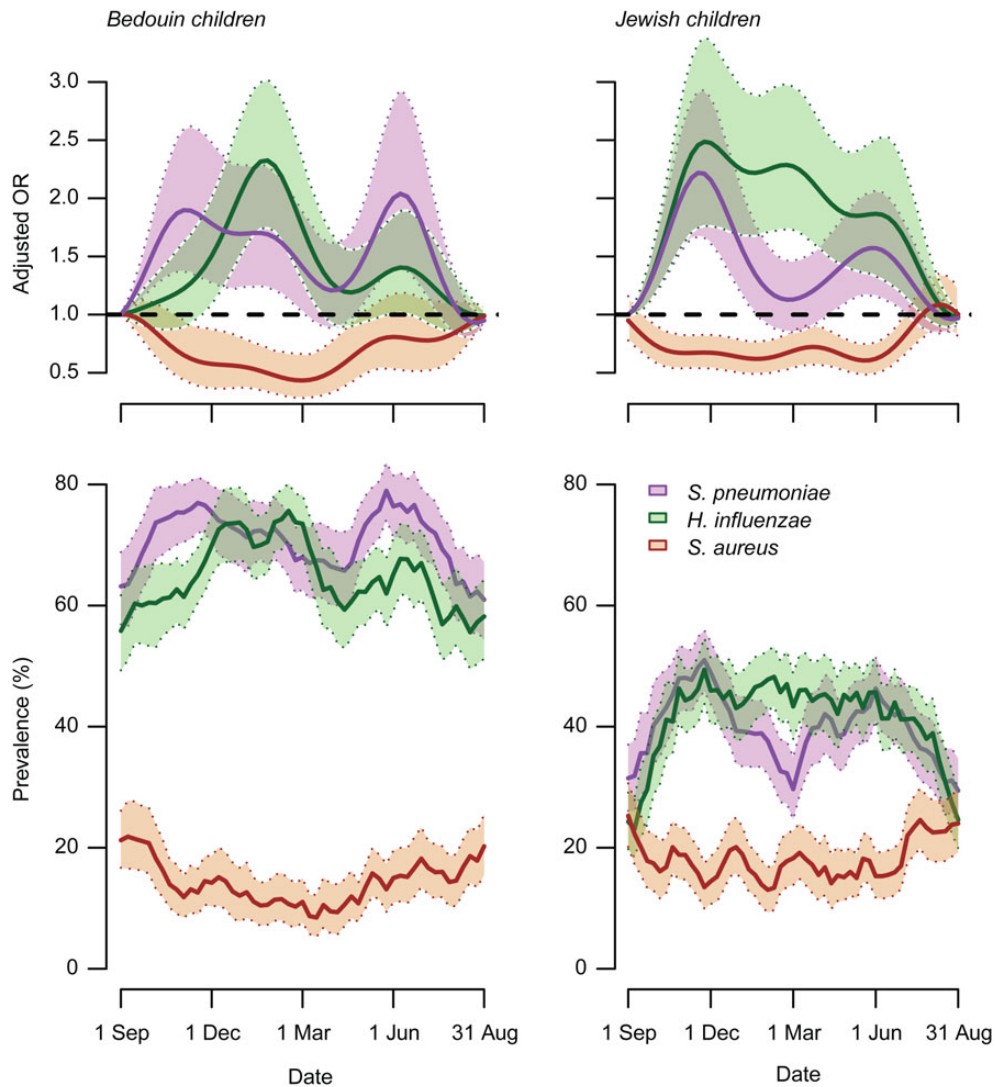


Figure 2. Seasonal variation in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* colonization among Bedouin and Jewish children. *Top*, Plots illustrate variation in carriage odds for each pathogen throughout the calendar year, with carriage during the first week of September as a reference. Seasonal trends are constructed from sine and cosine transformations of calendar week at which sampling occurred, considering 4-, 6-, and 12-month periods of oscillation. Odds ratios (ORs) are estimated via cluster-bootstrapped logistic regression adjusting for age, antibiotic receipt, child contacts, and ethnicity. Shaded regions delineate 95% confidence intervals (CIs). *Bottom*, Plots illustrate prevalence of carriage for each pathogen, aggregated with a 3-week moving average across calendar weeks. Shaded regions delineate 95% CIs.

more frequently than Jewish children. In Western Australia, earlier acquisition of *S. pneumoniae* and *H. influenzae* among Aboriginal infants (compared with non-Aboriginal infants) similarly corresponded to steeper declines in *S. aureus* carriage during the first 2 years of life, despite equal *S. aureus* carriage in the 2 populations at 1 month of age [18]. Previous *S. pneumoniae* and *H. influenzae* carriage were associated with longer times to *S. aureus* acquisition among US infants [30]. Beyond carriage patterns over the first 30 months of life described in the present study, decreases in *S. pneumoniae* and *H. influenzae* carriage coincide with increases in *S. aureus* carriage during later childhood and adolescence [5, 17, 31, 32].

S. aureus carriage exhibited a distinct seasonal pattern within both the Bedouin and Jewish populations, peaking in the

summer, when *S. pneumoniae* and *H. influenzae* carriage were at their lowest. Seasonal patterns in *S. aureus* carriage outside clinical settings have received little attention [16]. Higher summer incidence of community-acquired *S. aureus* infections, particularly skin and soft-tissue infections, including impetigo contagiosa, has been attributed to sweating during hot or humid weather, which increases the risk for disease in colonized individuals [15, 16]. We find that this seasonal pattern is also associated with a trend toward higher carriage in the summer, suggesting that transmission may be facilitated by the same hygiene factors predisposing *S. aureus* carriers to infection during warm weather. In our study, *S. pneumoniae* carriage peaked bi-annually, with a secondary low phase in March. This pattern affected antibiotic-susceptible and antibiotic-resistant pneumococcal

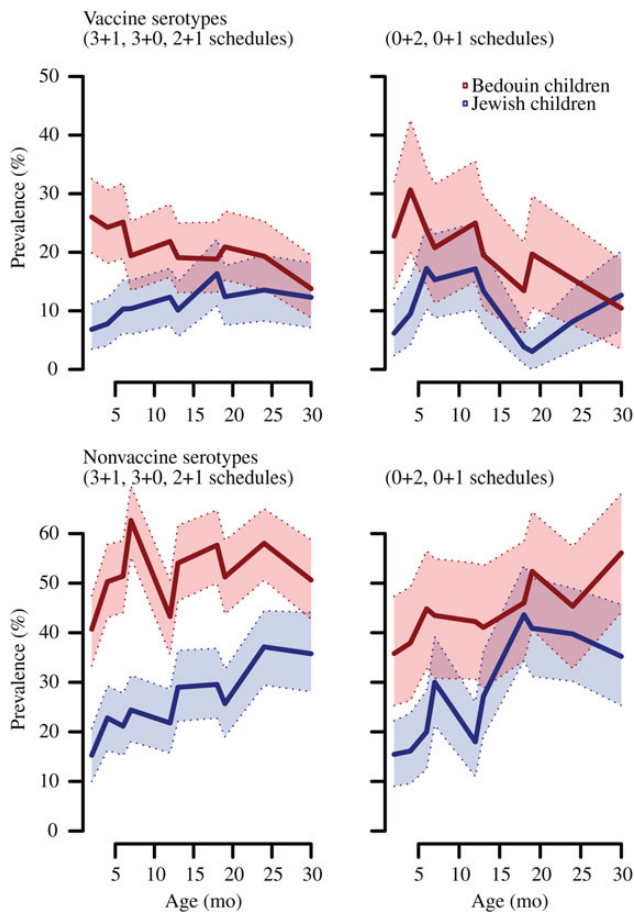


Figure 3. Vaccine and non-vaccine-serotype *Streptococcus pneumoniae* carriage among Bedouin and Jewish children. Plots illustrate prevalence of vaccine-serotype (top) and non-vaccine-serotype (bottom) carriage across study visit ages. Children are aggregated across study arms with primary-series schedules (left, “3 + 1” and “3 + 0” for 3-dose primary series at 2, 4, and 6 months with or without booster at 12 months; and “2 + 1” for 2-dose primary series at 4 and 6 months with booster at 12 months) and delayed-dosing schedules (right, “0 + 2” for doses at 12 and 18 months and “0 + 1” for 1 dose at 18 months). Shaded areas delineate 95% confidence intervals estimated by a cluster bootstrap of individuals.

strains alike, suggesting that midseason declines were not exclusively attributable to antibiotic prescribing.

Previous findings that the inverse association between *S. aureus* and *S. pneumoniae* occurs only in human immunodeficiency virus-uninfected children suggest that interspecies interference may be immune mediated [13, 14]. Indeed, nonspecific effectors elicited in response to one bacterium could play a role in clearing other microbiota. We noted significantly lower carriage of *S. aureus* among Bedouin children first at 6 months of age, although their carriage of *S. pneumoniae* and *H. influenzae* was markedly higher even at 2 months of age. This delayed effect of *S. pneumoniae* or *H. influenzae* exposure on *S. aureus* carriage suggests that acquired immune responses resulting from earlier carriage may mediate inverse interspecies associations with *S. aureus* carriage. For instance, interleukin 17-expressing CD4⁺ T cells triggered in response to *S. pneumoniae*

and *H. influenzae* facilitate phagocytic clearance of *S. aureus* [33–36]. A previous finding that *S. aureus* acquisition is delayed among infants who had previously carried other bacterial species further suggests acquired immunity as a determinant for polymicrobial carriage patterns [30]. In turn, positive associations between *S. pneumoniae* and *H. influenzae* may relate to mutualistic interactions between the species reported in animal models [37, 38].

Several previous studies assessed the effects of PCV on *S. aureus* and *H. influenzae* out of concern that immunological pressure from the vaccine would open a niche for these species. Most studies of healthy asymptomatic children have shown no interspecies effect on colonization [1]. However, intermittently higher nasopharyngeal *S. aureus* carriage was noted in the Netherlands during a precensure PCV7 trial [39], and increases in nasopharyngeal *S. aureus* and *H. influenzae* carriage coincided with the rollout of routine PCV7 administration in that country [40]. There have also been conflicting reports of an increased risk for *S. aureus* carriage in the ear canal or nasopharynx during acute otitis media among children who received PCV [41, 42]. In contrast to these studies, we assessed asymptomatic *S. aureus* colonization in the anterior nares, the dominant anatomical niche for healthy staphylococcal carriage. We found that nonvaccine pneumococcal serotypes replaced those targeted by PCV7, so that we observed no change in total carriage of *S. pneumoniae* or, in turn, *H. influenzae* and *S. aureus*. Serotype replacement in carriage has been observed in numerous settings where PCV has been implemented [1, 43–45] and may suggest that interactions underlying interspecies correlates of *S. pneumoniae* carriage are not specific to vaccine-targeted serotypes. Other pneumococcal factors, such as the pilus, have been suggested to mediate interspecies associations with *S. aureus* [46].

Our study has several limitations. By following children only to age 30 months, we did not see how declining *S. pneumoniae* and *H. influenzae* prevalence during later childhood related to *S. aureus* acquisition [5, 17, 31, 32]. Swabbing was too infrequent to identify all carriage episodes, which would have permitted us to investigate the impacts of previous or concurrent colonization on the rates that children acquired or cleared other species [30]. Whereas immunomodulation by viral infections contributes to variation in bacterial density and host susceptibility, we did not investigate how respiratory viruses influenced age and seasonal bacterial carriage patterns [47, 48]. Similarly, we assessed only 3 bacterial pathogens, although the upper respiratory tract microbiome is diverse and interactions with other species may be important determinants of carriage. Last, our analysis did not address exposures reported to influence bacterial carriage, such as breastfeeding and tobacco smoke, which have been found inconsistently to be associated with bacterial carriage [49, 50].

We found that, although *S. pneumoniae*, *H. influenzae*, and *S. aureus* follow distinct age and seasonal carriage patterns among

children, characteristic polymicrobial carriage patterns among these pathogens do not owe to confounding by age and season. Differences among Jewish and Bedouin children in the prevalence and age distribution of *S. aureus* carriage suggest that *S. pneumoniae* and *H. influenzae* exposure may impact staphylococcal colonization. PCV7 did not influence *H. influenzae* and *S. aureus* carriage, suggesting that bacterial interactions are not specific to pneumococcal serotypes contained in the vaccine. Multiple-species carriage should be monitored in trials of next-generation cellular and protein-based pneumococcal vaccines conferring pan-serotype protection, as interspecies effects may arise when vaccine-driven immunological pressure does not differentially affect pneumococcal serotypes. Clinical implications of polymicrobial carriage for otitis media, pneumonia, and invasive disease risk remain an important research area.

Supplementary Data

Supplementary materials are available at <http://jid.oxfordjournals.org>. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. D. M. W. reports receipt of investigator-initiated research funds from Pfizer by Yale University for other studies and has received consulting fees from Merck and Pfizer. R. D. has received grants and research support from Berna/Crucell, Wyeth/Pfizer, Merck, and Protea; has been a scientific consultant for Berna/Crucell, GlaxoSmithKline, Novartis, Wyeth/Pfizer, Merck, and Protea; has been a speaker for Berna/Crucell, GlaxoSmithKline, and Wyeth/Pfizer; and is a shareholder of Protea/NASVAX. G. R.-Y. has received consulting fees from Neopharm and Pfizer. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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