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Authors

Parker, Anna

Jackson, Nicole

Awasthi, Shevya

et al.

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Upper respiratory *Streptococcus pneumoniae* colonization among working-age adults with prevalent exposure to overcrowding

Anna M. Parker,¹ Nicole Jackson,² Shevya Awasthi,² Hanna Kim,² Tess Alwan,² Anne L. Wyllie,³ Katherine Kogut,⁴ Nina Holland,⁴ Ana M. Mora,⁴ Brenda Eskenazi,⁴ Lee W. Riley,² Joseph A. Lewnard^{1,2,5}

AUTHOR AFFILIATIONS See affiliation list on p. 17.

ABSTRACT Most pneumococcal disease occurs among infants and older adults and is thought to be driven by the transmission of *Streptococcus pneumoniae* from young children to these vulnerable age groups. However, pneumococcal disease outbreaks also affect non-elderly adults living or working in congregate, close-contact settings. Little is known about pneumococcal carriage in such populations. From July to November 2020, we collected saliva from low-income adult farmworkers in Monterey County, California, and tested for pneumococcal carriage following culture enrichment via quantitative PCR assays targeting the pneumococcal *lytA* and *piaB* genes. Participants were considered to carry pneumococci if *lytA* and *piaB* cycle threshold values were both below 40. Among 1,283 participants enrolled in our study, 117 (9.1%) carried pneumococci. Carriers tended more often than non-carriers to be exposed to children aged <5 years [odds ratio (OR) = 1.45 (0.95–2.20)] and overcrowding [OR = 1.48 (0.96–2.30) and 2.84 (1.20–6.73), respectively, for participants in households with >2–4 and >4 persons per bedroom vs ≤2 persons per bedroom]. Household overcrowding remained associated with increased risk of carriage among participants not exposed to children aged <5 years [OR = 2.05 (1.18–3.59) for participants living in households with >2 vs ≤2 persons per bedroom]. Exposure to children aged <5 years and overcrowding were each associated with increased pneumococcal density among carriers [*piaB* c_T difference of 2.04 (0.36–3.73) and 2.44 (0.80–4.11), respectively]. While exposure to young children was a predictor of pneumococcal carriage, associations of overcrowding with increased prevalence and density of carriage in households without young children suggest that transmission also occurs among adults in close-contact settings.

IMPORTANCE Although infants and older adults are the groups most commonly affected by pneumococcal disease, outbreaks are known to occur among healthy, working-age populations exposed to overcrowding, including miners, shipyard workers, military recruits, and prisoners. Carriage of *Streptococcus pneumoniae* is the precursor to pneumococcal disease, and its relation to overcrowding in adult populations is poorly understood. We used molecular methods to characterize pneumococcal carriage in culture-enriched saliva samples from low-income adult farmworkers in Monterey County, CA. While exposure to children in the household was an important risk factor for pneumococcal carriage, living in an overcrowded household without young children was an independent predictor of carriage as well. Moreover, participants exposed to children or overcrowding carried pneumococci at higher density than those without such exposures, suggesting recent transmission. Our findings suggest that, in addition to transmission from young children, pneumococcal transmission may occur independently among adults in overcrowded settings.

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Address correspondence to Joseph A. Lewnard, jLewnard@berkeley.edu.

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KEYWORDS *Streptococcus pneumoniae*, carriage, colonization, epidemiology, qPCR

The bacterial pathogen *Streptococcus pneumoniae* (pneumococcus) is a prominent cause of severe invasive infections as well as mucosal conditions such as acute otitis media, sinusitis, and non-bacteremic pneumonia among children and adults (1, 2). Commensal carriage of pneumococci in the upper respiratory tract is the source of transmission and a necessary precursor to pneumococcal diseases (3). Children aged 2–4 years are widely understood to serve as the primary reservoir for pneumococcal transmission. While carriage prevalence has been estimated at 30%–34% in recent studies of United States (US) children (4–6), estimates have spanned 17%–90% across settings with differing socioeconomic status, living conditions, and pneumococcal conjugate vaccine (PCV) access (7–9).

Whereas adults account for most pneumococcal disease burden in the US (10, 11) and may contribute to transmission across age groups, understanding of the prevalence and predictors of adult carriage remains limited (12). Studies employing conventional nasopharyngeal sampling techniques and culture-based identification of pneumococci have yielded low (<5%) estimates of carriage prevalence among adults, although novel molecular approaches enabling pneumococcal carriage detection from saliva have identified $\geq 20\%$ prevalence of adult carriage in some populations (13–16). Studies employing these higher-sensitivity methods for sampling and detection offer an opportunity to better understand pneumococcal epidemiology among adults, providing insight into risk factors for carriage acquisition and the role of adults in transmission within distinct communities.

Working-age adults exposed to crowded conditions have emerged as an important risk group for pneumococcal disease, with invasive pneumococcal disease outbreaks reported among miners (17), military recruits (18), shipyard workers (19), prisoners (20), displaced migrants (21), residents of homeless shelters (22), and others living or working within close, congregate settings (23). While these observations suggest that pneumococcal transmission may occur among adults under crowded conditions without the primary involvement of young children, prior studies reporting pneumococcal outbreaks in such settings have not investigated underlying carriage dynamics among adults. In the US, agricultural workers may comprise an at-risk group for such transmission due to the prevalent exposure to overcrowding, poor ventilation, and suboptimal hygienic conditions in their housing, transportation, and working environments (24). As part of a prior cross-sectional study among adult farmworkers within California's Salinas Valley, we collected saliva samples for pneumococcal carriage detection while administering SARS-CoV-2 testing in both clinical and community settings from July to November 2020 (25, 25, 26). We revisited data from this study for secondary analyses aiming to characterize potential risk factors and serotypes associated with adult pneumococcal carriage within the study communities.

RESULTS

Enrollment and inclusion

Of 1,309 participants who enrolled in the study, our analyses included 1,283 (1,099 farmworkers within the primary study group and 184 additional individuals who participated in the abridged supplemental study not restricted to farmworkers). We excluded 8 participants who did not meet the eligibility criteria (detailed in Materials and Methods), 3 participants who did not provide a saliva sample, and 15 participants whose saliva specimens did not meet the quality criteria (for reasons including label mismatch, risk of contamination, or insufficient saliva volume). Among the participants included, 566 (44.1% of 1,283) enrolled in clinical settings and 717 (55.9%) enrolled in outreach settings (Table 1; Table S1). Most participants spoke Spanish at home (1,055/1,283; 82.2%), had never attended high school (774/1,282; 60.4%), and lived in households earning <\$25,000 annually (622/1,212; 51.3%). As compared to participants recruited in clinical settings, a greater proportion of those recruited via outreach testing were born in

TABLE 1 Characteristics of the study population, by recruitment venue^a

Characteristics	Participants, no. (%)		
	All	Clinic sample	Outreach sample
	<i>N</i> = 1,283	<i>N</i> = 566	<i>N</i> = 717
Study population			
Primary study	1,099 (85.7)	551 (97.3)	548 (76.4)
Supplemental study	184 (14.3)	15 (2.7)	169 (23.6)
Age range (years)			
18–29	344 (26.8)	145 (25.6)	199 (27.7)
30–39	306 (23.9)	137 (24.2)	169 (23.6)
40–49	327 (25.5)	158 (27.9)	169 (23.6)
≥50	306 (23.9)	126 (22.2)	180 (25.1)
Sex			
Female	698 (54.4)	304 (53.7)	394 (55.0)
Male	585 (45.6)	262 (46.3)	323 (45.0)
Country of birth			
Mexico	1,012 (78.9)	484 (85.5)	528 (73.6)
United States	226 (17.6)	54 (9.5)	172 (24.0)
Other	45 (3.5)	28 (4.9)	17 (2.4)
Language spoken at home			
Spanish	1,055 (82.2)	457 (80.7)	598 (83.4)
English	118 (9.2)	25 (4.4)	93 (13.0)
Indigenous language ^b	110 (8.6)	84 (14.8)	26 (3.6)
Educational attainment (<i>N</i> = 1,282)			
Never attended school	63 (4.9)	48 (8.5)	15 (2.1)
Some primary school	460 (35.9)	225 (39.8)	235 (32.8)
Primary school completed	251 (19.6)	117 (20.7)	134 (18.7)
Some high school	148 (11.5)	66 (11.7)	82 (11.4)
High school completed	360 (28.1)	109 (19.3)	251 (35.0)
Annual household income (<i>N</i> = 1,212)			
<25,000	622 (51.3)	286 (53.5)	336 (49.6)
25,000–34,999	284 (23.4)	113 (21.1)	171 (25.3)
35,000–49,000	195 (16.1)	88 (16.4)	107 (15.8)
≥50,000	111 (9.2)	48 (9.0)	63 (9.3)
Washing machine in residence (<i>N</i> = 1,099) ^c			
No	408 (37.1)	214 (38.8)	194 (35.4)
Yes	691 (62.9)	337 (61.2)	354 (64.6)
H-2A visa (<i>N</i> = 946) ^c			
No	881 (93.1)	477 (96.0)	404 (90.0)
Yes	65 (6.9)	20 (4.0)	45 (10.0)
Years living in United States (<i>N</i> = 958) ^c			
<5 years	105 (11.0)	59 (11.7)	46 (10.1)
5–18 years	265 (27.7)	159 (31.6)	106 (23.3)
≥18 years	588 (61.4)	285 (56.7)	303 (66.6)
Field worker occupation (<i>N</i> = 1,089) ^c			
No	270 (24.8)	134 (24.8)	136 (24.8)
Yes	819 (75.2)	407 (75.2)	412 (75.2)
Workplace setting (<i>N</i> = 1,098) ^c			
Indoor and outdoor	260 (23.7)	134 (24.4)	126 (23)
Outdoor only	838 (76.3)	417 (75.6)	422 (77)
Body mass index (<i>N</i> = 1,071) ^c			
<18.5, underweight	4 (0.4)	2 (0.4)	2 (0.4)
18.5–24.9, normal	186 (17.4)	101 (19.0)	85 (15.7)
25–29.9, overweight	409 (38.2)	202 (38.0)	207 (38.3)

(Continued on next page)

TABLE 1 Characteristics of the study population, by recruitment venue^a (Continued)

Characteristics	Participants, no. (%)			
	All	Clinic sample	Outreach sample	
Cigarette smoking (N = 1,098) ^c	30–39.9, obese	427 (39.9)	205 (38.6)	222 (41.1)
	≥40, severely obese	45 (4.2)	21 (4.0)	24 (4.4)
Wearing a face covering when in proximity to others outside the home (N = 1,099) ^c	Never smoked	891 (81.1)	446 (81)	445 (81)
	Former smoker	158 (14.4)	86 (16)	72 (13)
	Current smoker	49 (4.5)	18 (3.3)	31 (5.7)
Housing type	Some of the time, rarely, or never	86 (7.8)	44 (8.0)	42 (7.7)
	All or most of the time	1,013 (92.2)	507 (92.0)	506 (92.3)
Household size (N = 1,282)	House	597 (46.5)	330 (58.3)	267 (37.2)
	Apartment	576 (44.9)	193 (34.1)	383 (53.4)
	Trailer/mobile home	45 (3.5)	23 (4.1)	22 (3.1)
	Hotel/motel	38 (3.0)	8 (1.4)	30 (4.2)
	Other	27 (2.1)	12 (2.1)	15 (2.1)
Persons per bedroom (N = 1,280)	0–2 others	222 (17.3)	100 (17.7)	122 (17.0)
	3–4 others	557 (43.5)	207 (36.6)	350 (48.8)
	≥5 others	503 (39.2)	258 (45.7)	245 (34.2)
Child aged <5 years in household	0–2 persons	854 (66.7)	337 (59.8)	517 (72.2)
	>2–4 persons	387 (30.2)	206 (36.7)	181 (25.3)
	>4 persons	39 (3.0)	21 (3.7)	18 (2.5)
SARS-CoV-2 infection (N = 1,278)	No	821 (64.0)	345 (61.0)	476 (66.4)
	Yes	462 (36.0)	221 (39.0)	241 (33.6)
	Negative	1,134 (88.7)	469 (83.2)	665 (93.1)
	Positive	144 (11.3)	95 (16.8)	49 (6.9)

^aSARS-CoV-2, severe acute respiratory syndrome coronavirus 2, as detected by clinical transcription-mediated amplification testing from oropharyngeal specimens. We indicate the total number of individuals providing answers to each question with missing data.

^bWe detail the characteristics of participants who reported speaking indigenous languages at home, versus other participants, in Table S11.

^cVariable collected among primary study participants only and not available for participants in the expanded study. We present disaggregated data for the primary and supplemental study populations in Table S1.

the US, had completed high school, lived in apartments or hotels (versus other housing types), and were not concurrently infected with SARS-CoV-2.

Pneumococcal detection

Applying quantitative PCR to culture-enriched saliva samples, we detected the pneumococcal *lytA* gene at cycle threshold (c_T) values below 40 in 342 participants (26.7% of 1,283; Table 2). Pneumococcal carriage (based on co-detection of *lytA* and *piaB* at $c_T < 40$) was present in 117 samples (9.1%), and higher-density pneumococcal carriage ($c_T < 35$ for *lytA* and *piaB*) was present in 53 samples (4.1%). The prevalence of pneumococcal carriage was 11.1% (63/566) among participants recruited in clinical settings and 7.5% (54/717) among participants recruited through outreach testing. The prevalence of higher-density pneumococcal carriage was 5.5% (31/566) and 3.1% (22/717) in clinical and outreach venues, respectively, while the prevalence of any *lytA*-positive oral streptococcal carriage was 29.3% (166/566) and 24.5% (176/717) in clinical and outreach venues, respectively (Table S2). Restricting the sample to participants with negative SARS-CoV-2 testing results, the prevalence of pneumococcal carriage, higher-density

TABLE 2 Detection of pneumococcal carriage in the study population^a

Outcome	Participants, n (%)		
	All	Clinic	Outreach
	<i>N</i> = 1,283	<i>N</i> = 566	<i>N</i> = 717
Any pneumococcal carriage	117 (9.1)	63 (11.1)	54 (7.5)
Higher-density pneumococcal carriage	53 (4.1)	31 (5.5)	22 (3.1)
Any oral streptococcal (<i>lytA</i> -positive) carriage	342 (26.7)	166 (26.3)	176 (24.5)
Sample restricted to individuals with negative SARS-CoV-2 test results	<i>N</i> = 1,134	<i>N</i> = 469	<i>N</i> = 665
Any pneumococcal carriage	85 (7.5)	37 (7.9)	48 (7.2)
Higher-density pneumococcal carriage	34 (3.0)	15 (3.2)	19 (2.9)
Any oral streptococcal (<i>lytA</i> -positive) carriage	279 (24.6)	116 (24.7)	163 (24.5)

^aSARS-CoV-2, severe acute respiratory syndrome coronavirus 2, as detected by clinical transcription-mediated amplification testing from oropharyngeal specimens. Pneumococcal detection was defined as *c_T* values below 40 for both the *lytA* and *piaB* targets. Higher-density pneumococcal carriage was defined as *c_T* values below 35 for both the *lytA* and *piaB* targets. Oral streptococcal carriage detection was defined as *c_T* values below 40 for *lytA* target.

pneumococcal carriage, and *lytA*-positive oral streptococcal carriage was 7.5% (85/1,134), 3.0% (34/1,134), and 24.6% (279/1,134), respectively, and did not differ appreciably according to the recruitment setting (<1% prevalence differences among participants enrolled in clinical and community settings).

Predictors of pneumococcal carriage

In comparison to non-carriers, pneumococcal carriers tended to more often be 30–39 years old and less often be 40–49 or ≥50 years old [odds ratio (OR) = 1.29 (95% confidence interval: 0.76–2.18), 0.77 (0.44–1.36), and 0.37 (0.18–0.75) for ages 30–39, 40–49, and ≥50 years, vs 18–29 years]. Carriers were more likely than non-carriers to speak indigenous languages in their households [OR = 3.94 (2.36–6.60)], less likely to have completed high school [OR = 0.48 (0.26–0.90)], and more likely to be engaged in field work tasks [OR = 2.02 (1.12–3.63)] (Tables 3 and 4). Pneumococcal carriers had also resided in the US for shorter periods of time than non-carriers [OR = 0.51 (0.27–0.96) for those living ≥18 years vs <5 years in the US], although this variable was inherently related to participant age. Speaking an indigenous language at home also predicted higher-density pneumococcal carriage [OR = 3.94 (2.36–6.60)], along with a lack of access to in-home laundry facilities [OR = 0.52 (0.28–0.94)].

Pneumococcal carriers were more likely than non-carriers to commute to work on employer-provided buses [OR = 1.96 (1.05–3.65)] and tended to commute with persons from outside their household more often than non-carriers [OR = 1.26 (0.76–2.09) and 2.36 (1.12–4.95) for participants commuting with 1–14 and ≥15 others from outside their household, as compared to participants who did not commute with persons from outside their household].

With regard to household exposures, pneumococcal carriers were more likely than non-carriers to be exposed to a child aged <5 years at home [OR = 1.45 (0.95–2.20)]; tended more often to live in households with >2–4 persons per bedroom [OR = 1.48 (0.96–2.30) vs <2 persons per bedroom] or >4 persons per bedroom [OR = 2.84 (1.20–6.73) vs <2 persons per bedroom]; and tended to live in households with 3–4 others [OR = 2.20 (1.04–4.64) vs <3 others] or ≥5 others [OR = 2.41 (1.15–5.07) vs <3 others; Table 4]. For the same exposures, greater effect sizes were apparent for the outcome of higher-density pneumococcal carriage.

We next aimed to determine whether the relationship between household overcrowding and pneumococcal carriage depended upon the presence of children in the household (Table 5; Table S3). Among participants residing within households without a child aged <5 years, those living in households with >2 persons per bedroom had 2.05 (1.18–3.59)-fold greater odds of pneumococcal carriage and 5.01 (1.96–12.83)-fold greater odds of higher-density pneumococcal carriage in comparison to those living in households with ≤2 persons per bedroom. Likewise, those living in households with ≥3 other inhabitants had 2.13 (0.98–4.63)-fold greater odds of pneumococcal carriage

TABLE 3 Characteristics of pneumococcal carriers and non-carriers^a

Characteristics	Participants, <i>n</i> (%)						
	No pneumococcal carriage		Pneumococcal carriage		Higher-density pneumococcal carriage		
	Primary study population	Expanded study population	Primary study population	Expanded study population	Primary study population	Expanded study population	
	<i>N</i> = 996	<i>N</i> = 1,166	<i>N</i> = 103	<i>N</i> = 117	<i>N</i> = 47	<i>N</i> = 53	
Age							
	18–29	245 (24.6)	311 (26.7)	29 (28.2)	33 (28.2)	14 (29.8)	14 (26.4)
	30–39	235 (23.6)	266 (22.8)	37 (35.9)	40 (34.2)	20 (42.6)	22 (41.5)
	40–49	264 (26.5)	299 (25.6)	26 (25.2)	28 (23.9)	10 (21.3)	12 (22.6)
	≥50	252 (25.3)	290 (24.9)	11 (10.7)	16 (13.7)	3 (6.4)	5 (9.4)
Sex							
	Female	518 (52.0)	632 (54.2)	58 (56.3)	66 (56.4)	22 (46.8)	25 (47.2)
	Male	478 (48.0)	534 (45.8)	45 (43.7)	51 (43.6)	25 (53.2)	28 (52.8)
Country of birth							
	Mexico or other	869 (87.2)	960 (82.3)	90 (87.4)	97 (82.9)	41 (87.2)	44 (83.0)
	United States	127 (12.8)	206 (17.7)	13 (12.6)	20 (17.1)	6 (12.8)	9 (17.0)
Language spoken at home							
	English	50 (5.0)	106 (9.1)	6 (5.8)	12 (10.3)	4 (8.5)	6 (11.3)
	Spanish	866 (86.9)	979 (84)	68 (66)	76 (65)	28 (59.6)	32 (60.4)
	Indigenous	80 (8.0)	81 (6.9)	29 (28.2)	29 (24.8)	15 (31.9)	15 (28.3)
Annual household income (USD) (<i>N</i> = 1,212)							
	<25,000	498 (52.3)	566 (51)	53 (58.9)	56 (54.9)	19 (45.2)	21 (44.7)
	≥25,000	455 (47.7)	544 (49)	37 (41.1)	46 (45.1)	23 (54.8)	26 (55.3)
Smoking status (<i>N</i> = 1,098)							
	Never smoked	804 (80.8)	-- ^b	87 (84.5)	--	38 (80.9)	--
	Current or former smoker	191 (19.2)	--	16 (15.5)	--	9 (19.2)	--
Years living in United States							
	<5 years	89 (10.3)	--	16 (17.8)	--	9 (22.0)	--
	5–18 years	237 (27.3)	--	28 (31.1)		15 (36.6)	
	≥18 years	542 (62.4)	--	46 (51.1)	--	17 (41.5)	--
H-2A visa (<i>N</i> = 946)							
	No	798 (93.2)	--	83 (92.2)	--	37 (90.2)	--
	Yes	58 (6.8)	--	7 (7.8)	--	4 (9.8)	--
Educational attainment (<i>N</i> = 1,282)			--				
	Less than high school	767 (77.1)	826 (70.9)	91 (88.3)	96 (82.1)	40 (85.1)	42 (79.3)
	High school diploma or higher	228 (22.9)	339 (29.1)	12 (11.7)	21 (17.9)	7 (14.9)	11 (20.7)
Child aged <5 years in household							
	No	633 (63.6)	753 (64.6)	58 (56.3)	68 (58.1)	21 (44.7)	25 (47.2)
	Yes	363 (36.4)	413 (35.4)	45 (43.7)	49 (41.9)	26 (55.3)	28 (52.8)
Household size (<i>N</i> = 1,282)							
	0–2 others	172 (17.3)	210 (18)	9 (8.7)	12 (10.3)	3 (6.4)	5 (9.4)
	3–4 others	424 (42.6)	503 (43.2)	46 (44.7)	54 (46.2)	20 (42.6)	23 (43.4)
	≥5 others	399 (40.1)	452 (38.8)	48 (46.6)	51 (43.6)	24 (51.1)	25 (47.2)
Persons per bedroom (<i>N</i> = 1,280)							
	0–2 persons	644 (64.7)	789 (67.7)	53 (52.5)	65 (56.5)	20 (43.5)	24 (46.2)
	>2–4 persons	322 (32.4)	345 (29.6)	40 (39.6)	42 (36.5)	21 (45.7)	23 (44.2)
	>4 persons	29 (2.9)	31 (2.7)	8 (7.9)	8 (7.0)	5 (10.9)	5 (9.6)

(Continued on next page)

TABLE 3 Characteristics of pneumococcal carriers and non-carriers^a (Continued)

Characteristics	Participants, <i>n</i> (%)					
	No pneumococcal carriage		Pneumococcal carriage		Higher-density pneumococcal carriage	
	Primary study population	Expanded study population	Primary study population	Expanded study population	Primary study population	Expanded study population
Washing machine in residence (<i>N</i> = 1,099)						
No	364 (36.5)	--	44 (42.7)	--	26 (55.3)	--
Yes	632 (63.5)	--	59 (57.3)	--	21 (44.7)	--
Field worker occupation (<i>N</i> = 1,089)						
No	256 (25.9)	--	14 (13.7)	--	5 (10.6)	--
Yes	731 (74.1)	--	88 (86.3)	--	42 (89.4)	--
Commuted to work with non-household members (<i>N</i> = 1,078)						
No	645 (66.0)	--	59 (58.4)	--	27 (58.7)	--
Yes	332 (34.0)	--	42 (41.6)	--	19 (41.3)	--
Commuted to work in employer-provided bus (<i>N</i> = 899)						
No	737 (90.2)	--	68 (82.9)	--	32 (84.2)	--
Yes	80 (9.8)	--	14 (17.1)	--	6 (15.8)	--
Number of people outside of household that you commute with (<i>N</i> = 912)						
0 others	552 (66.7)	--	48 (57.1)	--	24 (60.0)	--
1–14 others	224 (27.1)	--	26 (31.0)	--	10 (25.0)	--
≥15 others	52 (6.3)	--	10 (11.9)	--	6 (15.0)	--
Wearing a face covering when in proximity to others outside the home (<i>N</i> = 1,099)						
Some of the time, rarely, or never	78 (7.8)	--	8 (7.8)	78 (7.8)	5 (10.6)	--
All or most of the time	918 (92.2)	--	95 (92.2)	918 (92.2)	42 (89.4)	--

^aWe indicate the total number of individuals providing answers to each question with missing data.

^bDashes (–) indicate data not available for the expanded study population.

and 3.25 (0.73–14.37)-fold greater odds of higher-density pneumococcal carriage in comparison to those living in households with <3 others.

We also aimed to address the possibility that crowded housing and commuting exposures could be related to arrangements such as living in dormitory-style or employer-provided facilities. We repeated analyses of the association of commuting arrangements with pneumococcal carriage within the subgroup of participants who lived in households with ≤2 persons per bedroom. Within this subgroup, participants commuting to work in an employer-provided bus had 2.03 (0.66–6.27)-fold greater odds of pneumococcal carriage than those not commuting in an employer-provided bus, while those who commuted in vehicles with 1–14 and ≥15 others from outside their household had 1.22 (0.61–2.43)- and 1.71 (0.37–7.94)-fold greater odds of pneumococcal carriage than those who did not commute with persons from outside their household (Table 6; Table S4).

TABLE 4 Associations of pneumococcal carriage and high-density carriage with participant characteristics^a

Characteristic	Odds ratio (95% CI)				
	Any pneumococcal carriage		Higher-density pneumococcal carriage		
	Primary study population	Expanded study population	Primary study population	Expanded study population	
Age					
	18–29	ref.	ref.	ref.	ref.
	30–39	1.29 (0.76–2.18)	1.38 (0.84–2.26)	1.46 (0.71–3.01)	1.79 (0.89–3.63)
	40–49	0.77 (0.44–1.36)	0.80 (0.47–1.37)	0.61 (0.26–1.41)	0.79 (0.35–1.76)
	≥50	0.37 (0.18–0.75)	0.53 (0.28–0.98)	0.21 (0.06–0.76)	0.40 (0.14–1.30)
Sex					
	Female	ref.	ref.	ref.	ref.
	Male	0.82 (0.54–1.24)	0.88 (0.60–1.30)	1.18 (0.65–2.14)	1.25 (0.71–2.20)
Country of birth					
	Mexico or other	ref.	ref.	ref.	ref.
	United States	1.10 (0.59–2.06)	1.13 (0.67–1.90)	1.21 (0.49–2.98)	1.23 (0.57–2.65)
Language spoken at home					
	English or Spanish	ref.	ref.	ref.	ref.
	Indigenous	3.94 (2.36–6.60)	3.80 (2.28–6.33)	4.18 (2.08–8.40)	4.03 (2.01–8.08)
Household income					
	Below \$25,000	ref.	ref.	ref.	ref.
	\$25,000 or above	0.78 (0.50–1.21)	0.89 (0.59–1.35)	1.37 (0.73–2.57)	1.39 (0.76–2.52)
Marital status					
	Not married or living as married	ref.	ref.	ref.	ref.
	Married or living as married	1.38 (0.88–2.14)	1.38 (0.92–2.07)	1.91 (0.97–3.75)	1.99 (1.06–3.72)
Cigarette smoking					
	Never smoked	ref.	--	ref.	--
	Current or former smoker	0.78 (0.44–1.37)	--	1.04 (0.49–2.21)	--
Years living in United States					
	<5 years	ref.	--	ref.	--
	5–18 years	0.68 (0.35–1.34)	--	0.66 (0.27–1.60)	--
	≥18 years	0.51 (0.27–0.96)	--	0.36 (0.15–0.85)	--
H-2A visa					
	No	ref.	--	ref.	--
	Yes	1.00 (0.43–2.33)	--	1.30 (0.43–3.95)	--
Educational attainment					
	Less than high school	ref.	ref.	ref.	ref.
	High school diploma or higher	0.48 (0.26, 0.90)	0.59 (0.36, 0.97)	0.68 (0.30, 1.56)	0.76 (0.38, 1.52)
Child aged <5 years in household					
	No	ref.	ref.	ref.	ref.
	Yes	1.45 (0.95–2.20)	1.38 (0.93–2.05)	2.42 (1.32–4.43)	2.25 (1.28–3.98)
Household size					
	0–2 others	ref.	ref.	ref.	ref.
	3–4 others	2.20 (1.04–4.64)	1.96 (1.02–3.78)	2.89 (0.84–9.97)	2.00 (0.74–5.40)
	≥5 others	2.41 (1.15–5.07)	1.98 (1.03–3.83)	3.60 (1.06–12.27)	2.32 (0.86–6.22)
Persons per bedroom					
	≤2 people	ref.	ref.	ref.	ref.
	>2 people to 4 people	1.48 (0.96–2.30)	1.42 (0.94–2.16)	2.00 (1.06–3.79)	2.05 (1.13–3.74)
	Over 4 people	2.84 (1.20–6.73)	2.55 (1.09–5.95)	4.81 (1.62–14.29)	4.38 (1.50–12.79)
Washing machine in residence					
	No	ref.	-- ^b	ref.	--
	Yes	0.83 (0.55–1.27)	--	0.52 (0.28–0.94)	--
Field worker occupation					

(Continued on next page)

TABLE 4 Associations of pneumococcal carriage and high-density carriage with participant characteristics^a (Continued)

Characteristic	Odds ratio (95% CI)				
	Any pneumococcal carriage		Higher-density pneumococcal carriage		
	Primary study population	Expanded study population	Primary study population	Expanded study population	
Workplace setting	No	ref.	--	ref.	--
	Yes	2.02 (1.12–3.63)	--	2.63 (1.02–6.77)	--
Commuted to work with non-household members	Indoors and outdoor	ref.	--	ref.	--
	Outdoors only	1.67 (0.96–2.92)	--	1.46 (0.67–3.20)	--
Commuted to work in employer-provided bus	No	ref.	--	ref.	--
	Yes	1.35 (0.89–2.05)	--	1.31 (0.72–2.40)	--
Number of people outside of household that you commute with	No	ref.	--	ref.	--
	Yes	1.96 (1.05–3.65)	--	1.82 (0.74–4.50)	--
Wearing a face covering when in proximity to others outside the home	0 others	ref.	--	ref.	--
	1–14 others	1.26 (0.76–2.09)	--	0.95 (0.45–2.04)	--
	≥15 others	2.36 (1.12–4.95)	--	2.95 (1.14–7.61)	--
Wearing a face covering when in proximity to others outside the home	All or most of the time	ref.	--	ref.	--
	Some of the time, rarely, or never	1.12 (0.52–2.41)	--	1.68 (0.63–4.46)	--

^aOdds ratios are computed via conditional logistic regression models stratified by recruitment venue and SARS-CoV-2 infection status.

^bDashes (–) indicate data not available for the expanded study population.

TABLE 5 Associations of pneumococcal carriage and higher-density carriage with crowding among participants without residential exposure to children aged <5 years^a

Characteristic	Odds ratio (95% CI)				
	Any pneumococcal carriage		Higher-density pneumococcal carriage		
	Primary study population	Expanded study population	Primary study population	Expanded study population	
Household size	0–2 others	ref.	ref.	ref.	ref.
	3–4 others	2.10 (0.93–4.72)	1.95 (0.94–4.04)	2.93 (0.63–13.66)	2.22 (0.61–8.07)
	≥5 others	2.18 (0.91–5.23)	1.98 (0.89–4.41)	3.81 (0.78–18.64)	2.99 (0.78–11.51)
	≥3 others	2.13 (0.98–4.63)	1.96 (0.97–3.95)	3.25 (0.73–14.37)	2.48 (0.72–8.57)
Persons per bedroom	≤2 persons	ref.	ref.	ref.	ref.
	>2–4 persons	1.78 (0.98–3.23)	1.68 (0.95–2.97)	4.41 (1.67–11.83)	4.00 (1.64–9.74)
	≥4 persons	2.93 (0.86–9.90)	2.58 (0.77–8.59)	6.49 (1.15–36.63)	5.14 (0.94–28.00)
	>2 persons	2.05 (1.18–3.59)	1.92 (1.13–3.25)	5.01 (1.96–12.83)	4.42 (1.90–10.28)

^aOdds ratios are computed via conditional logistic regression models stratified by recruitment venue and SARS-CoV-2 infection status. Due to the limited statistical power within the subset of participants not exposed to children aged <5 years within their households, we present disaggregated estimates (per the analyses in Table 4) for three exposure categories as well as estimates for exposure to 0–2 vs ≥3 household members and exposure to households with ≤2 vs >2 persons per bedroom.

TABLE 6 Associations of pneumococcal carriage and higher-density carriage with commuting exposures among participants without exposure to household crowding^a

Characteristic	Odds ratio (95% CI)	
	Any pneumococcal carriage	Higher-density pneumococcal carriage
Commuted to work in employer-provided bus (N = 899)	No	ref.
	Yes	2.03 (0.66–6.27)
Number of people outside of household that you commute with (N = 912)	0 others	ref.
	1–14 others	1.22 (0.61–2.43)
	≥15 others	1.71 (0.37–7.94)

^aOdds ratios are computed via conditional logistic regression models stratified by recruitment venue and SARS-CoV-2 infection status. Commuting exposures were not collected for the extended population; therefore, analysis is limited to the primary study population.

Pneumococcal carriage density

Among participants carrying pneumococci, those living with a child aged <5 years, on average, had *piaB* c_T values 2.04 (0.36–3.73) units lower than those not living with a child aged <5 years (Fig. 1; Tables S5 and S6). Similarly, pneumococcal carriers exposed to household overcrowding (defined as >2 persons per bedroom in the household), on average, had *piaB* c_T values 2.44 (0.80–4.11) units lower than those not exposed to household overcrowding. We did not identify any participant characteristics or exposures associated with differences in *lytA* c_T values.

Serotype distribution

The most commonly detected serotypes among pneumococcal carriers were 15A ($n = 22$, 18.8%), 24F/A/B ($n = 14$, 12.0%), 10A ($n = 12$, 10.3%), and 23B ($n = 10$, 8.5%; Table 7). Among 78 participants with any serotype detected (66.7% of all 117 pneumococcal carriers), 13 (16.7%), 14 (17.9%), and 32 (41.0%) participants carried serotypes covered by PCV13, PCV15, and PCV20, respectively. The detection of a pneumococcal serotype was 33.2% (3.2%–67.9%) more likely among pneumococcal carriers with concurrent SARS-CoV-2 infection than among those without concurrent SARS-CoV-2 infection [26/32 (81.3%) and 52/85 (61.2%), respectively], in line with our prior finding of higher pneumococcal carriage density among participants with concurrent SARS-CoV-2 infection (26). Overall, serotype distributions did not differ appreciably among participants who were infected or not infected with SARS-CoV-2 ($P > 0.1$ by χ^2 test).

Symptoms

Among participants not infected with SARS-CoV-2, the odds of reporting any solicited symptoms or any respiratory symptoms did not differ appreciably according to pneumococcal carriage detection or carriage density (Tables S7 and S8). However, higher-density pneumococcal carriers had greater odds of reporting fever [OR = 4.72 (1.27–17.45)], sweating [OR = 4.42 (1.21–16.14)], and difficulty breathing [OR = 9.16 (2.36–35.48)] in comparison to participants who did not concurrently carry pneumococci. Within analyses that included all participants (matched on SARS-CoV-2 infection status), pneumococcal carriers had greater odds of reporting pressure in the ears [OR = 3.49 (1.32–9.23)], fever [OR = 2.63 (1.28–5.41)], chills [OR = 2.04 (1.03–4.02)], difficulty breathing [OR = 3.12 (1.34–7.23)], and shortness of breath [OR = 3.72 (1.39–9.94)]. The same symptoms were associated with higher-density pneumococcal carriage (Tables S9 and S10).

TABLE 7 Pneumococcal serotypes detected, stratified by SARS-CoV-2 infection status^a

Serotype	Participants, n (%)		
	All participants	SARS-CoV-2 negative participants	SARS-CoV-2 positive participants
	N = 117	N = 85	N = 32
15A	22 (18.8)	15 (17.6)	7 (21.9)
24F/A/B	14 (12)	8 (9.4)	6 (18.8)
10A	12 (10.3)	10 (11.8)	2 (6.3)
23B	10 (8.5)	7 (8.2)	3 (9.4)
22A/F	5 (4.3)	3 (3.5)	2 (6.3)
22A	3 (2.6)	2 (2.4)	1 (3.1)
22F	1 (0.9)	0 (0)	1 (3.1)
Indistinguishable 22A/F	1 (0.9)	1 (1.2)	0 (0)
9L/N	5 (4.3)	2 (2.4)	3 (9.4)
35F/47F	5 (4.3)	5 (5.9)	0 (0)
3	4 (3.4)	1 (1.2)	3 (9.4)
34	4 (3.4)	2 (2.4)	2 (6.3)
13	4 (3.4)	4 (4.7)	0 (0)
28A/F	4 (3.4)	3 (3.5)	1 (3.1)
11A/D/E	4 (3.4)	2 (2.4)	2 (6.3)
14	3 (2.6)	2 (2.4)	1 (3.1)
15B/C	3 (2.6)	2 (2.4)	1 (3.1)
23F	2 (1.7)	2 (2.4)	0 (0)
7F/A	2 (1.7)	1 (1.2)	1 (3.1)
16F	1 (0.9)	1 (1.2)	0 (0)
19A	1 (0.9)	0 (0)	1 (3.1)
6A/B	1 (0.9)	1 (1.2)	0 (0)
12F/44	0 (0)	0 (0)	0 (0)
8	0 (0)	0 (0)	0 (0)
38	0 (0)	0 (0)	0 (0)
1	0 (0)	0 (0)	0 (0)
20	0 (0)	0 (0)	0 (0)
Any serotype detected	78 (66.7)	52 (61.2)	26 (81.3)
Serotypes grouped according to PCV inclusion			
PCV13 serotypes	13 (11.1)	7 (8.2)	6 (18.8)
PCV15 serotypes	14 (12.0)	7 (8.2)	7 (21.9)
PCV20 (including 15B/C)	32 (27.4)	21 (24.7)	11 (34.4)
Number of serotypes detected per sample			
1	53 (45.3)	34 (40.0)	19 (59.4)
2	20 (17.1)	16 (18.8)	4 (12.5)
3	3 (2.6)	1 (1.2)	2 (6.3)
4	2 (1.7)	1 (1.2)	1 (3.1)

^aSerotype findings are presented only for specimens where serotype-specific *c_T* values were within the range of ±2 from the *piab* *c_T* value. Serotypes 4, 9V/9A, 17F, 21, 23A, 33A/33F, and 35B are excluded due to the low specificity of these probes in previous publications. Serogroup 6A/B was differentiated from 6A/B/C/D using conventional PCR methods.

DISCUSSION

Our findings provide insight into several facets of pneumococcal carriage among adults within a population with low socioeconomic status and prevalent exposure to overcrowding. Within our study, 7.5% of participants without SARS-CoV-2 infection carried pneumococci, including 7.9% of those recruited in clinics and 7.2% of those recruited in community settings. Our findings implicate exposure to young children (aged <5 years) as a key risk factor for pneumococcal carriage, consistent with other studies enrolling

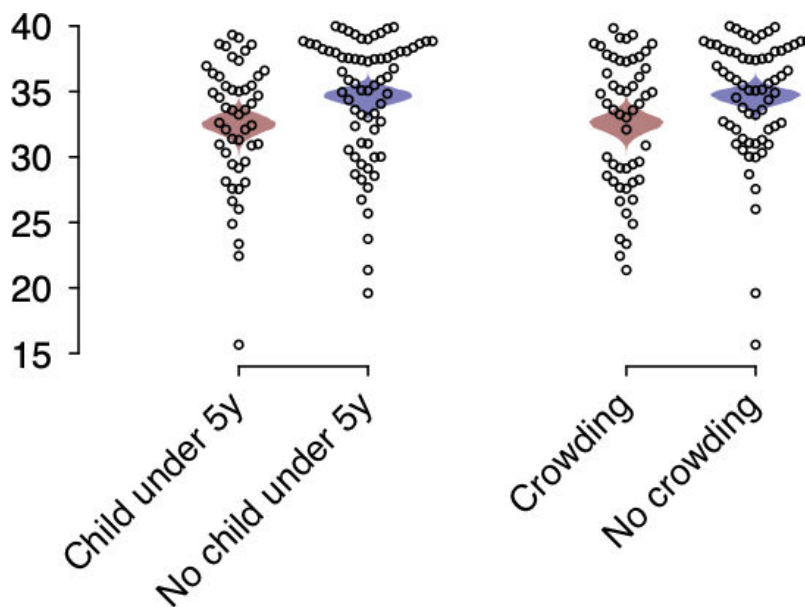


FIG 1 Factors predicting pneumococcal carriage density. We illustrate *piaB* c_T value distributions among participants found to carry pneumococci based on their exposure to children aged <5 years in the household and crowding in the household. Crowding is defined as living in a household with ≥ 2 persons per bedroom. Mean between-group differences in c_T values were -2.04 (-3.73 , -0.36) for households with or without children aged <5 years and -2.44 (-4.11 , -0.80) for crowded vs uncrowded households; including data from the expanded study population, mean between-group differences in c_T values were -2.08 (-3.85 , -0.33) for crowded vs uncrowded households and -2.17 (-3.95 , -0.41) for households with or without children aged <5 years. Shaded polygons illustrate the distributions of mean estimates within each stratum based on bootstrap resampling.

adults (16, 27). However, we also found that exposure to household overcrowding (>2 persons per bedroom) was independently associated with an increased risk of pneumococcal carriage, including among adults in households without young children. Each of these associations was strengthened when evaluating an outcome of higher-density pneumococcal carriage (*lytA* and *piaB* $c_T < 35$). Because higher-density carriage can be a transient state resulting from recent pneumococcal acquisition, these findings may indicate that individuals with residential exposure to young children and overcrowding acquire pneumococci with greater frequency. Taken together, our findings suggest that in addition to transmission from young children to adults, transmission among adults may be an important feature of pneumococcal epidemiology in populations with prevalent exposure to residential overcrowding. This finding from adult carriage data may help to explain observations such as the occurrence of pneumococcal disease outbreaks among otherwise healthy, non-elderly adults living or working in close, congregate settings (17–22).

Consistent with other indications of transmission among adults in crowded settings, pneumococcal carriage was also more common among participants who commuted to work in employer-provided buses or via other modes of transportation shared with ≥ 15 others from outside their household. Directionally similar findings persisted in analyses subset to participants who did not live in overcrowded households. Although underpowered due to the limited sample size, consistency of effect estimates within this subgroup suggests that the finding cannot owe exclusively to the fact that many individuals who commuted to work via such arrangements also lived in close, congregate settings such as employer-provided or dormitory-style housing. Crowded vehicles may, thus, represent a setting for pneumococcal transmission within our study population, who often rely on such means of transportation to reach remote work sites. This finding is consistent

with meningococcal outbreak investigations implicating school buses as settings for transmission (28).

Our study also identified several risk factors for pneumococcal carriage related to lower socioeconomic status within this community, including indigenous ethnic or cultural background (indicated by speaking an indigenous language at home), lower educational attainment, and engagement in fieldwork tasks (which receive lower pay in comparison to other agricultural occupations such as machine operation or truck driving). Individuals without access to in-home laundry facilities were also at increased risk of higher-density carriage. This finding may reflect similar socioeconomic status considerations or may result from transmission within laundromats (*lavanderías*), which represent important community gathering places within the study setting and remained open during the pandemic.

Because our study was undertaken during the early phases of the COVID-19 pandemic, our questionnaires also addressed behaviors aiming to reduce pathogen transmission. Whereas the use of face coverings was not associated with participants' risk of pneumococcal carriage, prior studies have suggested that face masks may reduce the risk of carriage acquisition among healthcare workers (29) and Hajj pilgrims (30). The effectiveness of this approach may depend, in part, upon the type of face coverings worn and the relative contributions of airborne, droplet, or fomite exposures to pneumococcal carriage acquisition. While associations of pneumococcal carriage with close-contact exposures such as crowded housing and commuting conditions in our study are more consistent with droplet than airborne routes of spread, the understanding of pneumococcal transmission mechanisms remains limited.

Overall, 11.1%, 12.0%, and 27.4% of pneumococcal carriers in our study were found to carry serotypes targeted by PCV13, PCV15, and PCV20, respectively. These findings are broadly in agreement with prior evidence (31) that PCV20 (in comparison to PCV15) offers a greater improvement in coverage of serotypes prevalent in mucosal carriage or infections of the upper respiratory tract over PCV13. Pediatric PCV coverage is not known within our specific study setting, although prior studies suggest that coverage may be high within similar populations. Coverage has been estimated at 92% among children residing in the US with parents born in Mexico (32) and 95% among children in Mexico (33). Because a low proportion of pneumococcal carriage was associated with PCV13-targeted serotypes within our sample, it is unlikely that there is a reservoir of vaccine-serotype pneumococcal circulation in the study communities associated with unvaccinated or undervaccinated children. As the US and Mexico first implemented PCV7 in 2000 and 2006, respectively, most adults within our sample were ineligible to have received PCVs as children.

Despite prevalent overcrowding and low socioeconomic status within our study population, our estimates of adult pneumococcal carriage prevalence are similar to others from studies using saliva-based sampling and molecular detection methods (13–16). Several factors may account for this finding. Most (64%) of the participants were not exposed to children aged <5 years at home. Although we did not test for respiratory viruses other than SARS-CoV-2, circulation of such viruses was heavily suppressed during the study period, including in California (34); it is, thus, unlikely that such testing would identify appreciable numbers of infections. As infection with these viruses is associated with increased pneumococcal carriage acquisition and carriage density (27, 35, 36), their absence may have contributed to lower pneumococcal carriage prevalence and density during the study period. Lastly, assessments of pneumococcal carriage were a secondary objective of our study, which primarily addressed SARS-CoV-2 infection among farm workers in clinical and community settings. While our findings reveal characteristics associated with pneumococcal carriage, our results should not be considered to provide generalizable measures of pneumococcal carriage prevalence among farm workers—some of whom may have avoided participation due to fear of SARS-CoV-2 testing and implications for employment, income, and visibility to government authorities.

Several further limitations of our study should be noted. First, due to the evaluation of pneumococcal carriage as a secondary endpoint, our study was not designed to support causal inference about pathways of pneumococcal transmission. Many variables, such as indigenous ethnicity, education, income, and fieldwork tasks, are collinear and may each imperfectly measure similar socioeconomic status characteristics rather than serve as independent predictors of carriage. Second, our design is cross-sectional and, thus, does not offer an opportunity to establish temporality between exposures and pneumococcal carriage acquisition. Longitudinal studies sampling household members and other close contacts are needed to elucidate transmission dynamics. Such studies may also provide insight into the temporal relationship between pneumococcal carriage acquisition and onset of respiratory symptoms, which our analysis could not address. Third, the living and working conditions of farmworkers are distinct from those of the general public; thus, associations may vary in differing communities. For example, exposures of interest such as overcrowding were prevalent within our study population. Studies enrolling more diverse participants (including individuals with higher socioeconomic status) may be positioned to identify greater effect sizes due to greater variation in exposure. Fourth, stronger associations of measured risk factors with higher-density pneumococcal carriage may relate to the greater specificity of this outcome in comparison to pneumococcal carriage detection at the *lytA* and *piaB* $c_T < 40$ threshold. Fifth, the infeasibility of colony-based pneumococcal detection methods for the assessment of adult carriage limited our ability to characterize serotype distributions in this study population. While restricting serotyping results to samples with c_T values within ± 2 of values observed for *piaB* helped to maximize specificity, we could not study all serotypes due to the non-specific association of some *cps* genes with other colonizing bacteria. Sixth, reliance on saliva samples alone without other accompanying upper respiratory specimens may have limited our ability to identify all pneumococcal carriers. Other studies using similar molecular approaches have reported 85%–87% sensitivity of saliva-only sampling relative to the combined use of saliva and nasopharyngeal/oropharyngeal or trans-nasal and trans-oral specimens (37, 38); a lack of gold standard prohibits the determination of specificity, although the use of a two-probe (*lytA* and *piaB*) qPCR protocol limits the risk of false positive detections in our study (39). Last, our study was not designed or powered to support inferential hypothesis testing; point estimates and confidence intervals should be interpreted as measures of effect sizes rather than hypothesis tests. Subgroup analyses, in particular, are underpowered and are intended to evaluate consistency in the directional nature of relationships observed within the larger sample rather than to support hypothesis testing.

In conclusion, our study identifies exposure to young children and residential overcrowding—including in households without young children—as important risk factors for pneumococcal carriage among adults in low-income agricultural communities within California. The association of these factors with higher carriage density further suggests that individuals encountering young children or crowded conditions within the residential environment acquire pneumococcal carriage with greater frequency. While confirming prior understanding that young children serve as a reservoir for the transmission of pneumococci, these findings also suggest that transmission among adults may play an important feature in pneumococcal epidemiology among populations exposed to overcrowding. Strategies to prevent transmission under such conditions, or to reduce individuals' risk of severe pneumococcal disease through vaccination, may be of value for certain populations.

MATERIALS AND METHODS

Study setting

The Salinas Valley in Monterey County, California, is home to an agricultural workforce of approximately 50,000 resident workers and 40,000 seasonal workers (24). Most are undocumented Latino migrants from Mexico and Central American countries, who often

reside in crowded multigenerational housing, including makeshift residences (40, 41). The local workforce also includes holders of temporary agricultural H-2A visas, many of whom receive employer-provided group housing within motels or dormitories (42). We undertook the parent study in partnership with Clínica de Salud del Valle de Salinas (CSVS), a Federally Qualified Health Center focused on providing health care for agricultural workers and their families within the region. At the time of our study, CSVS offered SARS-CoV-2 testing at no cost to all members of the community, both at their network of Salinas Valley clinics and off-site at community testing events held at housing complexes, agricultural fields, community health fairs, and other sites.

Shelter-in-place recommendations and other non-pharmaceutical interventions were in place during the study period. We have previously described compliance with such guidance within the study population in home, workplace, and community settings (43). Overall, 92% of participants reported routinely wearing face coverings when outside of their household in close proximity to others, 10% reported attending social gatherings outside of their household, and 5% reported attending indoor social gatherings, which were prohibited throughout the study period. Roughly half of participants reported fever or symptom screening at their workplaces. School closures were in force throughout Monterey County. While guidance for the reopening of childcare facilities was tied to county-level COVID-19 case reporting, informal childcare arrangements may have remained in place to enable continued workforce participation for parents.

Study design

Enrollment for the study occurred between 16 July and 30 November 2020. For the primary study, we invited non-pregnant farmworkers aged ≥ 18 years who spoke English or Spanish and who received SARS-CoV-2 testing via CSVS at clinical and non-clinical settings to participate in a study on risk factors for SARS-CoV-2 infection. To expand the study sample for analyses specifically focused on pneumococcal carriage and SARS-CoV-2 infection (26), we also invited adults of any occupational status, who were not pregnant, to complete an abbreviated version of the study procedures. Regardless of the version of the study into which they were enrolled, all participants provided an ~ 3 mL saliva sample at the site where they completed their SARS-CoV-2 testing with CSVS (whether in clinical or non-clinical settings) and responded to a questionnaire addressing sociodemographic characteristics, household, and workplace exposures and clinical symptoms. After collection, untreated saliva samples were frozen at -20°C immediately and then stored at -80°C until delivery to the laboratory for processing.

Molecular detection

We used previously validated protocols (38) for the molecular detection of pneumococcal carriage in saliva samples after a culture-enrichment step in which saliva samples were incubated on trypticase soy agar supplemented with 7% sheep's blood and 5 mg/L gentamicin (Supplementary Methods). After bacterial DNA isolation, we evaluated pneumococcal carriage based on the presence of *lytA* and *piaB* genes via qPCR. We defined adults with *lytA* and *piaB* c_T values < 40 as pneumococcal carriers; those with c_T values < 35 for both *lytA* and *piaB* were considered to carry pneumococci at higher density. Adults with *lytA* c_T values < 40 but *piaB* c_T values ≥ 40 were considered probable carriers of other *lytA*-positive oral Streptococci (44).

We accounted for SARS-CoV-2 infection status within statistical analyses (as described below) to correct for a potential synergistic relationship between SARS-CoV-2 and pneumococci, as identified within the primary study (26). To identify SARS-CoV-2 infection, oropharyngeal samples (collected at the same visit as study-related saliva samples) were tested via the qualitative Aptima nucleic acid transcription-mediated amplification assay (Hologic, Marlborough, MA, US).

Serotyping

We conducted molecular serotyping using DNA from bacterial culture-enriched samples using a previously validated quadruplex qPCR technique (45). To overcome the low specificity of serotyping results from colony-free detection methods (e.g., due to the presence of other bacteria encoding the serotype-specific *cps* genes), we included only results where serotype-specific c_T values fell within a range of ± 2 relative to the *piab* c_T value. We excluded detections of serotypes 4, 9V/9A, 17F, 21, 23A, 33A/33F, and 35B due to prior evidence of low assay specificity for these serotypes (24, 27).

Statistical analyses

We compared the distribution of risk factors among individuals according to pneumococcal carriage status. Risk factors included age, sex, country of birth, language spoken, household income, marital status, cigarette smoking, years residing in the US, H-2A visa status, educational attainment, exposure to children at home, household size and overcrowding, access to in-home laundry facilities, engagement in field work, commuting to work with persons from outside participants' household or via employer-provided buses, and use of face coverings when outside the home. We calculated ORs using conditional logistic regression, matching participants on the recruitment venue (clinical or outreach testing site) and concurrent SARS-CoV-2 infection (positive or negative) via regression strata. Individuals without pneumococcal carriage provided the reference group for analyses of pneumococcal carriage or higher-density pneumococcal carriage outcomes.

We repeated the analyses within subgroups to verify effect size estimates for several exposures where collinearity was expected to occur. Because households with greater numbers of residents have a greater chance of including at least one child aged <5 years, we separately computed ORs for the association of pneumococcal carriage with household size and household overcrowding within the stratum of participants not exposed to children aged <5 years at home. Similarly, living in employer-provided or dormitory-style housing could account for both overcrowding and commuting arrangements. We, thus, also computed ORs for the association of pneumococcal carriage with commuting variables within the stratum of participants living in households with <2 persons per bedroom, who were unlikely to be living in employer-provided housing or other dormitory-style arrangements.

Because higher carriage density may indicate recent acquisition of pneumococcal carriage, we also explored associations of the risk factors listed above with carriage density to better understand potential sources of transmission. We used linear regression models to measure adjusted associations of each exposure with c_T values for *piab* among individuals found to carry pneumococcus; we used *piab* as the primary target for measuring carriage density due to greater specificity as an indicator of pneumococcal carriage. The resulting effect measure can be interpreted as the adjusted mean difference in c_T values when comparing pneumococcal carriers across exposure categories. We also report findings with respect to c_T values for *lytA* as a supplemental analysis, noting that this measure may relate to the density of other *lytA*-positive oral Streptococci, even among pneumococcal carriers, due to its lower specificity.

Last, we aimed to assess whether pneumococcal carriage was related to the presence of concurrent respiratory symptoms. We stratified the analysis among the entire study population and among individuals with negative SARS-CoV-2 testing results; potential associations between symptoms and pneumococcal interactions with SARS-CoV-2 infection were addressed in prior analyses (26). We defined individuals experiencing respiratory symptoms as those who reported non-productive cough, productive cough, blocked nose, runny nose, sneezing, hoarseness, a tickling sensation in the throat, sore throat, painful sinuses, difficulty breathing, wheezing, or shortness of breath. We used conditional logistic regression models, stratified on recruitment clinical or outreach recruitment venue, to calculate ORs for outcomes of pneumococcal carriage and higher-density pneumococcal carriage. We also used linear regression to evaluate

the mean difference in *lytA* and *piaB* c_T values among pneumococcal carriers who experienced or did not experience symptoms, consistent with the density analyses described above.

For all analyses, we present ORs with accompanying 95% confidence intervals to describe effect sizes.

We conducted analyses using R (version 4.1.1; R Foundation for Statistical Computing, Vienna, Austria). Data for replication of the analyses are available via GitHub (46).

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J.A.L. conceived the study. J.A.L., A.L.W., and R.L. designed the study protocol. J.A.L., A.M.M., B.E., K.K., and L.W.R. managed the study. A.M.P., N.P., S.A., H.K., T.A., and N.H. were responsible for sample receipt, processing, testing, and generating the data. A.M.P. performed the analyses and interpreted the data. A.M.P. and J.A.L. drafted the manuscript. All authors amended and commented on the final manuscript.

AUTHOR AFFILIATIONS

¹Division of Epidemiology, School of Public Health, University of California, Berkeley, California, USA

²Division of Infectious Diseases & Vaccinology, School of Public Health, University of California, Berkeley, California, USA

³Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, Connecticut, USA

⁴Center for Environmental Research & Community Health, School of Public Health, University of California, Berkeley, California, USA

⁵Center for Computational Biology, College of Engineering, University of California, Berkeley, California, USA

AUTHOR ORCIDs

Nicole Jackson  <http://orcid.org/0000-0002-0202-3658>

Anne L. Wyllie  <http://orcid.org/0000-0001-6015-0279>

Joseph A. Lewnard  <http://orcid.org/0000-0002-8505-8839>

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AUTHOR CONTRIBUTIONS

Joseph A. Lewnard, Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review and editing.

ETHICS APPROVAL

The study protocol was approved by the Office for the Protection of Human Subjects at the University of California, Berkeley. Participants provided informed consent for all study procedures.

ADDITIONAL FILES

The following material is available [online](#).

Supplemental Material

Supplemental material (Spectrum00879-24-S0001.pdf). Supplemental methods; Tables S1 to S11.

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