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Contact with young children is a major risk factor for pneumococcal colonization in older adults

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Abstract

Important questions remain about the sources of transmission of pneumococcus to older adults in the community. This is critical for understanding the potential effects of using pneumococcal conjugate vaccines (PCVs) in children and older adults. For non-institutionalized individuals, we hypothesized that the most likely source of adult-to-adult transmission is within the household. We designed a longitudinal study to sample adults ≥60 years of age living in the same household (New Haven, CT, USA), without younger residents in the household. Saliva samples and social and health questionnaires were obtained every 2 weeks for a period of 10 weeks. DNA extracted from culture-enriched saliva was tested using qPCR for pneumococcus genes *piaB*, *lytA*, and serotype. Across two study seasons (November 2020–August 2021, November 2021–September 2022), 121 individuals from 61 households completed all six visits; 62 individuals were enrolled in both seasons. Overall, 52/1088 (4.8%) samples tested positive for pneumococcus, with 27/121 (22.3%) individuals colonized at least once. Several individuals were colonized at multiple time points; two individuals were colonized at 5/6 time points and two at all six. In 5 instances, both household members were carriers in the same season, though not necessarily at the same time. Pneumococcal carriage was substantially higher among individuals who had contact with children (10.0% vs. 1.6%). Contact with young children was the most important factor that influenced pneumococcal acquisition rates. While there were several instances where both adult household members were colonized at the same time or at sequential visits, these individuals typically had contact with children. As such, PCV immunization can directly protect older adults who have contact with children.

Keywords: pneumococcus; saliva; surveillance; carriage; transmission

Introduction

Streptococcus pneumoniae (pneumococcus) is an important human commensal and cause of respiratory infection and invasive disease. Pneumococcus resides primarily in the upper respiratory tract and is transmitted via aerosolized droplets. Studies frequently report children as the main reservoir of transmission of pneumococcus in the community (Walter et al. 2009, Wyllie et al. 2016a, 2021, 2023, Weinberger et al. 2019, Flasche et al. 2020). It is clear that vaccinating infants with pneumococcal conjugate vaccines (PCVs) leads to sharp reductions in disease caused by vaccine-targeted serotypes in unvaccinated age groups. However, some PCV serotypes (such as 3 and 19A) have persisted as causes of disease in the adult population (Grant et al. 2023), despite PCVs being used at high rates among infants. This has led to questions about whether these serotypes might be transmitted by age groups other than children.

If substantial pneumococcal transmission occurs between adults, then vaccination of older adults could have an additional benefit of reducing transmission and subsequent disease. However, quantifying carriage among adults is challenging. Surveys of pneumococcal colonization in older adults have reported vastly different estimates, depending on the sampling and testing methodology used. Using culture-based detection of pneumococcus from nasopharyngeal swabs (Satzke et al. 2013), carriage is rarely detected (<5%). When saliva samples are tested using qPCR, higher rates of colonization are detected (Krone et al. 2015). Regardless of the method used, no study has directly evaluated rates of transmission among community-dwelling adults, with prior studies focused on households where either children or adults could be the source of transmission (Melegaro et al. 2004, Mosser et al. 2014, Tramuto et al. 2017, Thindwa et al. 2021).

The risk of pneumococcal colonization in older adults is likely influenced by numerous factors, including contact patterns, living conditions, and underlying individual health. In this study, we aimed to evaluate the importance of within-household transmission between adults and risks associated with pneumococcal acquisition among community-dwelling older adults.

Methods

Ethics

This study was approved by the Institutional Review Board at Yale School of Medicine (Protocol ID #2000026100). Demographic data and samples were collected after the study participant had acknowledged that they had understood the study protocol and provided digitally signed informed consent, collected in the Research

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Electronic Data Capture (REDCap) electronic data capture tools hosted at Yale University (Harris et al. 2009, 2019). All participant information and samples collected were assigned anonymized study identifiers.

Study design

Using a longitudinal household sampling design, household pairs (e.g. married couples) were enrolled if both individuals were aged 60 years or above and no additional individuals under the age of 60 years were residing in the household. If an individual reported symptoms of respiratory illness at the time of consenting or had received antibiotics or pneumococcal vaccination within the past four weeks, the enrollment of that household pair into the study was delayed by up to four weeks. There were no exclusion criteria based on underlying health status. Enrollment was completed over the course of the two autumn/winter seasons of 2020/2021 and 2021/2022. Saliva samples and demographic data were collected every 2 weeks for six total visits (10 week study inclusion), as previously described (Wyllie et al. 2023). At the final visit, study participants also provided a urine sample. Participants from the first season were invited to participate again in the second season.

Sample processing and pneumococcal detection

On arrival at the lab, raw saliva samples were culture-enriched on TSAII plates with 5% sheep's blood and 10% gentamicin, as previously described (Wyllie et al. 2023). DNA was extracted from 200 μl of each culture-enriched sample using a modified protocol of the MagMAX Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher Scientific) on the KingFisher Apex (Thermo Fisher Scientific) (Wyllie et al. 2023). Extracted DNA was tested by qPCR on a CFX96 Touch (Bio-Rad) using primers and probes specific for two pneumococcal genes: *piaB* (Wyllie et al. 2016b) and *lytA* (Carvalho et al. 2007), as previously described (Wyllie et al. 2023). Since the *lytA* gene is not specific to pneumococcus, with homologues present in many other alpha-hemolitic *streptococci* (Hislop et al. 2023), and due to the *lytA* qPCR exhibiting a slightly lower sensitivity than the more specific *piaB* qPCR assay (Wyllie et al. 2017, Hislop et al. 2023), a sample was only classified as positive for pneumococcus when reporting a *piaB* cycle threshold (Ct) value of <40 (Wyllie et al. 2023). Samples that reported a weak Ct value of 35–40 were tested again. If upon re-testing the sample tested negative (Ct > 40), we re-tested the sample for a third time as a tiebreaker to determine positivity (two out of three tests with Ct < 40) (Wyllie et al. 2023). Using the same qPCR protocol, all samples were also tested for SP2020 (Tavares et al. 2019), which has been proposed as an additional target to further increase the specificity of detection of pneumococcus.

DNA samples were subsequently pooled, then tested in each of seven multiplexed PCR assays targeting a total of 36 pneumococcal serotypes (see Supplementary Information) (Azzari et al. 2010, Sakai et al. 2015, Olwagen et al. 2017, Velusamy et al. 2020). DNA samples testing *piaB*-positive were pooled by four. Where possible, samples from the same person were pooled together. From each pool, 8 µl of DNA was tested in a total PCR reaction volume of 25 µl, containing 12.5 µl NEB Luna Mastermix and 4.5 µl of primer/probes (see Supplementary Information). All samples from any pool generating a serotype-specific Ct value <40 were re-tested individually in that serotyping assay. The serotype of an individual sample was assessed based on the concordance between the *piaB* Ct value and the serotype-specific Ct value (ΔCt = 3).

In season 1 (S1), samples testing negative for pia*B* were pooled by 10. Samples that also tested negative for *lytA* were pooled separately from those that tested positive for *lytA*. This testing of negative samples was conducted to evaluate rates of confounding (i.e. a serotype-specific Ct value of <40, despite DNA templates being qPCR negative for *piaB*) within each assay and to inform its reliability.

Strain isolation

Saliva samples that tested positive for *piaB* with Ct < 28 were revisited by culture (Wyllie et al. 2023) and/or magnetic bead-based separation (York et al. 2023) in an attempt to isolate pure pneumococci. Pneumococcal isolates were serotyped by latex agglutination and qPCR, then stored at –80°C.

Detection of respiratory viruses

All saliva samples were also tested for the presence of SARS-CoV-2, influenza A/B, and respiratory syncytial virus (RSV). Lysates were prepared from 50 µl of each saliva sample, which was heated at 95°C for 5 min (Allicock et al. 2023) before testing in a modified "SalivaDirect" PCR assay (Vogels et al. 2020), expanded for multiplexed detection of these viruses (Allicock et al. 2023).

Detection of pneumococcus using urine antigen detection

On arrival at the lab, urine samples were aliquoted into PIPES buffer. Aliquots were stored frozen at –80°C until batch shipping on dry ice to the reference laboratory of Pfizer Vaccine Research (Pearl River, NY). Upon receipt, samples were stored at –80°C until batch testing could be performed. All samples were tested according to the manufacturer's protocol using the serotype-specific urine antigen detection (UAD) assays, which target 24 of the 100 known pneumococcal serotypes (Pride et al. 2012, Kalina et al. 2020), and the BinaxNOW® test, which targets a pan-pneumococcal antigen to determine the presence of any pneumococcus serotype not covered by the UAD.

Statistical analysis of dynamics of acquisition and clearance

To take advantage of the longitudinal nature of the study, we evaluated factors associated with the acquisition rate of pneumococcus. To do this, we fitted a continuous-time Markov model, using the msm package (Jackson 2011) in R. The model had two states (colonized and uncolonized), with an acquisition rate (λ) governing the rate of transition from uncolonized to colonized and a clearance rate (μ) that captures the rate of transition from colonized to uncolonized. We evaluated the association between covariates (e.g. recent contact with children of different age groups) and the acquisition rate (λ) using a proportional hazards framework (fitted with the msm package). We also tested whether the colonization status of the household partner at the previous time point was associated with the acquisition rate. Each covariate was tested one at a time; due to sample size limitations, it was not possible to perform a multivariate analysis. The parameter estimates for the covariates are reported as hazard ratios (HR). Due to the gap in sampling between seasons and the short mean duration of colonization, data from individuals who were re-enrolled in S1 and S2 were treated as distinct participants. Because we were not able to determine serotype for many carriers, and because there were no clear instances where an individual switched serotypes or had different serotypes within the household, we tracked pneumococcal colonization status but did not account for serotype in this analysis.

Table 1: Characteristics of the study population.

	(N = 121)
Age (years)	
Mean (SD)	70.9 (5.98)
Median [min, max]	71.0 [60.0, 86.0]
Missing	1 (0.8%)
Gender	
F	62 (51.2%)
M	59 (48.8%)
Race	
Asian	3 (2.5%)
Black or African American	3 (2.5%)
White	105 (86.8%)
Missing	10 (8.3%)
Ethnicity	
Hispanic or Latino	1 (0.8%)
Non-Hispanic	116 (95.9%)
Missing	4 (3.3%)
Ever smoked	
No	83 (68.6%)
Yes	27 (22.3%)
Missing	11 (9.1%)
Diabetes	
No	101 (83.5%)
Yes	8 (6.6%)
Missing	12 (9.9%)
Asthma	
No	100 (82.6%)
Yes	5 (4.1%)
Missing	16 (13.2%)
Influenza vaccine	
No	4 (3.3%)
Yes	103 (85.1%)
Missing	14 (11.6%)
Pneumococcal vaccine[a]	
No	34 (28.1%)
Yes	75 (62.0%)
Missing	12 (9.9%)
Education level	
High Sshool	11 (9.1%)
Undergraduate (Bbchelor or Aasociate)	44 (36.4%)
Graduate	50 (41.3%)
Unknown	16 13.2%)

[a]Includes PPSV23 or PCV13.

Results

Population characteristics

From November 2020 to September 2022, 121 individuals from 61 households were sampled; 62 individuals were enrolled in both seasons. In S1, individuals were sampled between November 2020 and August 2021. In S2, individuals were sampled between November 2021 and September 2022. One household was composed of a single individual who was enrolled in the study due to residing in a living facility for older adults.

The mean age of study participants was 70.9 years (range: 60–86) (Supplementary Fig. 1). Of the study participants, 85.2% were white (Table 1). Among the study participants who responded to the question, 77.6% held a bachelor's degree or higher. After experiencing a high rate of survey incompleteness during S1, we updated our data capture to ensure more complete data in S2.

Of the 1,091 samples collected, three were not tested due to low collection volume ($n = 1$) or a weather-related delay (two weeks) in transporting samples to the lab ($n = 2$). Four samples from one individual were not collected because that participant was hospitalized. Three samples from three individuals were not obtained because the participant was unable to produce saliva at that sampling moment.

In 2020/2021, all individuals tested negative for SARS-CoV-2. In 2021/2022, 12 individuals tested positive, one of which also tested positive for pneumococcus at the same sampling moment (#61, serotype 22F/A). None of the samples tested positive for influenza or RSV.

Prevalence of pneumococcal carriage

Overall, 52/1088 (4.8%) samples tested positive for pneumococcus based on *piaB*, with 27/121 (22.3%) individuals colonized on at least one time point. By study season, 14/95 (14.7%) individuals were colonized on at least one time point in S1, and 14/88 (15.9%) individuals were colonized at least once in season 2 (Fig. 1). In 2/48 (4.2%) households in S1 and 3/44 (6.8%) households in S2, both members were carriers, though not necessarily at the same time point. There were no meaningful differences in prevalence across seasons or by demographics (Table S1).

When samples were positive for both *piaB* and *lytA*, there was good concordance in the bacterial density (Ct value) (Fig. 2). This was also observed when testing samples for SP2020 (Supplementary Fig. 2). However, concordance between *lytA* and SP2020 was weaker, with high rates of samples positive for only one of these targets, indicating a reduced specificity of both the *lytA* (Hislop et al. 2023) and SP2020 qPCR assays (Supplementary Fig. 3), and it supports our reasoning to rely solely on *piaB* for determining sample positivity for pneumococcus. As expected for a study cohort of individuals without pneumonia, none of the urine samples collected tested positive for pneumococcal antigens using either the UAD or the BinaxNOW® test.

Pneumococcal carriage and serotype patterns across study seasons

Several individuals were colonized at multiple time points, including two individuals who were colonized throughout the 10-week sampling period in one season (S1 #3, serotype 17F and S1 #41, serotype 15B/C; Fig. 3). The household pair of S1 #44 (S1 #45) also tested qPCR positive for 15B/C at one of the same sampling moments. Two individuals were colonized at five of the six time points (S1 #33, serotype 15B/C and S2 #25, serogroup 6). Participant S2 #25 also participated in the first study season and was positive for pneumococcus on 3/6 sampling moments (S1 #92), with the later two visits of S1 also being positive for serogroup 6 (we were unable to resolve a serotype for the first sampling moment they tested positive). This individual reported daily contact with children 2–59 months and 5–9 years of age in both seasons.

In line with other reports (Wyllie et al.2014, 2016a, Krone et al. 2015, Miellet et al. 2023b), testing *piaB*-negative DNA samples demonstrated that many serotyping assays were subject to confounding by non-pneumococcus *streptococci* (see Supplementary Information). We observed high rates of false positivity with the assays targeting serotypes 9V/A (Velusamy et al. 2020), 12FAB/44/46 (Pimenta et al. 2013), 17 (Olwagen et al. 2017), 20 (Velusamy et al. 2020), 21 (Velusamy et al. 2020), 23FAB (Olwagen et al. 2017), 28F/A (Olwagen et al. 2017), and 34 (Velusamy et al. 2020) (much lower Ct values as compared to *piaB* or signal detected in *piaB*-negative DNA templates), and as such, all results from these assays were excluded. Interestingly, two individuals from the same household (#28 and #29) tested positive for both *lytA* and serotype 21 (Velusamy et al. 2020) (but negative for *piaB*

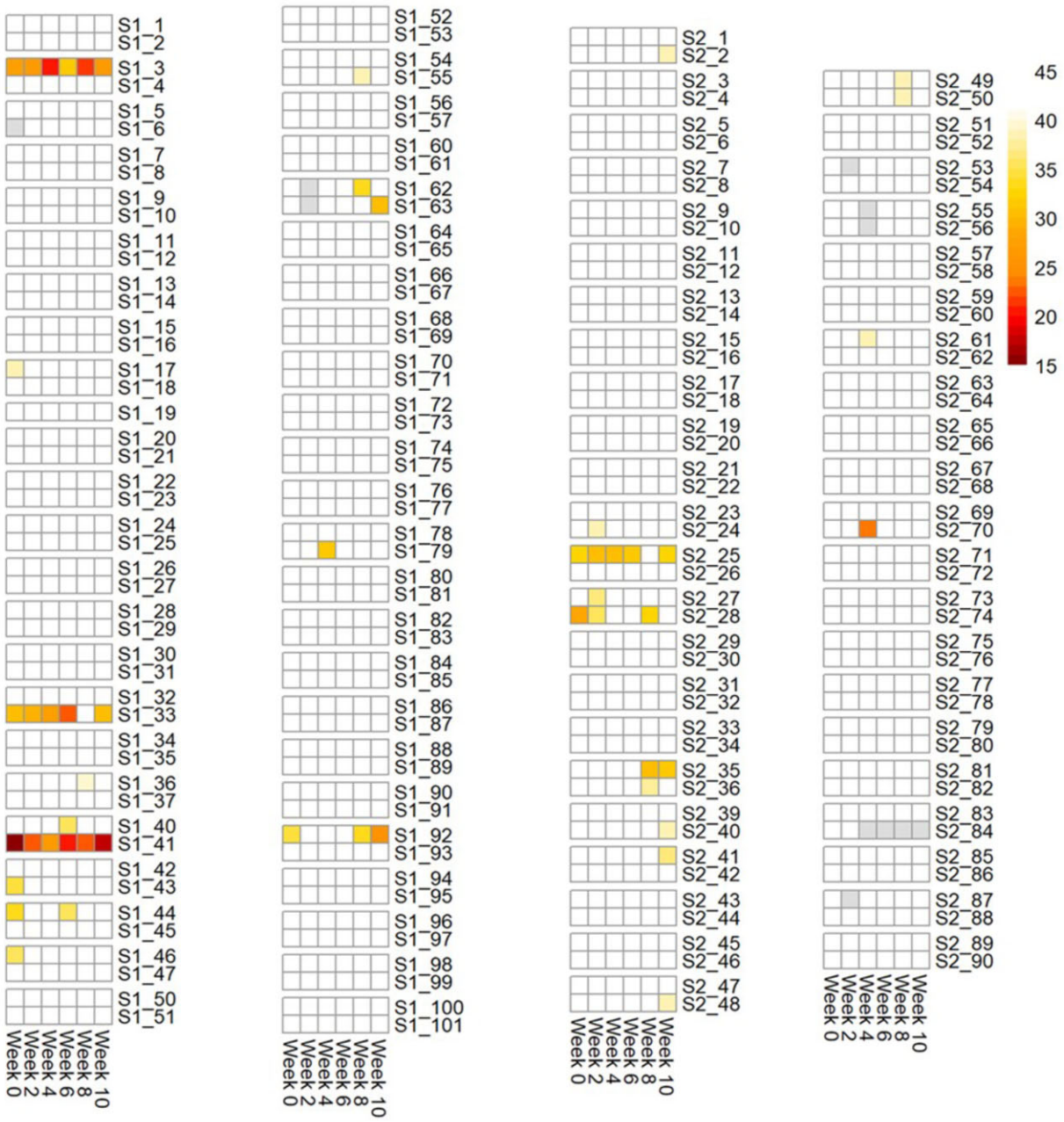

Figure 1. Heatmap of sample positivity for the pneumococcus gene, *piaB*. Overall, 52/1088 (4.8%) samples tested qPCR positive, with 28/183 (15.3%) individuals colonized on at least one time point. Darker colors indicate lower Ct values (higher concentration of pneumococcus). Gray boxes indicate samples that were not tested or missing. Each row represents an individual, each column represents a time point. Individuals in the same household are grouped together. For participants who were re-enrolled, the study ID does not match between S1 and S2.

and SP2020) at all six sampling moments. Moreover, two samples from S2 #35 tested positive for serotype 28F/A with a Ct value concordant with their *piaB* and *lytA* Ct values (within Ct = 2), yet we cannot be sure whether this represents genuine detection of a serotype 28F/A pneumococcus or a *Streptococcus* spp. with homologous genes present. We were able to replace the assays targeting serotype 9V/A (Olwagen et al. 2017), 17 (Velusamy et al. 2020), and 28F/A (Sakai et al. 2017), though 9V/A proved only marginally more specific, so results from this assay were also excluded.

For 13 sampling moments from 12 individuals, we could not resolve a serotype due to the incomplete serotype coverage of our qPCR assays.

Point prevalence was higher among those with contact with children (descriptive statistics)

The point prevalence of pneumococcal carriage was substantially higher among individuals who reported contact with children as compared to those who had no recent contact with children (10.0% vs. 1.6%, respectively; Table 2). Participants who reported recent contact with <5-year-olds and 5–9 year olds had point prevalences of 13.8% and 14.1%, respectively; those reporting contact with children >10 years had a prevalence of 7.1%. While the numbers were sparse, further subdividing the <5 year-old population found high prevalence among those reporting contact with children aged <12 months (13.8%), 12–23 months (10.7%), and 24–

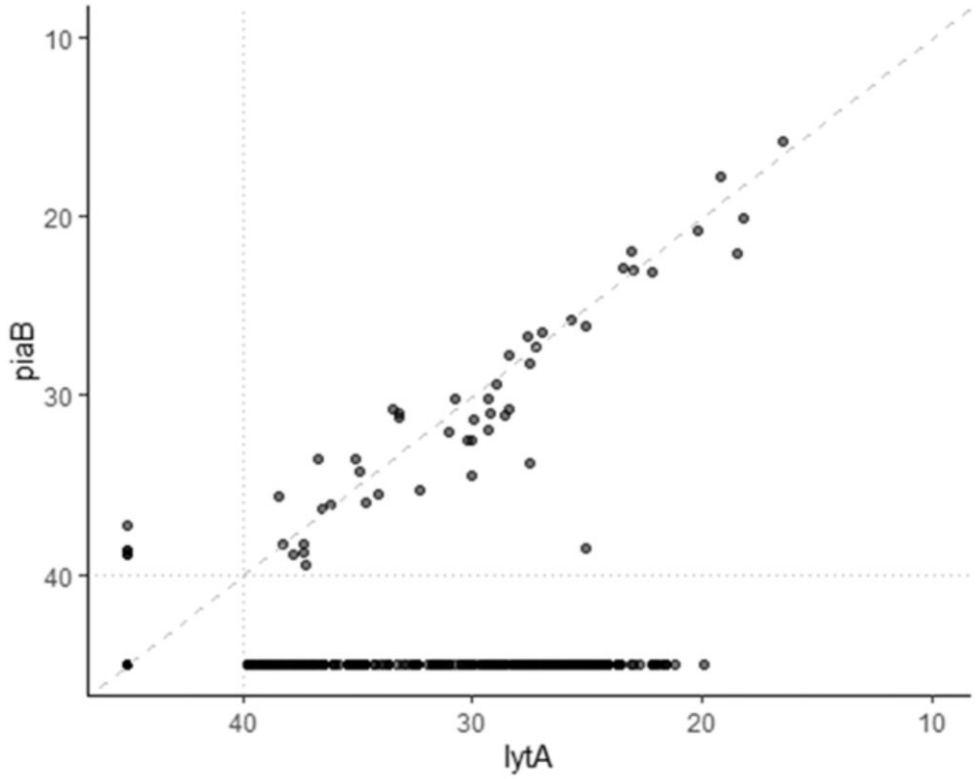

Figure 2. Concordance between *lytA* and *piaB* qPCR values. Ct values are shown, with a value of <40 considered positive for the gene target.

59 months (17.3%). While the numbers were small, those who had contact with children daily or every few days had the highest prevalence (15.7% and 14.0%, respectively). Those who had contact once or twice a month or no contact tended to have lower prevalence compared with those who had daily contact (4.5% and 1.8% compared with 15.7%, respectively).

Acquisition and clearance rates of pneumococcal colonization

The repeated sampling scheme used in this study allowed us to estimate characteristics of colonization episodes, including the acquisition rate, duration of colonization, and risk factors for acquisition and clearance. The average time to acquire pneumococcus was 358 days (95% confidence interval [CI]: 230 days, 556 days), indicating roughly one detected colonization episode per year. The mean duration of detectable colonization was 17.7 days (95% CI: 11.4, 27.3 days). Acquisition rate did not vary by sex. The acquisition rate was substantially higher among individuals who reported contact with children <10 years of age (HR: 3.0, 95% CI: 1.3, 7.2), and the risk was particularly high among the subset of adults who had contact with children at least every few days (HR: 6.0, 95% CI: 2.3, 15.2). There was not an association between the colonization status of the household partner at the previous time point and the acquisition rate (HR: 1.4, 95% CI: 0.18, 10.3).

Discussion

There are ongoing policy discussions about the use of PCVs in the adult population. Much of this discourse focuses on whether vaccinating older adults is important in populations where children are vaccinated at high rates. Additionally, as higher valency adult-specific PCVs are licensed and introduced into communities around the world, it is important to have baseline data of serotype colonization in children and older adults and an understanding of potential pneumococcal transmission among adults and from older adults to children. In this study, we found that among community-dwelling older adults, pneumococcal carriage prevalence was high. While there were households in which an individual was positive for pneumococcus across numerous sampling moments and instances where both adults in the household carried pneumococcus around the same time, there was no clear evidence of adult–adult transmission in this relatively small community-based study. Rather, carriage was highest among those who had frequent contact with young children. This suggests that the main benefit of adult PCV immunization is to directly protect older adults who are exposed to children, who still carry and transmit some vaccine-type pneumococci despite successful pediatric national immunization programs. In contrast, PCV immunization of older adults living in the community may not have a major impact on onward transmission to other adults; whether adult-to-child transmission occurs was not evaluated in our study.

With the study period coinciding with the COVID-19 pandemic, we were able to explore risk factors for pneumococcal carriage in a period when strict transmission mitigation measures were in place and eased over time. Other than continued contact with families, study participants reported few activities outside of the home, adhering to the social distancing recommendations in place (Wyllie et al. 2023). This study setting allowed greater resolution into household transmission, removing many of the possible external sources that could be expected from social activities in the absence of social distancing. We found that carriage rates remained consistent across both study seasons, despite a return to community activities in the second season, and an in-

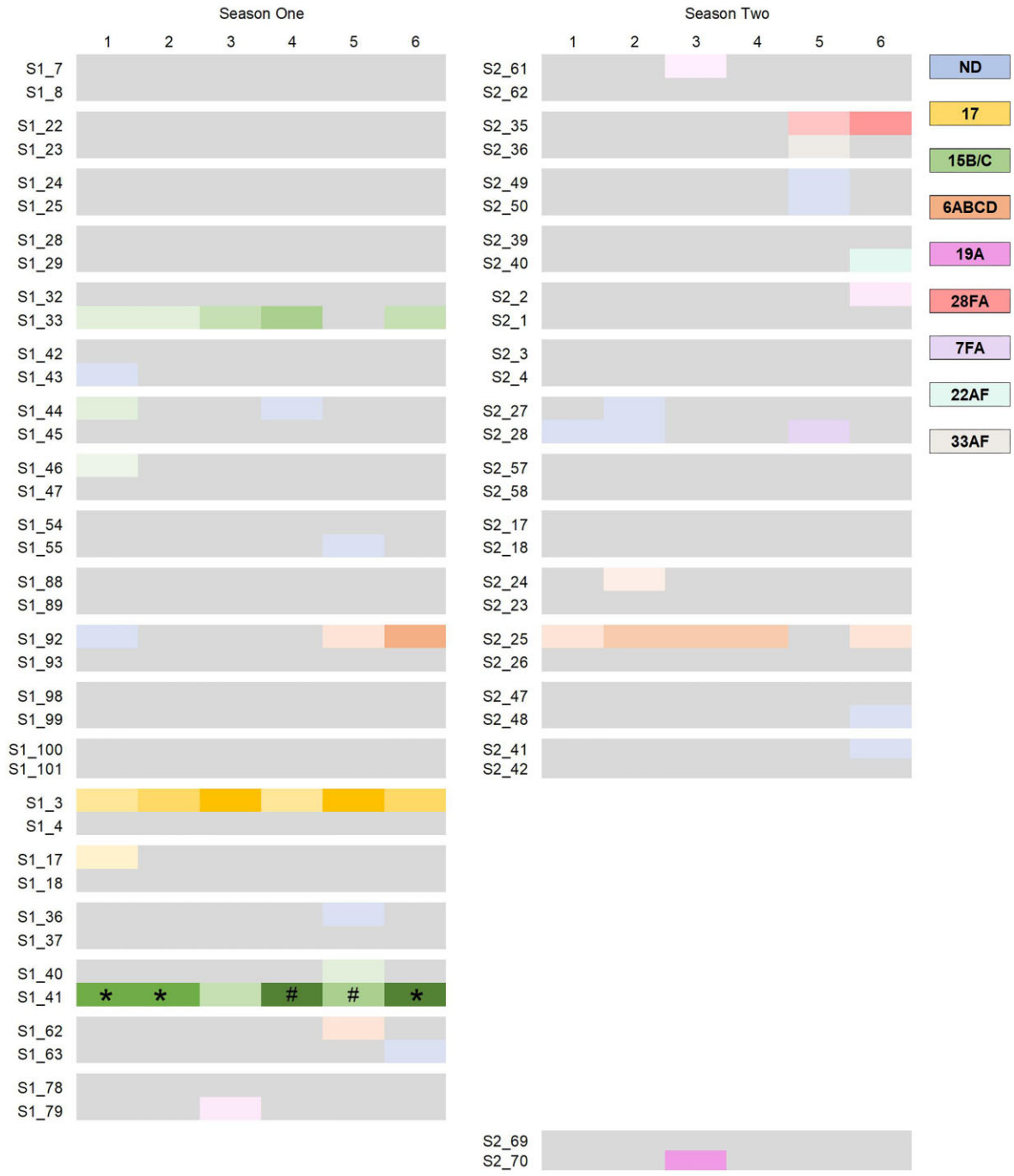

Figure 3. Heatmap of sample positivity for pneumococcal serotype across seasons when tested using multiplex qPCR assays (see Supplementary Information). Darker colors indicate lower Ct values (higher concentration of serotype-specific signal). Gray boxes indicate sampling moments for which the individual tested negative for pneumococcal gene, *piaB*. Isolates were obtained by culture from samples denoted with * and by magnetic bead separation from samples denoted with #. Each row represents an individual, and each column represents a time point. Individuals in the same household are grouped together. Results for study participants and households that participated in both study seasons are shown side by side with the corresponding study ID. Households with results only in one column participated only in that one study season. ND: For 13 sampling moments from 12 individuals, we could not resolve a serotype due to the incomplete serotype coverage of our qPCR assays.

creased circulation of respiratory viruses in the local community. Other studies have reported a persistence of pneumococcal carriage in children across the COVID-19 pandemic (Danino et al. 2021, Willen et al. 2021). Here, in addition to persistent carriage across the pandemic periods studied, the overall number of individuals that we identified as colonized at least once in this study was similar to that in a cohort of community-dwelling individuals aged 65–88 years that we sampled, though under a different study design and sampling scheme, over the autumn/winter seasons of 2018/2019 and 2019/2020 (22.3% vs. 25.6%, respectively) (Hislop et al. 2023). In contrast to our findings, carriage among older adults in Denmark was reported to decline (Tinggaard et al. 2023). Interestingly, it was also reported that in Denmark, distancing recommendations, including the distancing from children, were strictly adhered to (Jørgensen et al. 2021), unlike in our population (Wyllie et al. 2023). A limitation of our study, however, was that despite enrolling from a diverse population or study participants were skewed towards white individuals with higher education, mean-

Table 2: Percent of samples positive for pneumococcus by contact with children.

	n, samples tested	*n*, *piaB* positive	%, *piaB* positive
OVERALL	**1088**	**52**	**4.8**
Contact with children: any			
No	575	9	1.6
Yes	402	40	10.0
Missing/no response	111	3	2.7
Contact with children: <5 years			
No	777	22	2.8
Yes	195	27	13.8
Missing/no response	116	3	2.6
Contact with children: <12 months			
No	885	37	4.2
Yes	87	12	13.8
Missing/no response	116	3	2.6
Contact with children: 12–23 months			
No	897	41	4.6
Yes	75	8	10.7
Missing/no response	116	3	2.6
Contact with children: 24–59 months			
No	874	32	3.7
Yes	98	17	17.3
Missing/no response	116	3	2.6
Contact with children: 5–9 years			
No	788	23	2.9
Yes	184	26	14.1
Missing/no response	116	3	2.6
Contact with children: >10 years			
No	804	37	4.6
Yes	168	12	7.1
Missing/no response	116	3	2.6
Intensity of contact: per day			
<4 h (morning or afternoon)	252	27	10.7
4–8 h (full day)	105	11	10.5
8+ h (day care, overnight)	31	2	6.5
No contact/missing/no response	700	12	1.7
Intensity of contact: frequency			
Daily	51	8	15.7
Every few days	171	24	14.0
Once or twice a month	157	7	4.5
No contact/missing/no response	709	13	1.8

ing that results may not be representative across the entire greater New Haven community (US Census Bureau). Additionally, with a third of the study participants from S1 re-enrolling into S2, this further impacted the representation of the wider community.

Across the entire study, there were clear differences in colonization prevalence and acquisition rates based on recent contact with young children. While there were several instances where both household members were colonized at the same time or at sequential visits, these individuals also typically had contact with children. As such, we cannot be certain as to whether instances in which the same serotype was detected within the household pair represented adult-to-adult transmission or acquisition from the children they were in contact with. The frequency and intensity of contact with children also mattered. Higher carriage prevalence was observed among those who reported daily contact (15.7%) or contact every few days (14.0%) as compared to those who reported to have contact with children only once or twice a month (4.5%) or no contact (1.8%).

The current gold standard recommendations for the detection of pneumococcus (Satzke et al. 2013) are insensitive when applied for carriage detection in adults (Wyllie et al. 2016a, Miellet et al. 2023b, Krone et al. 2015). As such, more sensitive methods of pneumococcal carriage surveillance must be established and appropriately applied (Miellet et al. 2023a). Saliva, when tested using qPCR, facilitates more sensitive and thus informative detection of pneumococcus in older adults (Krone et al. 2015, Miellet et al. 2021). Therefore, in the current study, we tested saliva samples collected over two study seasons and confirmed higher carriage rates than typically reported when culture-based methods are applied to nasopharyngeal swabs. We also explored several methods for serotyping the samples. We primarily used qPCR, adapting assays that had been described elsewhere (see Supplementary Table 1) into a multiplex format. While we obtained some unambiguous findings when we focused on subjects with a higher density of pneumococcal colonization (Ct < 35) and were able to isolate and confirm the serotype present using traditional culture-based methods in samples with higher abundance, we were unable to resolve a primary serotype for all samples, particularly those at a lower abundance. This relatively low abundance of pneumococcus in positive samples also made it difficult to isolate pneumococcus by culture. Moreover, we observed a high prevalence of non-pneumococcal streptococci in our samples as evident through high rates of positivity in the *lytA* qPCR assay (see Supplementary Fig. 2), while negative for *piaB* (Hislop et al. 2023). These streptococci can have capsules that are identical to pneumococcal capsules in their structure and

whose capsular biosynthesis genes are nearly identical to pneumococcal genes (Skov Sørensen et al. 2016). Therefore, all qPCR assays were tested on a set of samples that were *lytA*-positive and *piaB*-negative (indicating presence of *Streptococcus* spp., but not pneumococcus) to determine the background rate of detection. As such, we detected a high degree of non-specificity in many of the serotyping assays that have been published by others. Further validation and optimization work is required to establish more robust and reliable methods for the serotyping of pneumococci present in polymicrobial samples. Despite the improved sensitivity of detection of carriage with these methods, it is still possible that we have underestimated overall carriage prevalence because we did not sample the entire upper airway or beyond the surface of the upper airway.

With next-generation PCVs being introduced into older adult populations around the world, it is crucial to establish baseline data on serotype colonization in adults as well as children and to understand pneumococcal transmission pathways to and among adults. While a main goal of the current study was to quantify the effect of having a positive household contact on transmission, the number of households where both individuals were positive was extremely low, and it was contact with children that was most highly associated with colonization in this population of older adults. In addition to diagnostic testing differences, differing rates of contact with children could also help explain the differing pneumococcal colonization prevalences reported from different populations of older adults around the world. In other contexts, such as nursing homes, more adult–adult transmission likely occurs, as this would explain nursing home-based pneumococcal disease outbreaks (Guimbao et al. 2002). To adequately investigate this, however, the establishment and appropriate application of more sensitive methods for pneumococcal carriage surveillance are imperative. Despite some limitations, the results of our study contribute to our understanding of pneumococcal carriage and can inform future policy decisions and preventive strategies aimed at reducing pneumococcal disease burden in children and adults.

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Author contributions

A.L.W., A.A., and D.M.W. conceived the study. A.L.W., LR.G., A.A., BD.G., and D.M.W. designed the study protocol. A.L.W., D.Y.-C., R.A.-P., and D.M.W. managed the study. D.Y.-C. collected the data. D.Y.C., M.S.H., S.M., L.W., and P.W. were responsible for sample receipt, processing, and testing. A.L.W. and D.M.W. performed the analyses and interpreted the data. A.L.W. and D.M.W. drafted the manuscript. All authors amended and commented on the final manuscript. This study was conducted as a collaboration between Yale School of Public Health (A.L.W. and D.M.W.) and Pfizer. The study protocol was designed by the Yale researchers in consultation with Pfizer. The decision to publish was made by the Yale researchers in consultation with Pfizer; all authors agree with the decision to publish and with the results of the study.

Supplementary data

Supplementary data is available at *FEMSMC Journal* online.

Conflict of interest: A.L.W. has received consulting and/or advisory board fees from Pfizer, Merck, Diasorin, PPS Health, Primary Health, Co-Diagnostics, and Global Diagnostic Systems for work unrelated to this project, and is Principal Investigator on research grants from Pfizer, Merck, and NIH RADx UP to Yale University and from NIH RADx, Balvi.io and Shield T3 to SalivaDirect, Inc.. D.M.W. has received consulting fees from Pfizer, Merck, GSK, and Matrivax for work unrelated to this project and is the Principal Investigator on research grants and contracts with Pfizer and Merck to Yale University. This work has been previously presented in part at ESCMID Global 2024; the 13th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD-13), Cape Town, South Africa; and IDWeek 2023, Boston, MA, USA.

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