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Clinical Infectious Diseases

MAJOR ARTICLE

Unmasking Pneumococcal Carriage in a High Human Immunodeficiency Virus (HIV) Prevalence Population in two Community Cohorts in South Africa, 2016–2018: The PHIRST Study

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Background. Longitudinal pneumococcus colonization data in high human immunodeficiency virus (HIV) prevalence settings following pneumococcal conjugate vaccine introduction are limited.

Methods. In 327 randomly selected households, 1684 individuals were enrolled and followed-up for 6 to 10 months during 2016 through 2018 from 2 communities. Nasopharyngeal swabs were collected twice weekly and tested for pneumococcus using quantitative *lytA* real-time polymerase chain reaction. A Markov model was fitted to the data to define the start and end of an episode of colonization. We assessed factors associated with colonization using logistic regression.

Results. During the study period, 98% (1655/1684) of participants were colonized with pneumococcus at least once. Younger age (<5 years: adjusted odds ratio [aOR], 14.1; 95% confidence [CI], 1.8–111.3, and 5–24 years: aOR, 4.8, 95% CI, 1.9–11.9, compared with 25–44 years) and HIV infection (aOR, 10.1; 95% CI, 1.3–77.1) were associated with increased odds of colonization. Children aged <5 years had fewer colonization episodes (median, 9) than individuals ≥5 years (median, 18; $P <$ .001) but had a longer episode duration (<5 years: 35.5 days; interquartile range, 17–88) vs. ≥5 years: 5.5 days (4–12). High pneumococcal loads were associated with age (<1 year: aOR 25.4; 95% CI, 7.4–87.6; 1–4 years: aOR 13.5, 95% CI 8.3–22.9; 5–14 years: aOR 3.1, 95% CI, 2.1–4.4 vs. 45–65 year old patients) and HIV infection (aOR 1.7; 95% CI 1.2–2.4).

Conclusions. We observed high levels of pneumococcus colonization across all age groups. Children and people with HIV were more likely to be colonized and had higher pneumococcal loads. Carriage duration decreased with age highlighting that children remain important in pneumococcal transmission.

Keywords. *Streptococcus pneumoniae*; carriage; pneumococcus colonization; community cohort; South Africa.

Lower respiratory tract infections (LRTI) account for approximately 3 million deaths annually in all ages worldwide and are the leading cause of death in low-income countries, causing approximately 75 deaths per 100 000 population in 2016 [1]. Despite interventions, *Streptococcus pneumoniae* remains the leading bacterial cause of community-acquired LRTI. Asymptomatic colonization of *S. pneumoniae* (referred to as carriage) occurs in the human nasopharynx and is often a beneficial immunogenic event but can be a precursor for invasive disease [2–4]. Carriage is thought to be a source of pneumococcal disease transmission among close contacts [2]. This is particularly important in high human immunodeficiency virus (HIV)-prevalence settings because people with HIV (PWH) are more likely to develop severe pneumonia and have a higher mortality rate because of *S. pneumoniae* than HIV-uninfected individuals [5, 6]. In South Africa, it is estimated that approximately 13% of

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the population (7.5 million people) were PWH at the end of 2019 [7].

In 2009, the 7-valent pneumococcal conjugate vaccine (PCV) was introduced into South Africa's routine childhood immunization program in a 2 + 1 schedule and was replaced by 13-valent PCV in 2011 [8]. When comparing the period from 2005–2008 to 2011–2012, incidence rates of invasive pneumococcal disease (IPD) declined [9]. IPD caused by PCV7 serotypes declined by 89% (32.1 to 3.4 cases per 100 000 person-years) in children aged <2 years and 57% (3.7 to 1.6 cases per 100 000 person-years) in adults aged 25 to 44 years during this period. Overall incidence of IPD declined from 9.9 cases per 100 000 person-years in 2005 to 4.4 cases per 100 000 person-years in 2015 [9]. Furthermore, a decline in incidence of IPD caused by vaccine serotypes has been reported after the introduction of PCV in South Africa [10]. In South Africa, a 70% reduction in IPD caused by PCV7 serotypes, and a 66% reduction in the additional 6 serotypes targeted by PCV13, was observed 4 years after PCV13 introduction [10]. In a cross-sectional South African study, a 12% decline in pneumococcal carriage was observed in children after PCV introduction [11]. However, longitudinal carriage studies focusing on the rates of pneumococcal colonization in the post-PCV era are limited. We aimed to determine factors associated with pneumococcal colonization, duration of colonization, and density in the nasopharynx over time in a household community cohort study.

METHODS

This study was nested within the prospective household cohort study of influenza, respiratory syncytial virus (PHIRST), and other respiratory pathogens community burden and transmission dynamics in South Africa from 2016 to 2018 as described previously [12, 13]. Individuals were enrolled each year and followed-up from May through October in 2016 (because of a late start in the first year of the study) and January through October in 2017 and 2018. A total of 327 households were enrolled at 2 community sites in South Africa, Klerksdorp (urban site) and Agincourt (rural site), from 2016 through 2018, with a new cohort of households enrolled each year (100 households in 2016, 109 households in 2017, and 118 households in 2018). HIV status was determined by evidence of documented test results, or treatment with antiretroviral therapy. To be classified as HIV-uninfected, individuals had to have a documented negative HIV result in the 6 months before recruitment; study teams offered HIV testing after counseling at enrollment. Individuals newly diagnosed with HIV were referred to their local clinic for antiretroviral therapy. PCV vaccination records were obtained from the Road-to-Health cards of individuals aged <5 years [12]. Nasopharyngeal (NP) flocked swabs were collected twice weekly from a total of 1684 individuals (542 in 2016, 577 in 2017, and 565 in 2018). Among the 1684 individuals, 79 (5%) were lost to follow-up (53 [67%] left the study sites, 21 [27%] withdrew prematurely, and 5 [6%] died) but remained in the analysis. The NP swabs collected were placed in PrimeStore Molecular Transport Medium (Longhorn Vaccines & Diagnostics, San Antonio, Texas, USA) and shipped in cooler boxes to the National Institute for Communicable Diseases in Johannesburg. Total nucleic acids from 200 µL NP samples were extracted using a MagNA Pure 96 system with the MagNA Pure 96 DNA and Viral NA Small Volume Kit and "Viral NA Plasma SV" protocol (Roche, Mannheim, Germany) and eluted to a volume of 100 µL. The human ribonuclease *P (RNAseP)* gene was used as a control to assess the quality of sample collection procedures. A quantitative, in-house, real-time polymerase chain reaction (PCR) targeting the autolysin gene (*lytA*) was used to identify *S. pneumoniae* using universal cycling conditions [14]. An initial cycle threshold value of ≤40 were considered positive and no replicates were performed. A standard curve using serially diluted DNA extracts from a known starting quantity of American Type Culture Control control (ATCC 49619) was included with every PCR run to calculate pneumococcal density (DNA copies/mL).

Data Analysis

Individuals with ≤10 follow-up visits when NP swabs were collected were excluded from this analysis. The proportion of pneumococcal colonization was defined as the number of individuals colonized with pneumococcus at least once during the period of twice-weekly swabbing. Pneumococcal colonization was stratified by year, site, sex, age group, and HIV infection status. We selected 3 time points corresponding to the first visit in May, July, and September each year reflecting pneumococcal colonization point prevalence in autumn, winter, and spring, respectively. A Markov model was constructed to estimate clearance and acquisition events from the observed data thus consecutive episodes of colonization. The model adjusted for misclassification of samples based on probabilistic ascertainment as well as age and HIV status and is described in more detail elsewhere [15]. Using the results obtained from the Markov model, the median number of episodes per person and the median duration of a colonization episode were estimated by age group. We used the χ^2 or Fisher exact test for comparison of categorical variables.

A subanalysis was implemented to assess factors associated with high pneumococcal colonization density. This was determined by calculating the median pneumococcal density over an episode and the median pneumococcal density, by individual, over all pneumococcal episodes. Based on evidence showing that pneumococcal pneumonia is associated with a colonization density ≥1000 copies/mL, we categorized those with pneumococcal colonization into high- (median of ≥1000 copies/mL) and low-density (median of <1000 copies/mL) [16].

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Unconditional logistic regression was performed to assess factors associated with pneumococcal colonization and density of pneumococcal carriage. For multivariable models, we assessed all variables that were significant at $P < .1$ on the univariate analysis or *a priori* (HIV status) and dropped nonsignificant factors ($P \geq .05$) with manual backward elimination. We assessed pairwise interactions by the inclusion of product terms for all variables remaining in the final multivariable additive model. Carriage duration was left truncated and right censored at the first and last NP collection point for each year, respectively. We performed the analysis using Stata version 14 (StataCorp LLC, College Station, Texas, USA). Statistical significance was assessed at $P < .05$.

Ethics

Ethical approval for the PHIRST study (15080) and laboratory testing of *S. pneumoniae* (M210367) was obtained from the University of the Witwatersrand Human Research Ethics Committee.

RESULTS

In 327 households, 1684 individuals were enrolled and followed-up for 6 to 10 months [12]. The study population comprised 60% (1009/1684) females. HIV status was known for 97% (1628/1684) of participants and 15% (249/1684) were PWH. Individuals aged <5, 5–14, 15–44, and ≥45 years comprised 17% (279/1684), 32% (547/1684) 35% (590/1684), and 16% (268/1684), respectively. Of PWH with known $CD4^+$ counts, 15/214 (7%) had a $CD4^+$ count of less than 200 cells/mm^3 [17]. PCV vaccination information was available for 78.5% (219/279) of participants aged <5 years among those, 96.8% (212/219) of individuals were fully vaccinated for their age.

There were 122 113 potential follow-up visits during the study period; 105 783 (87%) follow-up visits were conducted, and 105 686 (99.9%) (23 693 in 2016, 41 679 in 2017, and 40 314 in 2018) NP specimens were collected and tested for *S. pneumoniae* and *RNAseP*. The proportion testing positive for *RNAseP* was 91% (93 456/102 379). We detected *S. pneumoniae* in 36.7% (38 829/105 686) of NP specimens (39.3% (9310/23 693) in 2016, 37.6% (15 672/41 679) in 2017, and 34.3% (13 847/40 314) in 2018) as shown in Supplementary Figure 1. The seasonal point prevalence for the 3 years was 36.1% (578/1599) in May, 40.7% (614/1508) in July, and 40.5% (645/1591) in September ($P = .98$).

Proportion of Individuals With Pneumococcal Colonization

We assessed the proportion of individuals with pneumococcal colonization for 1684 individuals over all 3-year cohorts and found that 98% (1655/1684) of individuals had at least 1 colonization episode (Table 1). No significant difference in colonization was observed by sex or site. However, individuals enrolled in 2017 were 3.6 times (adjusted odds ratio [aOR], 3.6; 95% confidence interval [95% CI], 1.2–11.3) more likely to be colonized with pneumococcus at least once during the study period than individuals enrolled in 2016. Younger age (<5 years: [278/279]; 99.6%), aOR 14.1, 95% CI, 1.8–111.3 and 5–24 years: [811/820; 98.9%], aOR 4.8, 95% CI, 1.9–11.9, compared with 25–44 years [303/317; 98.1%]) was associated with increased odds of colonization; PWH were 10-fold more likely to be colonized with pneumococcus at least once than HIV-uninfected individuals after adjusting for age (OR, 10.1; 95% CI, 1.3–77.1).

Number of Colonization Episodes

After fitting the Markov model to the data, there were 1625 participants whose HIV status and information were available. Of these, 1585 (97.5%) had at least 1 carriage episode. The median (interquartile range [IQR]) number of colonization episodes per individual during the follow-up periods (6–10 months) was 5 episodes (3–7 episodes) (Figure 1). The median (IQR) carriage duration for an episode was 7 days (4–19 days). Children aged <5 years had fewer colonization episodes than individuals aged ≥5 years (<5 years: 9 episodes vs. ≥5 years: 18 episodes; $P < 0.001$) but their duration of carriage was longer (<5 years: median [IQR] 35.5 days [17–88 days] vs. ≥5 years: 5.5 days [4–12 days]; $P < 0.001$ (Figure 2).

Pneumococcal Colonization Density

Individual median pneumococcal colonization density, over an episode, was 619 DNA copies/mL (IQR 133.5–4286.5). We observed that the median log pneumococcal density decreased with an increase in age ($P < .01$) (Figure 3). Following categorization of pneumococcal density into low (<1000 copies/mL) and high (≥1000 copies/mL) loads, we found that high pneumococcal loads were associated with younger age (<1 year: aOR 25.4, 95% CI, 7.4–87.6; 1–4 years: aOR 13.5, 95% CI, 8.3–22.9; 5–14 years: aOR 3.1, 95% CI, 2.1–4.4 vs. 45–65-year-old patients). On univariate analysis, PWH had a lower odds of high pneumococcal load of OR 0.7, 95% CI, .5–.9; however, after adjusting for age in the multivariable model, PWH were at greater risk for high pneumococcal loads (aOR 1.7, 95% CI, 1.2–2.4) (Table 2).

DISCUSSION

Ten years after PCV introduction in settings with high vaccine coverage, we observed a high proportion of people experiencing at least one episode of pneumococcal colonization. While nearly everyone was colonized at some point during the study period, younger children and PWH were more likely to be colonized with the pneumococcus. Furthermore, younger individuals (<5 years) had fewer episodes of

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Table 1. Proportion of Individuals Colonized With Pneumococcus at Least Once During the Follow-up period, PHIRST Study, South Africa, 2016–2018 (N = 1684)

Characteristics	Proportion of Pneumococcal Colonization n/N (%)	Univariate Analysis		Multivariable Analysis[b]	
		OR (95% CI)	*P* Value[a]	aOR (95% CI)	*P* Value[a]
Overall	1655/1684 (98.3)	…	-	…	…
Year					
May–October 2016	529/542 (97.6)	Reference	N/A	Reference	N/A
January–October 2017	572/577 (99.1)	2.8 (1.0–7.9)	.05	3.6 (1.2–11.3)	**.03**
January–October 2018	554/565 (98.1)	1.2 (.5–2.8)	.61	1.5 (.6–3.5)	.38
Site					
Rural	841/849 (99.1)	2.7 (1.2–6.2)	**.02**	2.0 (.9–4.7)	.11
Urban	814/835 (97.5)	Reference	N/A	Reference	N/A
Sex					
Male	660/675 (97.8)	Reference	N/A	…	…
Female	995/1009 (98.6)	1.6 (.8–3.4)	.20	…	…
Age group, years					
<5	278/279 (99.6)	12.8 (1.7–98.3)	**.01**	14.1 (1.8–111.3)	**.01**
5–24	811/820 (98.9)	4.2 (1.8–9.8)	**<.01**	4.8 (1.9–11.9)	**<.01**
25–44	303/317 (95.6)	Reference	N/A	Reference	N/A
≥45	263/268 (98.1)	2.4 (.9–6.8)	.09	2.4 (.8–7.0)	.12
HIV status					
Negative	1354/1379 (98.2)	Reference	N/A	Reference	N/A
Positive	248/249 (99.6)	1.5 (–.4 to 3.5)	.14	10.1 (1.3–77.1)	**.03**

Abbreviations: aOR, adjusted OR; HIV, human immunodeficiency virus; N/A, not applicable; OR, odds ratio.

[a]Bold font denotes statistical significance ($P < .05$). Only variables found to be statistically significant ($P < .1$) in univariate analysis or included *a priori* were assessed in the multivariable model.

[b]Year, site, and age group ($P < .1$ on univariate analysis) were evaluated in the multivariable model. Only *a priori* variables and variables remaining significant in the final model are shown in the table.

pneumococcal carriage but were colonized with pneumococcus for longer duration. Younger age (<5 years) and HIV infection were associated with high colonization densities (≥1000 copies/mL).

Colonization point prevalence ranged from 36% in autumn to 41% in winter and spring. Cross-sectional studies report that 45% of Canadian children ≤18 years and 37% of children in Vientiane (2013–2018) were pneumococcal carriers [18, 19]. A

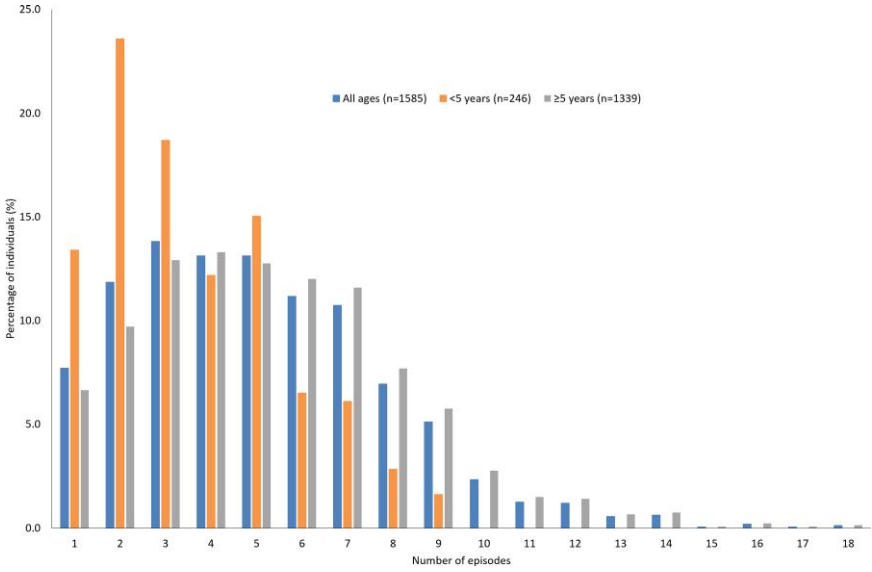


Figure 1. Distribution of the percentage of individuals that carried the pneumococcus at least once during the study period by number of pneumococcal colonization episodes and age group, PHIRST study, South Africa, 2016–2018 (N = 1585).

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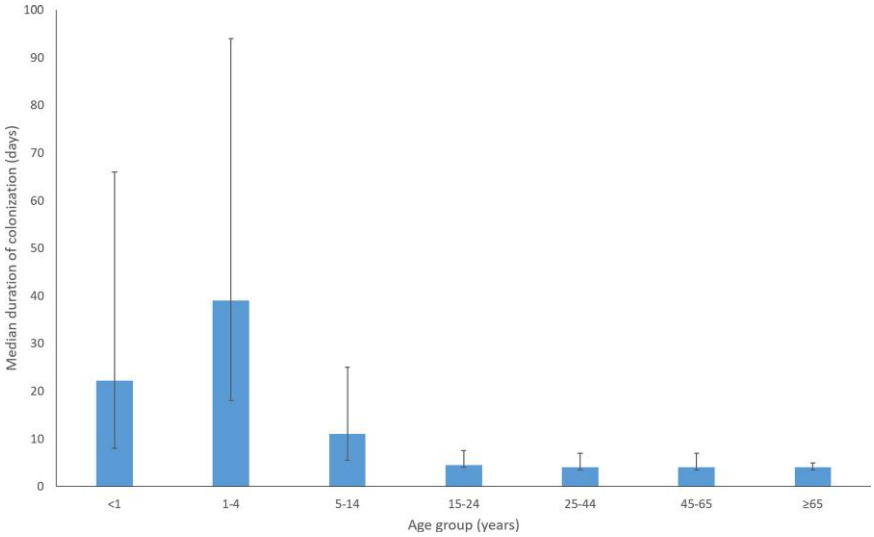


Figure 2. Median pneumococcal carriage duration by age group, with error bars indicating interquartile range, PHIRST study, South Africa, 2016–2018 (N = 1585).

meta-analysis of 29 studies showed that the point prevalence ranged from 0% to 39% in individuals aged 60 years and older [20]. In our study, 38% of specimens tested were positive for *S. pneumoniae*. Althouse et al. reported 36% of swabs, longitudinally collected once a month for 6 months, from Navajo and White Mountain Apache families in the United States were positive for pneumococcus from 2006 to 2008 [21]. A possible reason for similar results between our study and theirs despite their less-frequent sampling could be due to the long carriage duration observed in our study. In other cohort studies, the detection rate in NP specimens ranged from 25% to 86% with the highest detection seen in The Gambia [22–24]. Differences by study may be attributed to the age of the study populations enrolled, the number of individuals in a household, the frequency of specimen collection, and the method used to detect pneumococcus.

Some studies have shown a decrease in colonization and others reported colonization remained the same after PCV. A longitudinal study in The Gambia showed that all children carried pneumococcus at least once when swabs were collected twice monthly for 6 months with a high proportion of colonization in both children and adults; however, this was before routine pneumococcal immunization [24, 25]. In Peru, 92% of children were colonized at least once when NP specimens were collected monthly for approximately 6 months [23]. We found a high proportion of colonized individuals. Colonization levels vary across studies, likely attributed to sampling strategy, the population sampled, and immunization levels of the study population. In Peru, they have shown that carriage rates remained high and did not change after PCV7 introduction [23]. In the United Kingdom, where samples were collected monthly for 10 months, 58% of participants were colonized with pneumococcus after PCV introduction [26]. Similarly, in the Drakenstein study in South Africa, pneumococcal carriage was 54% in infants after PCV introduction [27].

Although colonization rates were high in all ages, there were differences by age. Young children (<5 years of age) were more likely to be colonized with pneumococcus than older individuals (25–44 year olds). This is not surprising because this association is well-documented, dating back to the 1950s [28–30]. Pneumococcal colonization rates decreasing with increasing age has been documented in The Gambia [25], Uganda [31], Kenya [32], United Kingdom [26], Nigeria [33], and the United States [21]. However, serotyping data are important to determine whether this decrease in colonization is due to vaccine serotypes. We observed PWH were more likely to be colonized with pneumococcus than HIV-uninfected individuals. Other South African studies report adults with HIV had a higher pneumococcal carriage prevalence than HIV-uninfected adults [34, 35].

We found children were colonized with pneumococcus for longer periods than adults and as a corollary, the number of episodes differed by age, with children having fewer episodes than adults, similar to findings by Masters et al. [29]. A limitation to our study is the data are left truncated and right censored; therefore, we are not certain how long the pneumococcus may have been carried before the first visit or after the last visit. Abdullahi et al. showed that pneumococcus clearance rate increased with age [36]. In our study, the median duration of a carriage episode was over a month in children aged <5 years, similar to studies from Peru and Kenya [23, 36].

Previous studies have shown the importance of high pneumococcal carriage density in the development of invasive

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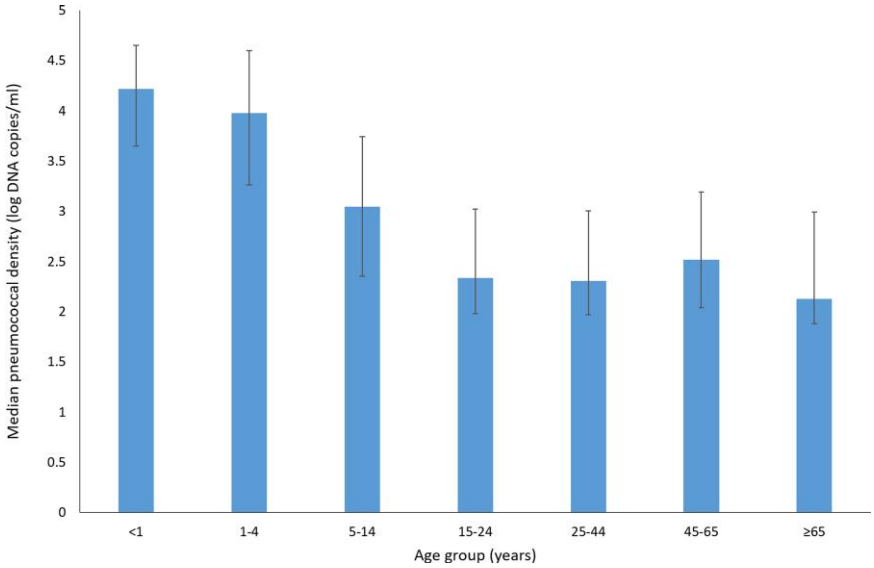


Figure 3. Median pneumococcal colonization density with interquartile range by age group, PHIRST study, South Africa, 2016–2018 (N = 1585).

Table 2. Comparison of Characteristics of Individuals With Low and High Pneumococcal Colonization Density, PHIRST Study, South Africa, 2016–2018 (N = 1585)

	Median Pneumococcal Colonization Density		Univariate Analysis		Multivariable Analysis[b]	
Characteristic	Low Pneumococcal Density (<1000 Copies/mL) n/N (%)	High Pneumococcal Density (≥1000 Copies/mL) n/N (%)	OR (95% CI)	*P* Value[a]	aOR (95% CI)	*P* Value[a]
Overall	893/1585 (56.3)	692/1585 (43.7)	N/A	N/A	…	…
Year						
2016	281/514 (54.7)	233/514 (45.3)	Reference	N/A	…	…
2017	321/549 (58.5)	228/549 (41.5)	0.9 (.7–1.1)	.21	…	…
2018	291/522 (55.8)	231/522 (44.3)	0.9 (.7–1.2)	.73	…	…
Site						
Agincourt	433/813 (53.3)	380/813 (46.7)	1.3 (1.1–1.6)	**.01**	…	…
Klerksdorp	460/772 (59.6)	312/772 (40.4)	Reference	N/A	…	…
Sex						
Male	313/626 (50.0)	313/626 (50.0)	1.5 (1.2–1.9)	**<.01**	…	…
Female	580/959 (60.5)	379/959 (39.5)	Reference	N/A	…	…
Age group, years						
<1	3/30 (10.0)	27/30 (90.0)	21.9 (6.4–75.3)	**<.01**	25.4 (7.4–87.6)	**<.01**
1–4	38/215 (17.7)	177/215 (82.3)	11.3 (7.1–18.2)	**<.01**	13.5 (8.3–21.9)	**<.01**
5–14	255/529 (48.2)	274/529 (51.8)	2.6 (1.8–3.7)	**<.01**	3.1 (2.1–4.4)	**<.01**
15–24	189/257 (73.5)	68/257 (26.5)	0.9 (.6–1.3)	.5	1.0 (0.6–1.5)	.9
25–44	222/297 (74.8)	75/297 (25.3)	0.8 (.5–1.2)	.4	0.8 (.5–1.2)	.2
45–65	134/189 (70.9)	55/189 (29.1)	Reference	N/A	Reference	N/A
>65	52/68 (76.5)	16/68 (23.5)	0.7 (.4–1.4)	.4	0.8 (.1–1.6)	.6
HIV status						
Negative	737/1338 (55.1)	601/1338 (44.9)	Reference	N/A	Reference	N/A
Positive	156/247 (63.2)	91/247 (36.8)	0.7 (.5–.9)	**0.02**	1.7 (1.2–2.4)	**<.01**

Abbreviations: aOR, adjusted OR; HIV, human immunodeficiency virus; N/A, not applicable; OR, odds ratio.

[a]Bold font denotes statistical significance ($P < .05$).

[b]Site, sex, age group, and HIV status ($P < .1$ on univariate analysis) were evaluated in the multivariable model. Only *a priori* variables and variables remaining significant in the final model are shown in the table.

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pneumococcal disease and transmission of the pneumococcus between individuals [16, 37]. We found young age and HIV infection were associated with a high pneumococcal density. Even with the decline in pneumococcal carriage of vaccine serotypes after PCV introduction, research has shown the persistent colonization of vaccine-serotypes 19F and 23F in South Africa [10]. Our data coupled with previous data suggest that after PCV introduction, even in populations with high PCV coverage, children may still play an important role in transmitting pneumococcus [37, 38]. Even though disease has decreased in children after PCV, children may still be at risk of disease if not immunized or if they have comorbidities [38]. This highlights the importance of HIV infection in transmitting pneumococcus because PCV vaccine efficacy is lower in children with HIV versus those uninfected [38]. Serotyping of pneumococcus and mathematical modeling could describe this in detail to gain an understanding of the transmission dynamics within the household and communities in South Africa.

A strength of our study was that we unlikely missed episodes of colonization, which affords us the ability to gain a higher resolution into pneumococcus over time, an advantage over cross-sectional or case-related studies. However, a limitation is that NP specimens were tested for pneumococcus using a real-time PCR, which has reduced specificity because of the genetic exchange that occurs between pneumococcus and *Streptococcus* spp. such as *S. pseudopneumoniae and S. mitis* [39]. Tavares et al. showed that using the *lytA* gene with the *SP2020* gene to identify *S. pneumoniae* improved specificity from 99.5% to 100% [40]. We identified pneumococcus by targeting the *lytA* gene only, potentially misidentifying 0.5% of *Streptococcus* spp. as *S. pneumoniae*. We observed a difference in the proportion of colonization in 2017 compared with 2016, which may be attributed to the difference in the duration of the follow-up period. Participants were followed up for a longer period in 2017 compared with 2016 (10 vs 6 months), which may have underestimated the number of episodes identified in 2016. Also, individuals that were lost to follow-up remained in the analysis, and we are unable to determine whether these individuals would have been colonized had they not withdrawn. Another limitation is that serotyping data for this study were not available; hence, the number of pneumococcal episodes may be underestimated and the duration of carriage may be overestimated because duration of colonization differs by serotype and multiple episodes of colonization with different serotypes could have been incorrectly classified as a single episode [32]. To help address this, we applied a Markov model to the follow-up data. Comparing the pneumococcal density in the nasopharynx over time is complex because the bacterial load is dependent on the volume of nasal secretions, the quality of the specimen taken, and variation in sample collection technique used. However, in a publication in which the concentration of human DNA was used to control for this limitation, the authors found that flocked swabs are efficient at collecting a standard volume of sample from the nasopharynx [41].

In our study cohort, we found high levels of pneumococcal colonization across all ages. Children aged <5 years carried pneumococcus for longer periods. PWH and children <5 years were associated with high pneumococcal densities. These findings highlight that young children may potentially impact transmission and remain important in the transmission of pneumococcus even after PCV introduction.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. C. C., M. M., T. M., J. M., F. K. T., O. H., N. W., N. A. M., K. K., A. v. G., and S. T.: Conception or design of protocol. All authors: Acquisition, analysis or interpretation of data for the work. M. C., N. W., O. H., F. K. T., and A. v. G.: Laboratory testing of samples. L. L., M. M., K. M., and F.W.: Enrolment and follow-up of participants. M. C., N. W., S. T., C. C., and A. v . G.: Drafting the work or revising it critically for important intellectual content. All authors: Final approval of the version to be published. All authors: Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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