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MAJOR ARTICLE

Temporal Changes in Nasopharyngeal Pneumococcal Colonization Density Associated With Respiratory Syncytial Virus and Influenza in a South African Household Cohort Study, 2016–2018

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Background. Respiratory syncytial virus (RSV) and influenza infections are associated with increased pneumococcal colonization and disease risk. We assessed the impact of RSV and influenza on pneumococcal colonization density and factors influencing density changes during viral infection.

Methods. Over 3 years, 1658 individuals from 325 households were enrolled, with nasopharyngeal swabs collected twice weekly for pneumococcus, RSV, and influenza A/B detection by real-time polymerase chain reaction. We analyzed samples from 2 weeks before, during, and 2 and 8 weeks after infection. Pneumococcal density was compared across infection periods by *t* tests, and multivariable regression identified factors influencing density changes.

Results. Pneumococcal density increased during RSV infection (log mean before vs during infection, 9.3 vs 10.2 genomic copies/mL; $P < .01$) but showed no significant overall increase with influenza (log mean before vs during infection, 9.6 vs 9.9 genomic copies/mL; $P = .2$). However, the following were correlated with increased pneumococcal density: higher influenza viral loads (cycle threshold [Ct] value <25: coefficient, 2.8; 95% CI 1.4–4.2) and RSV viral loads (viral Ct value <25: coefficient, 2.5 [95% CI, 1.1–3.9; $P < .01$]; viral Ct value of 25–29: coefficient, 1.1 [95% CI, .1–2.2; $P = .04$]; vs viral Ct value of 30–34). Participants who were underweight had lower pneumococcal density differences (coefficient, −1.8; 95% CI, −3.5 to −.1; $P = .04$) than those with a normal body mass index.

Conclusions. RSV infection, especially with higher viral loads, increases pneumococcal colonization, while individuals who are underweight exhibit lower density changes.

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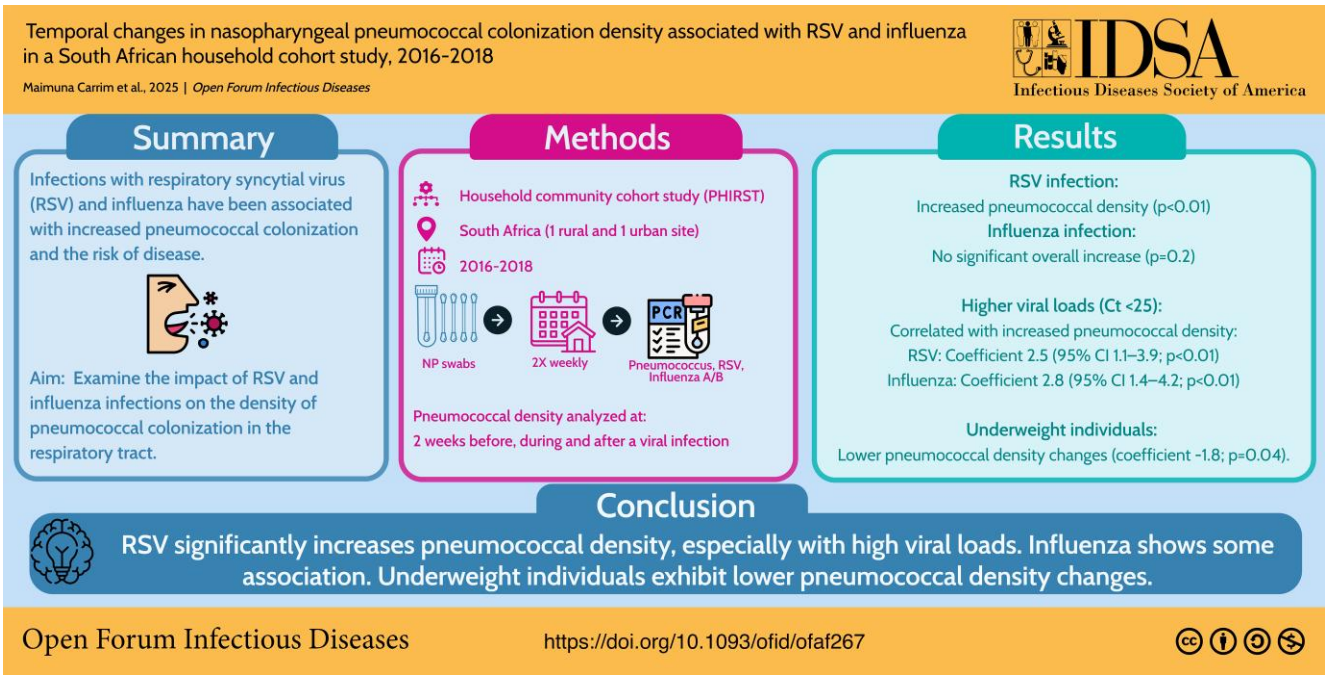
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Graphical Abstract



This graphical abstract is also available at Tidbit: https://tidbitapp.io/tidbits/temporal-changes-in-nasopharyngeal-pneumococcal-colonization-density-associated-with-rsv-and-influenza-in-a-south-african-household-cohort-study-2016-2018

Keywords. community cohort; influenza; pneumococcus colonization; RSV; *Streptococcus pneumoniae*.

Streptococcus pneumoniae, or the pneumococcus, colonizes the nasopharynx of healthy individuals, where it can persist without causing disease [1]. However, a weakened immune system or the presence of another respiratory pathogen can cause the proliferation and translocation of the pneumococcus from the nasopharynx to the lower respiratory tract, leading to severe infections such as pneumonia, meningitis, and bacteremia [2, 3].

Studies have shown that respiratory syncytial virus (RSV) infections increase the risk of pneumococcal disease [4], including a higher risk of developing pneumococcal pneumonia [5–7]. Research suggests that this may be due to macrophage polarization or impairment of an individual's immune system, or it may be a result of enhancing the virulence of the pneumococcus, specifically, by RSV binding to the penicillin-binding protein of the pneumococcus and increasing the bacterial adherence and infection of human respiratory epithelium [5–7].

Influenza infections in humans were associated with greater odds of pneumococcal carriage [8–10]. Similar to RSV, the interaction of the pneumococcus with influenza virus may worsen the disease outcome of an infected person [4, 10–13]. During the 1918 influenza pandemic, there was a significant increase in pneumococcal pneumonia cases, which were a major contributor to the high mortality rate observed [14]. A study found that experimental infection with influenza in animal models resulted in greater pneumococcal load in the nasopharynx [15]. This increase in pneumococcal load was accompanied by changes in the nasopharyngeal environment, including greater inflammation and decreased bactericidal activity, likely contributors to increased pneumococcal invasion [16, 17].

We aimed to determine (1) whether RSV or influenza infection is associated with a temporal increase in pneumococcal nasopharyngeal colonization density and (2) which factors are associated with changes in pneumococcal density during a RSV or influenza infection episode in a household community cohort in South Africa.

Our study was a household community cohort study encompassing individuals of all ages from urban and rural settings in South Africa. This study design allowed us the opportunity to observe the dynamic changes in the pneumococcus over time and to investigate factors influencing changes in pneumococcal density during RSV or influenza infections. Furthermore, we enrolled persons irrespective of symptoms, which is in contrast to previous studies that primarily focused on hospitalized patients with acute respiratory illness. Our study enhances the generalizability of findings to broader epidemiologic contexts.

METHODS

Study Design

As part of a larger study named "a prospective household cohort study of influenza, respiratory syncytial virus and other

respiratory pathogens community burden and transmission dynamics in South Africa" (PHIRST), household cohorts were enrolled at 2 community sites in South Africa [18, 19]. The sites were an urban site (Klerksdorp) and a rural site (Agincourt) with a new cohort of households enrolled each year (100 households in 2016, 108 households in 2017, and 117 households in 2018). The cohorts of households were followed up from May through October 2016 and from January through October in 2017 and 2018 [18, 19]. Nonhospitalized individuals, irrespective of symptoms or antibiotic use, were enrolled in the study. Baseline data, including demographics and having a chronic medical condition, were collected from all enrolled persons. Symptom data were collected from patients at the same time as sample collection. Symptoms included at least 1 of the following: fever (self-reported or measured tympanic temperature ≥38 °C), cough, difficulty breathing, sore throat, nasal congestion, chest pain, muscle aches, headache, vomiting, or diarrhea. Body mass index (BMI) was calculated by dividing the weight (in kilograms) by the squared height (in meters) of the person. Thereafter, adults were classified as underweight with a BMI <18.5, normal weight with a BMI of 18.5 to 24.9, overweight with a BMI of 25.0 to 29.9, and obese with a BMI ≥30. For children, we employed the BMI classification as stipulated by the World Health Organization [20].

Pneumococcal conjugate vaccine records were obtained from the Road-to-Health cards for participants aged <5 years. HIV status was determined by evidence of documented test results, treatment with antiretroviral therapy, or rapid test done as part of the study. Individuals were classified as HIV negative if they presented documented negative HIV results within 6 months before recruitment. Those without HIV results were classified as having HIV status unknown.

Inclusion and Exclusion Criteria for Enrollment Into the PHIRST Study

For the PHIRST study, the inclusion and exclusion criteria for enrollment and consent have been described in detail [18]. In brief, the PHIRST study enrolled households with ≥3 members who shared at least 4 meals a week, and >80% of approached households consented to participate. Exclusions applied if the household head was a minor or unavailable after 3 attempts for permission. Participants aged ≥18 years provided written consent, children aged 7 to 17 years provided assent, and children aged <18 years required parent or guardian consent. Exclusions during the study period included moving from study sites, refusal of sample collection, withdrawal from the study, or household member deaths or unavailability for swabbing resulting in <3 members in the household. For this analysis, we included only those individuals who tested positive for influenza or RSV at least once during the study period and tested positive for pneumococcus before and during the viral infection.

Specimen Collection and Laboratory Testing

Nasopharyngeal flocked swabs were collected twice weekly, placed in 1.5 mL of PrimeStore Molecular Transport Medium (Longhorn Vaccines & Diagnostics), and shipped at 4 to 8 °C to the National Institute for Communicable Diseases in Johannesburg. Total nucleic acids were extracted from 200 µL of sample and eluted into 100 µL of elution buffer by an automated magnetic bead MagNA Pure 96 system with the MagNA Pure 96 DNA and Viral NA Small Volume Kit and the Viral NA Plasma SV protocol (Roche). Quantitative, in-house, singleplex real-time polymerase chain reaction (PCR) targeting the autolysin gene (*lytA*) was used to identify *S pneumoniae* through universal cycling conditions, with each 25- µL reaction containing 5 µL of total nucleic acids [21]. Samples with a cycle threshold (Ct) value ≤40 were considered positive and were included in the analysis. A standard curve with serially diluted DNA extracts from a known starting quantity of American Type Culture Control (ATCC 49619) was included with every PCR assay to calculate pneumococcal density (genomic copies per milliliter). In an article published by Carvalho et al in which the assay was validated, they describe that the limit of detection of this assay was <10 genomic copies/mL [21]. For RSV and influenza detection, we performed reverse transcription real-time PCR (RT-qPCR) with the FTD Flu/RSV detection assay (Fast Track Diagnostics), with the RT-qPCR Ct value used as a proxy for viral load (ie, a low Ct value implied high viral load and vice versa). To assess whether viral load is associated with a change in pneumococcal density, we used the result of the swab with the lowest Ct value during the infection to assess the viral load. Influenza-positive samples were subtyped by the US Centers for Disease Control and Prevention (CDC) subtyping kit (available through the International Reagent Resource Program).

Statistical Analysis

We defined an RSV or influenza virus infection as at least 1 RT-qPCR (Ct value <37) nasopharyngeal swab positive for the virus, irrespective of symptoms. For influenza, we considered an infection to be new when the individual tested positive for a different virus subtype or the same subtype >2 weeks after the last day of previous positivity; all other instances were considered the same infection. These criteria were used as participants could test negative and then positive again due to fluctuations in viral load or specimen quality [19, 22]. For our analysis, we did not stratify RSV by subtype. A study interval was defined as all pneumococcal-positive swabs tested 2 weeks (4 visits) before a viral infection (RSV or influenza; "before") and all pneumococcal-positive swabs tested during a viral infection (during). The "after" period was defined as starting from the first RSV/influenza-negative swab collected after the last RT-qPCR–positive swab. We calculated the mean across swabs collected 2 weeks (4 visits) before, during, and 2 weeks

after infection for the 2-week time point. Similarly, for the 8-week time point, we used swabs from 8 weeks (16 visits) before, during, and 8 weeks after infection.

If an individual was positive for RSV and thereafter influenza or vice versa, each infection was regarded as a separate episode and included in the analysis for the virus. The duration of a viral infection in days was determined for each participant by multiplying the number of visits with positive results by 2.5 days, since swabs were collected every 2 to 3 days for an RSV and influenza infection. Thereafter, these data were used to determine the mean duration of the infection for each virus. Furthermore, we computed the mean pneumococcal density for each study interval per person. Subsequently, the logarithmic mean of the density for each participant was determined by the previously computed mean. We compared the log mean pneumococcal density by time (during vs before, during vs after, and after vs before) in relation to an RSV or influenza infection with a *t* test and a 95% CI. In a multivariable linear regression model, we determined the factors associated with a change in pneumococcal density—specifically, the difference in the log mean pneumococcal density before an RSV or influenza infection and that during the viral infection. We assessed all variables that were significant at $P < .2$ on univariate linear regression analysis or a priori (age group, HIV status, RSV, and influenza) and dropped nonsignificant factors in the multivariable model ($P \geq .05$) with manual backward elimination. We assessed pairwise interactions by the inclusion of product terms for all variables remaining in the final multivariable additive model. We also accounted for clustering by study site and within the household via mixed effects models. We performed the analysis in Stata version 14 (StataCorp LLC), R Foundation for Statistical Computing (R Core Team), and RStudio: Integrated Development for R (RStudio Team). Statistical significance was assessed at $P < .05$.

Ethics

The protocol was registered on http://clinicaltrials.gov on 6 August 2015 (NCT02519803) and was approved by the University of the Witwatersrand Human Research Ethics Committee (150808), and the US CDC's institutional review board relied on the local review (6840). This activity was reviewed by the CDC and was conducted consistent with applicable US federal law and CDC policy. Approval of laboratory testing for *S pneumoniae* (M210367) was obtained from the University of the Witwatersrand Human Research Ethics Committee.

RESULTS

In the PHIRST study, 325 households were enrolled comprising 1658 individuals (Figure 1). With twice-weekly follow-ups, 105,686 samples were tested for *S pneumoniae*, RSV, and influenza, of which 38 829 (36.7%) were positive for the pneumococcus, 1002 (0.9%) were positive for RSV, and 1265 (1.2%) were positive for influenza (n = 597 [0.6%], influenza A; n = 665 [0.6%], influenza B; n = 3 [<0.1%], influenza A and B). During the study, 1079 of 1442 (74.8%) individuals were infected with RSV or influenza: 462 (32.0%) were positive for RSV, 314 (21.8%) for influenza A, and 303 (21.0%) for influenza B. Of those positive for a virus, 46.7% (504/1079) had at least 1 symptom (35.7% [165/462], RSV; 58.3% [183/314], influenza A; 51.5% [156/303], influenza B).

Among individuals positive for either RSV or influenza during the study period, 530 (49.1%) were colonized with the pneumococcus before and during the infection (Figure 1, Table 1) and were included in our analysis. Among those colonized with the pneumococcus and infected with RSV or influenza, 55% (291/530) were female, 54% (286/530) were from the rural setting, 93% (472/509) were HIV uninfected, and 84% (194/231) were fully vaccinated for their age. Furthermore, 54.0% (286/530) reported at least 1 symptom during the infection (42.4% [98/231], RSV; 66.4% [97/146], influenza A; 59.5% [91/153], influenza B).

The mean duration of a viral infection in individuals colonized with the pneumococcus was 7.8 days (95% CI, 6.5–9.0) for RSV, 6 days (95% CI, 5.5–6.8) for influenza A, and 6.8 days (95% CI, 5.8–7.8) for influenza B. When we looked at the mean pneumococcal density by days that a sample was collected in relation to the first day positive, there was an increase in the density of pneumococcus at the first day in the viral infection, and the density weaned thereafter (Figure 2*A*). However, for influenza virus A and B, we did not observe a clear increase before or on the first day positive (Figure 2*B* and 2*C*).

We observed a significant increase in the log mean pneumococcal colonization density (expressed as log genomic copies per milliliter) before vs during an RSV infection (log mean before vs during, 9.3 vs 10.2; $P < .01$; Figure 3*A*). Conversely, there was no significant increase in the log mean pneumococcal density before and during an influenza infection (log mean before vs during, 9.6 vs 9.9 genomic copies/mL; $P = .2$; Figure 3*B*). Furthermore, findings were similar when we analyzed influenza A and B separately (influenza A infection: log mean before vs during, 9.5 vs 9.8 genomic copies/mL [$P = .3$]; influenza B infection: log mean before vs during, 9.7 vs 10.0 genomic copies/mL [$P = .4$]; Figure 3*C* and 3*D*).

Two weeks following an RSV infection, pneumococcal density remained elevated as compared with previral infection levels (log mean before vs 2 weeks after, 9.3 [95% CI, 8.9–9.8] vs 10.3 [95% CI, 9.9–10.7]; $P < .01$). This elevation in pneumococcal density persisted 2 months after an RSV infection (log mean during vs 2 months after, 10.3 [95% CI, 9.9–10.6] vs 11.0 [95% CI, 10.7–11.3]; $P < .01$).

When ascertaining factors associated with changes in pneumococcal carriage density during an RSV infection, we found that an increase in pneumococcal density was observed for

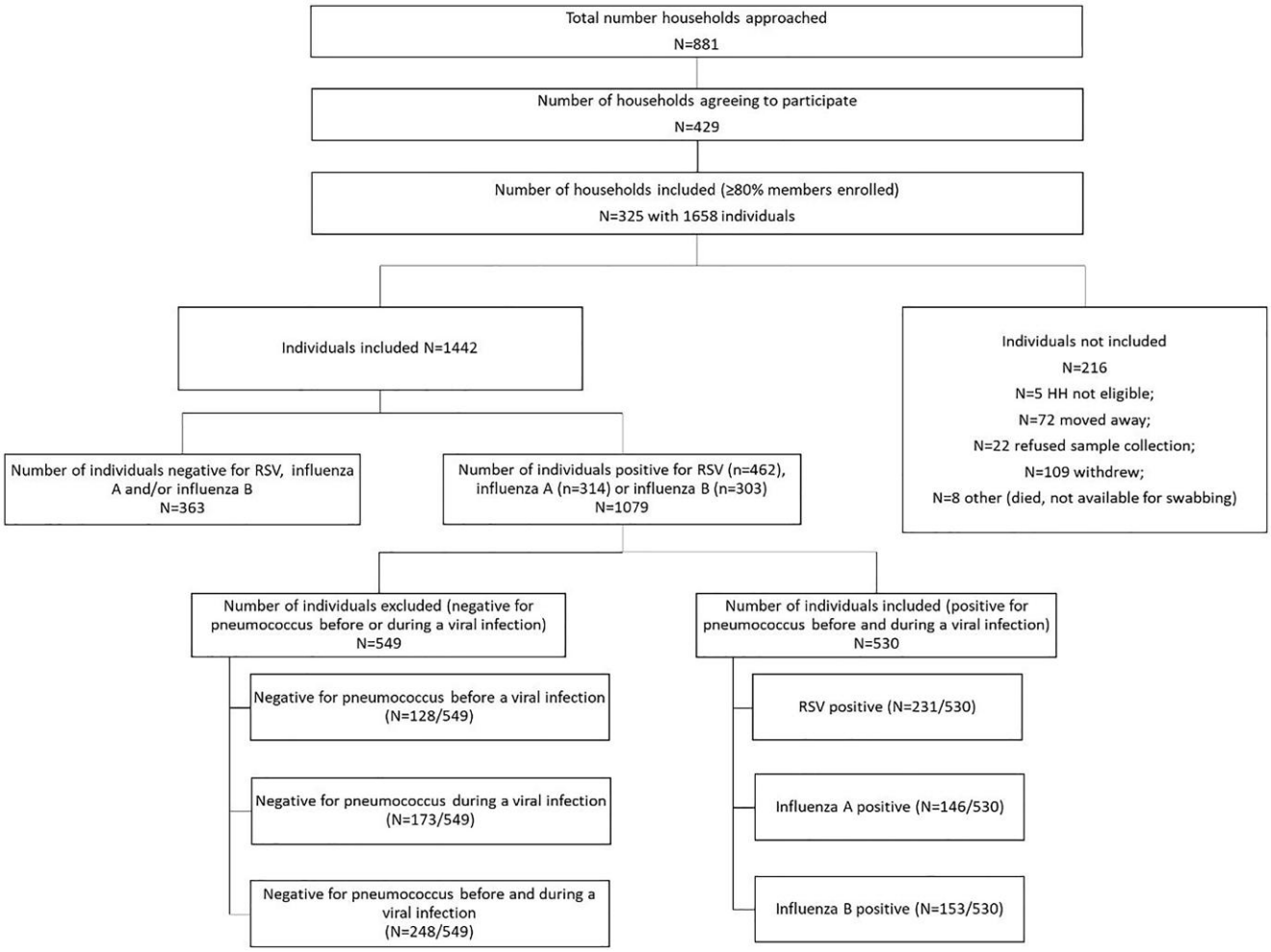


Figure 1. A flowchart depicting the number of participants enrolled, tested, and included in the analysis—PHIRST study, South Africa, 2016–2018. Abbreviation: RSV, respiratory syncytial virus.

individuals with a higher viral load (viral Ct value <25: coefficient, 2.5 [95% CI, 1.1–3.9; $P < .01$]; viral Ct value of 25–29: coefficient, 1.1 [95% CI, .1–2.2; $P = .04$]; vs viral Ct value of 30–34; Table 2). Furthermore, those who were underweight were less likely to have an increase in density (coefficient, −1.8; 95% CI, −3.5 to −.1; $P = .04$) than those with a normal BMI.

Even though there was no statistically significant increase in the pneumococcal density in the presence of influenza, we found that a higher mean influenza viral load (low Ct value) was associated with a significant change in pneumococcal density during an influenza infection as compared with before an influenza infection (viral Ct value <25: coefficient, 2.8 [95% CI, 1.4–4.2; $P < .01$]; viral Ct value of 25–29: coefficient, 1.2 [95% CI, .2–2.3; $P = .03$]; vs viral Ct value of 30–34; Table 3).

DISCUSSION

Previous research shows that respiratory viral infections such as RSV and influenza increase pneumococcal colonization density, but studies have predominantly been limited to hospitalized populations. Our household cohort study examined pneumococcal households, providing insights into how these viral infections influence pneumococcal colonization density over time.

We observed significant changes in the density of the pneumococcus in the nasopharynx before vs during an RSV infection. Furthermore, we found that 2 weeks after an RSV infection, pneumococcal density remained elevated. Pneumococcal carriage density increased in individuals with higher viral loads during RSV and influenza infections as compared with before the infections. Individuals who were underweight were less likely to have an increase in pneumococcal density in the presence of RSV when compared with those with a normal BMI.

The demographic characteristics of individuals colonized with pneumococcus and infected with RSV or influenza were similar to those of the overall PHIRST study population. This suggests that differences in outcomes are less likely to be driven by demographic variability and more likely to reflect the factors under investigation.

Table 1. Demographic Characteristics of All Individuals Enrolled in the PHIRST Study and Those Colonized With Pneumococcus During an Infection With RSV or Influenza A or B, South Africa, 2016–2018

Characteristic	PHIRST Study Population	Infection With Pneumococcal Colonization			
		RSV and Influenza	RSV	Influenza A	Influenza B
Year					
2016	542/1658 (33)	154/530 (29)	76/231 (33)	30/146 (20)	48/153 (31)
2017	558/1658 (34)	185/530 (35)	76/231 (33)	74/146 (51)	35/153 (23)
2018	558/1658 (34)	191/530 (36)	79/231 (34)	42/146 (29)	70/153 (46)
Age, y					
<1	34/1658 (2)	19/530 (4)	10/231 (4)	5/146 (3)	4/153 (3)
1–4	240/1658 (15)	212/530 (40)	97/231 (42)	61/146 (42)	54/153 (35)
5–14	546/1658 (33)	216/530 (41)	92/231 (40)	51/146 (35)	73/153 (48)
15–24	264/1658 (16)	30/530 (6)	9/231 (4)	11/146 (8)	10/153 (7)
25–44	311/1658 (19)	29/530 (6)	11/231 (5)	9/146 (6)	9/153 (6)
≥45	263/1658 (16)	24/530 (4)	12/231 (5)	9/146 (6)	3/153 (2)
Sex					
Female	991/1658 (60)	291/530 (55)	132/231 (57)	76/146 (48)	83/153 (54)
Male	667/1658 (40)	239/530 (45)	99/231 (43)	70/146 (52)	70/153 (46)
Site					
Rural	841/1658 (51)	286/530 (54)	140/231(61)	73/146 (50)	73/153 (48)
Urban	817/1658 (49)	244/530 (46)	91/231 (39)	73/146 (50)	80/153 (52)
HIV status					
Uninfected	1363/1609 (85)	472/509 (93)	206/222 (93)	135/141 (96)	131/146 (90)
PWHIV	246/1609 (15)	37/509 (7)	16/222 (7)	6/141 (4)	15/146 (10)
PCV vaccination[a]					
Fully vaccinated	221/274 (81)	194/231 (84)	85/107 (79)	58/66 (88)	51/58 (88)
Partially vaccinated	7/274 (2)	4/231 (2)	2/107 (2)	0/66 (0)	2/58 (4)
Unknown	46/274 (16)	33/231 (14)	20/107 (19)	8/66 (12)	5/58 (8)
Chronic medical conditions[b]					
Yes	50/1658 (3)	11/530 (2)	5/231 (2)	1/146 (1)	5/153 (3)
No	1608/1658 (97)	519/530 (98)	226/231 (98)	145/146 (99)	148/153 (97)

Data are presented as No. (%).

Abbreviations: PCV, pneumococcal conjugate vaccine; PHIRST, a prospective household cohort study of influenza, respiratory syncytial virus and other respiratory pathogens community burden and transmission dynamics in South Africa; PWHIV, people with HIV; RSV, respiratory syncytial virus.

[a]Participants younger than 5 years with available data.

[b]Self-reported history of asthma, lung disease, heart disease, stroke, spinal epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease, or diabetes.

A US study showed that infection with common respiratory viruses, such as enterovirus, RSV, adenovirus, rhinovirus, and human coronavirus, were associated with an increase in pneumococcal density (approximately 3 Ct values lower in individuals with a virus vs without a virus), while viruses such as human parainfluenza virus, human metapneumovirus, and influenza virus were not [23]. Similar to our study, this supports the concept that pneumococcal density in a person infected with a virus may be pathogen specific [23]. Additionally, to this study, Bennet et al showed that *S pneumoniae* carriage density was associated with RSV infection but not with influenza. Comparable to our results, a Kenyan study investigating children with acute respiratory infections found a positive correlation between RSV infections and greater pneumococcal nasopharyngeal carriage density [24]. The increase in pneumococcal density in the presence of RSV was also shown in animal models as described by Manna et al [25]. Miellet et al observed that in their study population the pneumococcal density was 8-fold higher in individuals with influenza infection after influenza-like illness onset as compared with influenza-negative controls [26]. In our study, we found an increase in the carriage density of the pneumococcus during an influenza infection vs before; however, this increase was not statistically significant. For influenza, we may have been underpowered to detect a significant difference. Miellet et al collected saliva from participants >60 years old with acute influenza-like illness, which were tested on a multiplex ligation-dependent probe amplification assay for the detection of influenza. Variability across studies could also be attributed to the difference in the study population, study participants' age, the test used, the sample type tested, and the time that the study was conducted. The nonsignificant difference with an influenza infection and the significant difference with RSV infection may be pathogen specific. We cannot ignore that the disparities in pneumococcal carriage density may be linked to other inherent differences among individuals and their immune response

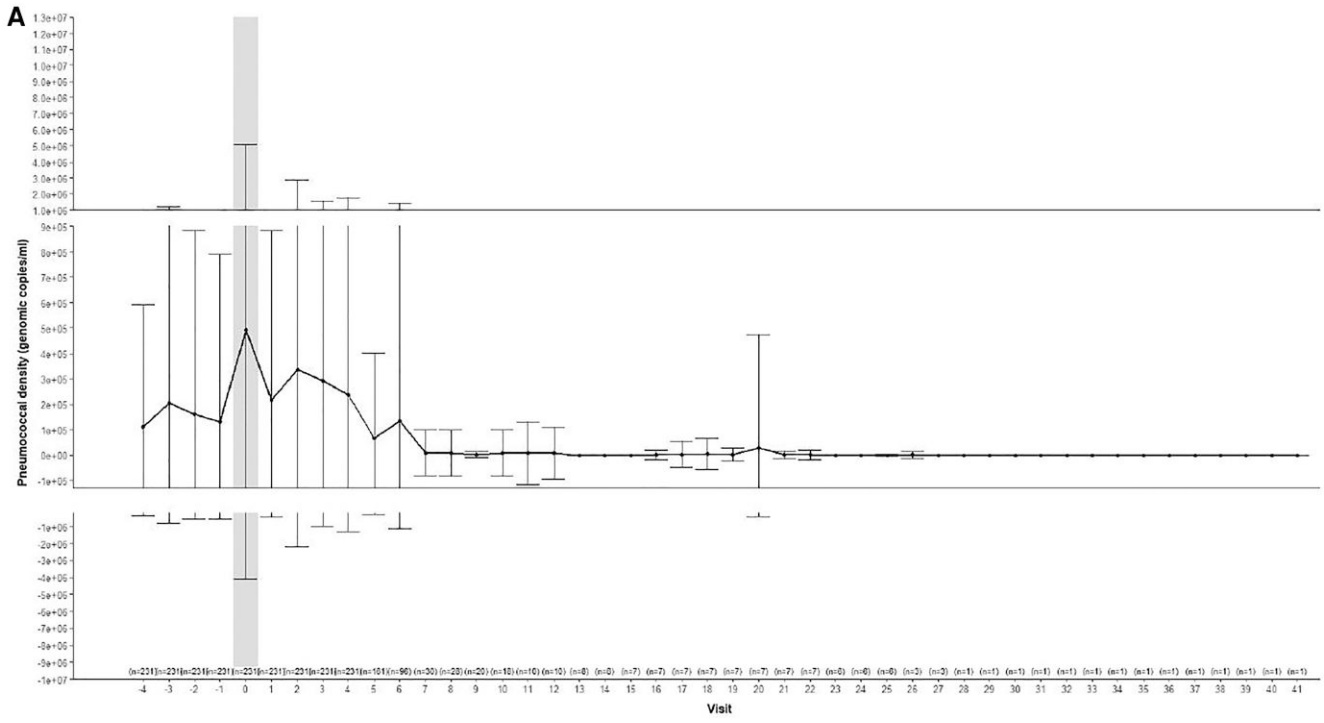


Figure 2. The mean pneumococcal density (genomic copies per milliliter) of all individuals with (*A*) respiratory syncytial virus (n = 231), (*B*) influenza A virus (n = 146), and (*C*) influenza B virus (n = 152) by visit in relation to the first visit positive for the virus, PHIRST study, South Africa, 2016–2018. Error bars depict 95% CI per point. Gray shaded block indicates the first day of a respiratory virus episode.

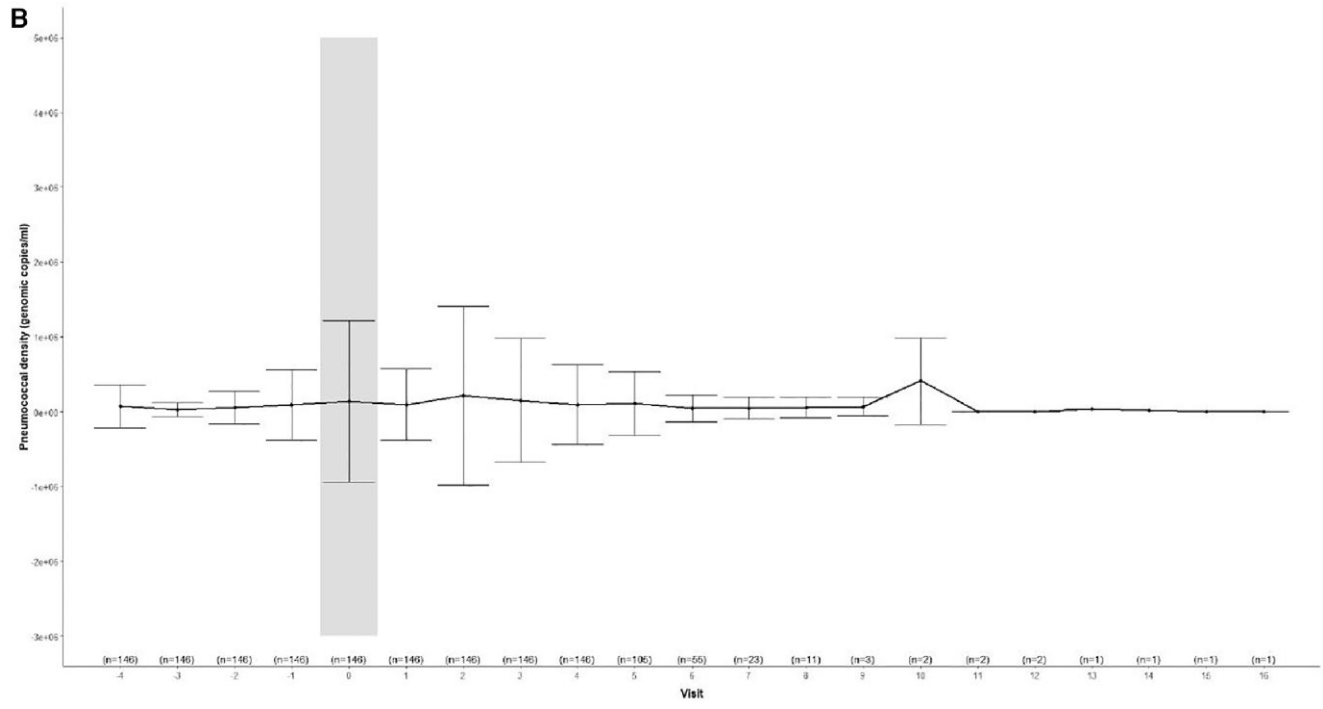


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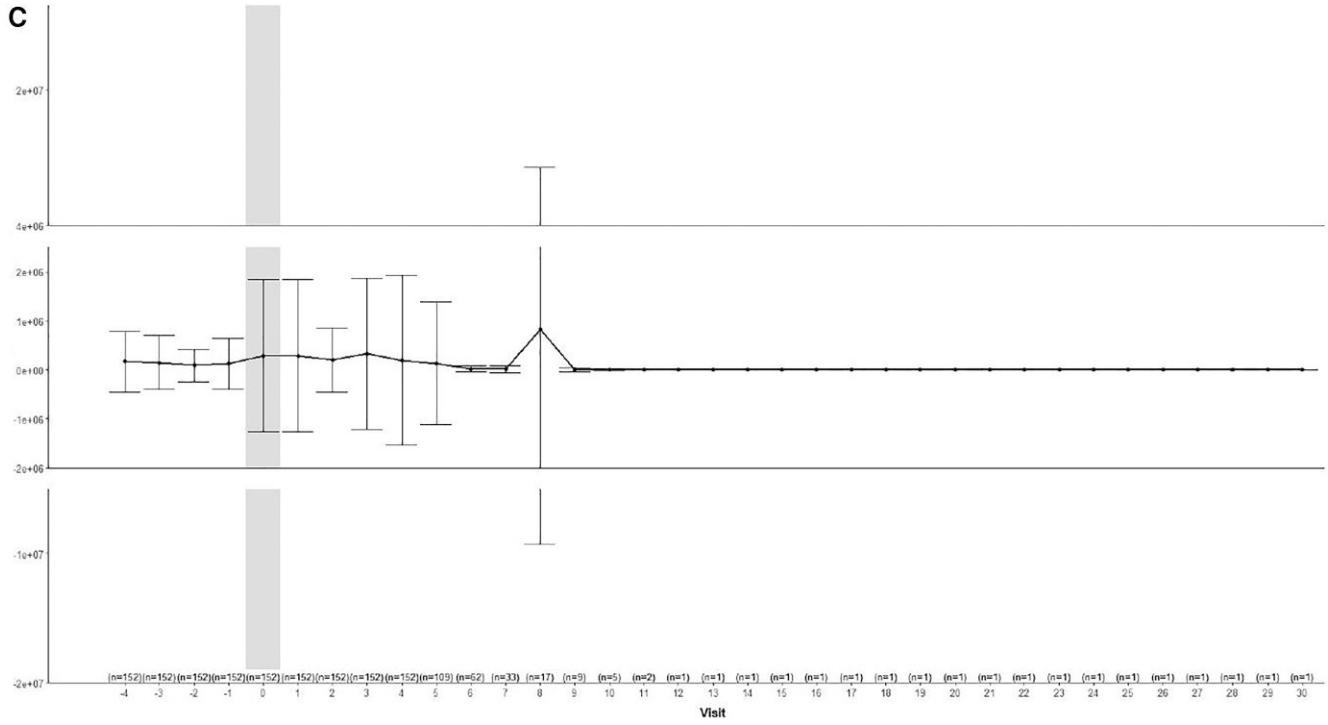


Figure 2. Continued

against viral infection [26, 27]. We further found that pneumococcal density remained elevated long after an RSV infection (ie, up to 2 months). Yet, since we tested only for RSV and influenza, we cannot exclude that the elevation might have been due to an infection with respiratory viruses not tested.

Miellet et al found that the synergistic relationship between respiratory viruses and pneumococcus may be related to host factors rather than the direct interaction of the virus with the pneumococcus [26]. Thus, we investigated the factors associated with changes in pneumococcal density in the nasopharynx during viral infection. To our knowledge, no similar studies have examined the factors associated with an increase in pneumococcal density in the presence of influenza or RSV in individuals irrespective of symptoms. We found a correlation between higher viral loads, as indicated by a lower Ct value, and greater pneumococcal density. When a virus infects the respiratory epithelial cells, it can compromise the integrity of the epithelial layer, creating a favorable environment for bacterial colonization and proliferation [7, 28–30]. This compromised epithelial layer impedes the immune system's ability to effectively clear bacterial invaders, thereby fostering conditions conducive to bacterial growth, and pneumococcal density increases [28]. Influenza virus, in particular, has been shown to deplete alveolar macrophages, which impair pneumococcal clearance [31]. Similarly, RSV infection can induce inflammation and mucosal damage, facilitating bacterial adherence and colonization, thereby raising pneumococcal carriage density [7]. Moreover, the higher viral load likely exacerbates the disruption of the epithelial layer, raising the host's susceptibility to greater bacterial colonization density. However, more research examining factors associated with changes in pneumococcal density in the presence of a virus in healthy individuals over time is needed for meaningful comparisons to be made.

A limitation of our study is that we did not have controls, although each participant served as one's control since we looked at the data before, during, and after an infection in the same individual. Another limitation is the use of qPCR for pneumococcus identification, which, while highly sensitive, can have specificity challenges due to genetic exchange with other *Streptococcus* spp, such as *S pseudopneumoniae* and *S mitis* [32]. However, culture also has limitations, including potential misclassification due to nonviable or low-density carriage. While Tavares et al reported that combining *lytA* and *SP2020* improved specificity from 99.5% to 100% [33], recent studies have raised concerns about the specificity of *SP2020*, as findings have not been consistently replicated across carriage studies. By exclusively targeting *lytA*, our approach may have led to minor misclassification. An alternative marker, *piaB*, has demonstrated higher specificity for pneumococcal identification, and future studies should consider using a combination of genetic targets, including *piaB*, to enhance the accuracy of pneumococcal detection [34].

Pneumococcal pneumonia is associated with high colonization density, and previous studies have shown that greater

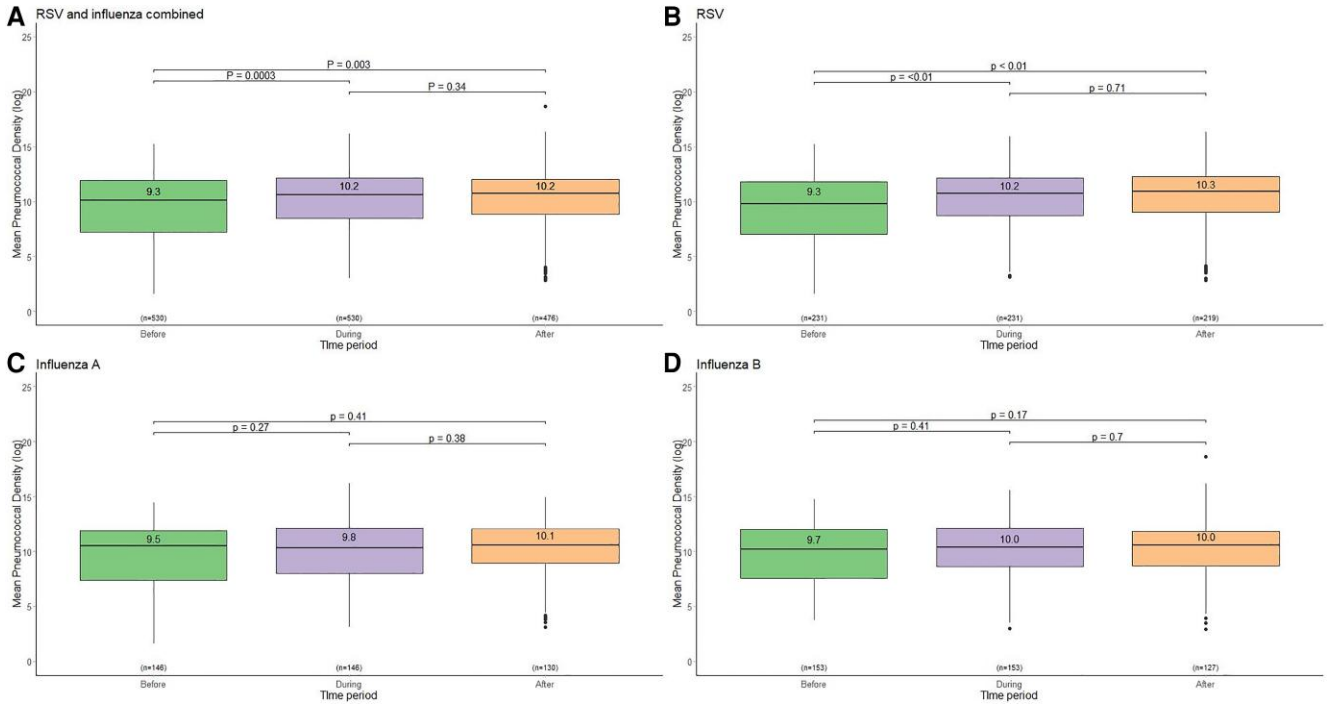


Figure 3. Box and whisker plots comparing the log mean pneumococcal density before, during, and after an RSV or influenza infection, PHIRST study, South Africa, 2016–2018: *A*, RSV and influenza combined (n = 530); *B*, RSV only (n = 231); *C*, influenza A (n = 146); *D*, influenza B (n = 153). Data are presented as median (line), interquartile range (box), and standard deviation (error bars). Horizontal lines on the top of each figure show the comparison of log mean density by period and the corresponding *P* value by *t* test. Abbreviation: RSV, respiratory syncytial virus.

pneumococcal carriage density plays a key role in the development of invasive disease and the transmission of pneumococcus between individuals [35–37]. The observed increases in pneumococcal density during viral infections may also have implications for transmission and community spread [35–37]. While our study was not designed to assess transmission directly, our findings contribute to the understanding of factors influencing pneumococcal density, which may in turn affect transmission dynamics. Furthermore, we did not serotype the pneumococcal-positive specimens, which limits our ability to analyze serotype-specific relationships between viral codetection and pneumococcal carriage density. Future analyses to explore pneumococcal transmission dynamics are required; however, this will require the inclusion of serotyping data to differentiate pneumococcal strains and accurately assess transmission events.

Another constraint in our study was the omission of examination of pneumococcal density in the context of concurrent coinfection with multiple viruses. Thus, we cannot exclude that the change in pneumococcal density could be affected by the coinfection with other viruses besides RSV and influenza that were not tested for.

A further limitation of our study is that we did not analyze the data by RSV and influenza subtype, which may have influenced our findings. Different viral strains may have varying effects on pneumococcal carriage and density, and without strain identification, we could not assess these interactions. Data from the larger PHIRST study indicate that influenza A (H1N1) was the predominant circulating strain in 2016 and 2018, while A (H3N2) was dominant in 2017 [18]. Additionally, seasonal variability in circulating influenza strains may have introduced unmeasured differences in pneumococcal dynamics, potentially masking strain-specific effects.

Nevertheless, the strength of our study was the consistent sampling of participants (twice weekly) irrespective of symptoms, enabling us to identify episodes of viral infection and explore the fluctuations in pneumococcal carriage density within individuals over time. Furthermore, our study was a community cohort study in which people of all ages were enrolled from an urban and rural site, which afforded us the ability to determine several factors associated with changes in pneumococcal density during an RSV or influenza infection, making our results more generalizable in our setting. In addition, our study was a community-level household cohort study, unique to previous studies that primarily examined the relationship between *S pneumoniae* carriage and viral codetection among hospitalized patients with acute respiratory illness.

Our results emphasize the complex interplay of viral infections and microbial dynamics, displaying a distinct impact on pneumococcal density. This further highlights the unique dynamics of the pneumococcal response to viral infections,

Table 2. Factors Associated With a Change in Pneumococcal Carriage Density Before vs During an RSV Infection, PHIRST Study, South Africa, 2016–2018 (n = 231)

Characteristic	No.[a]	Log Mean Density		Log Mean Density Differences[b] (95% CI)	Univariate Regression Analysis		Multivariable Regression Analysis	
		Before	During		Coefficient (95% CI)	*P* Value	Coefficient (95% CI)	*P* Value
Enrollment year								
2016	76	10.0	10.4	0.4 (–.3, 1.1)	1 [Reference]			
2017	76	9.2	10.5	1.3 (.4, 2.1)	0.9 (–.3, 2.0)	.1		
2018	79	8.8	9.8	1.0 (.1, 1.8)	0.5 (–.6, 1.7)	.4		
Age, y								
<1	10	11.1	11.0	–0.1 (–1.7, 1.5)	–1.0 (–3.2, 1.2)	.4	–1.9 (–4.3, .5)	.1
1–4	97	10.4	11.0	0.6 (–.1, 1.3)	–0.4 (–1.4, .5)	.4	–1.0 (–2.1, .04)	.06
5–14	92	9.0	10.0	1.0 (.2, 1.8)	1 [Reference]		1 [Reference]	
15–24	9	7.4	8.7	1.3 (–.5, 3.1)	0.4 (–1.9, 2.8)	.7	0.7 (–1.9, 3.2)	.6
25–44	11	6.2	7.9	1.7 (–.1, 3.4)	0.7 (–1.5, 2.8)	.5	0.6 (–1.8, 2.9)	.6
≥45	12	5.9	8.1	2.2 (–.1, 4.5)	1.2 (.9, 3.3)	.3	1.1 (–1.3, 3.5)	.4
Sex								
Female	132	9.4	9.9	0.6 (.1, 1.3)	1 [Reference]			
Male	99	9.2	10.6	1.1 (.4, 1.9)	0.4 (–.5, 1.3)	.4		
HIV status								
Uninfected	206	9.4	10.3	0.9 (.4, 1.4)	1 [Reference]		1 [Reference]	
PWHIV	16	7.6	8.8	1.2 (.2, 2.2)	0.1 (–1.4, 1.6)	.4	–0.8 (–2.8, 1.2)	.4
Chronic medical condition[c]								
Yes	5	9.6	12.1	2.5 (.01, 5.0)	1.6 (–1.40, 4.7)	.3		
No	226	9.3	10.2	0.8 (.4, 1.3)	1 [Reference]			
BMI,[d] kg/m^2								
Underweight	18	10.9	10.1	–0.8 (–2.7, 1.0)	**–1.7 (–3.4, –.04)**	**.04**	**–1.8 (–3.5, –.1)**	**.04**
Normal	177	9.5	10.4	0.9 (.4, 1.4)	1 [Reference]		1 [Reference]	
Overweight	14	7.1	9.5	2.3 (.01, 4.6)	1.4 (–.5, 3.3)	.1	0.3 (–1.1, 2.3)	.8
Obese	20	7.6	9.2	1.6 (.2, 3.1)	0.8 (–.8, 2.4)	.3	0.1 (–.6, 2.0)	.9
Symptoms[e]								
Yes	98	9.1	10.6	1.5 (.8, 2.3)	**1.1 (.2, 2.0)**	**.02**	0.9 (–.9, 1.8)	.07
No	133	9.5	9.9	0.4 (–.2, 1.0)	1 [Reference]		1 [Reference]	
RSV infection duration, d								
<10	203	9.2	10.1	0.8 (.4, 1.3)	1 [Reference]			
≥10	28	10.0	11.2	1.1 (–.2, 2.4)	0.3 (–1.1, 1.7)	.7		
Mean Ct value of the RSV								
<25	42	9.3	11.6	2.3 (1.2, 3.4)	**2.1 (.9, 3.4)**	**.01**	**2.5 (1.1, 3.9)**	**<.01**
25–29	94	9.3	10.4	1.1 (.4, 1.8)	0.9 (–.09, 1.9)	.07	**1.1 (.1, 2.2)**	**.04**
30–34	79	9.3	9.5	0.2 (–.5, 1.0)	1 [Reference]		1 [Reference]	
≥35	16	9.2	8.3	–0.9 (–2.8, .9)	–1.2 (–3.0, .6)	.2	–0.6 (–2.5, 1.4)	.6

Bold denotes statistical significance ($P < .05$). Only variables found to be statistically significant ($P < .2$) in univariate analysis or included a priori (age group and HIV status) were assessed in the multivariable model. Only a priori variables and variables remaining significant in the final model are shown in the table.

Abbreviations: BMI, body mass index; Ct, cycle threshold; PHIRST, a prospective household cohort study of influenza, respiratory syncytial virus and other respiratory pathogens community burden and transmission dynamics in South Africa; PWHIV, people with HIV; RSV, respiratory syncytial virus; WHO, World Health Organization.

[a]Number of individuals colonized with pneumococcus and infected with RSV.

[b]Log mean density differences were calculated by subtracting the log mean pneumococcal density before an RSV or influenza infection from that during an RSV or influenza infection.

[c]Self-reported history of asthma, lung disease, heart disease, stroke, spinal epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease, or diabetes.

[d]We defined BMI categories as follows. Underweight: age <18 years, weight for age or BMI <–2 SD of the WHO child growth standards; age ≥18 years, BMI <18.5 kg/m^2. Overweight: age <18 years, BMI >+1 and ≤+2 SD of the WHO growth standards; age ≥18 years, BMI ≥25 and <30 kg/m^2. Obese: age <18 years, BMI >+2 SD of the WHO growth standards; age ≥18 years, BMI ≥30 kg/m^2.

[e]Symptoms included at least 1 of the following: fever (self-reported or measured tympanic temperature ≥38 °C), cough, difficulty breathing, sore throat, nasal congestion, chest pain, muscle aches, headache, vomiting, or diarrhea.

particularly in individuals with high viral loads. The outcome of our research bears relevance to public health, offering insights that can inform vaccination strategies for bacterial and viral infections, such as the development of combination vaccines that provide broader protection against respiratory infections. Furthermore, vaccination strategies could be optimized to coincide with periods of heightened pneumococcal density following viral infections.

Table 3. Factors Associated With a Change in Pneumococcal Carriage Density Before and During an Influenza Infection, PHIRST Study, South Africa, 2016–2018 (n = 299)

		Log Mean Density			Univariate Regression Analysis		Multivariable Regression Analysis	
Characteristic	No.[a]	Before	During	Log Mean Density Differences[b] (95% CI)	Coefficient (95% CI)	*P* Value	Coefficient (95% CI)	*P* Value
Year								
2016	78	9.7	9.9	0.2 (–.7, 1.1)	1 [Reference]			
2017	109	9.1	9.3	0.2 (–.4, .8)	0.1 (–1.0, 1.0)	.9		
2018	112	10.1	10.4	0.3 (–.2, .9)	0.1 (–.9, 1.1)	.9		
Age, y								
<1	9	11.5	11.2	–0.3 (–4.1, 3.5)	–1.1 (–3.3, 1.1)	.3	–2.4 (–4.9, .1)	.06
1–4	115	10.3	10.6	0.3 (–.3, .9)	–0.5 (–1.4, –.3)	.2	–1.0 (–2.0, .02)	.06
5–14	124	9.4	10.0	0.6 (.4, 1.2)	1 [Reference]		1 [Reference]	
15–24	21	8.9	8.5	0.4 (–1.7, .8)	–1.0 (–2.5, .5)	.2	0.4 (–2.2, 2.9)	.8
25–44	18	7.7	7.1	–0.7 (–2.6, 1.2)	–1.5 (–3.1, .1)	.06	0.9 (–1.4, 3.2)	.4
≥45	12	7.5	6.6	–0.9 (–3.2, 1.3)	–1.7 (–3.6, 1.2)	.08	1.1 (–1.1, 3.4)	.3
Sex								
Female	159	9.6	9.8	0.2 (.4, .7)	1 [Reference]			
Male	140	9.6	10.0	0.4 (–.2, 9.4)	0.2 (–.5, 1.0)	.5		
HIV status								
Uninfected	266	9.7	10.0	0.3 (–.1, .7)	1 [Reference]		1 [Reference]	
PWHIV	21	8.1	8.5	0.4 (–1.2, 1.9)	0.1 (–1.4, 1.6)	.9	–0.9 (–2.9, 1.1)	.4
Chronic medical condition[c]								
Yes	6	8.7	11.4	2.7 (–.2, .6)	**2.4 (–.2, 5.0)**	**.08**	2.1 (–1.1, 5.4)	.2
No	293	9.6	9.8	0.2 (–1.4, 6.9)	1 [Reference]		1 [Reference]	
BMI,[d] kg/m^2								
Underweight	32	10.8	10.1	–0.7 (–1.9, .4)	–1.0 (–2.2, –.3)	.1		
Normal	215	9.7	10.0	0.3 (–.1, .8)	1 [Reference]			
Overweight	31	8.9	9.3	0.4(–.8, 1.6)	0.3 (–1.0, 1.5)	.7		
Obese	21	8.3	9.0	0.7 (–.9, 2.2)	0.2 (–1.2, 1.7)	.8		
Symptoms[e]								
Yes	188	9.7	10.3	0.4 (–.01, .9)	0.4 (–.4, 1.2)	.3		
No	111	9.5	9.4	–0.1 (–.7, .6)	1 [Reference]			
Virus type								
Influenza A	146	9.5	9.8	0.3 (–.2, .8)	1 [Reference]			
Influenza B	153	9.7	9.9	0.2 (–.3, .8)	–0.04 (–.8, .7)	.9		
Virus infection duration, d								
<10	271	9.6	9.7	0.1 (–.3, .5)	1 [Reference]		1 [Reference]	
≥10	28	9.8	11.5	1.7 (.6, 2.7)	**1.5 (.3, 2.8)**	**.02**	0.4 (–1.0, 1.8)	.6
Mean Ct value of the virus								
<25	67	10.0	9.9	–0.1 (–.8, .7)	0.1 (–1.0, 1.1)	.9	**2.8 (1.4, 4.2)**	**<.01**
25–29	139	9.7	10.4	0.7 (.1, 1.3)	0.8 (–.5, 1.7)	.07	**1.2 (.2, 2.3)**	**.03**
30–34	83	9.2	9.1	–0.1 (–.7, .5)	1 [Reference]		1 [Reference]	
≥35	10	9.6	9.2	–0.4 (–2.9, .9)	–0.3 (–2.4, 1.8)	.7	0.8 (–2.8, 1.1)	.4

Bold denotes statistical significance ($P < .05$). Only variables found to be statistically significant ($P < .2$) in univariate analysis or included a priori (age group and HIV status) were assessed in the multivariable model. Only a priori variables and variables remaining significant in the final model are shown in the table.

Abbreviations: BMI, body mass index; Ct, cycle threshold; PHIRST, a prospective household cohort study of influenza, respiratory syncytial virus and other respiratory pathogens community burden and transmission dynamics in South Africa; PWHIV, people with HIV; RSV, respiratory syncytial virus; WHO, World Health Organization.

[a]Number of individuals colonized with pneumococcus and infected with influenza.

[b]Log mean density differences were calculated by subtracting the log mean pneumococcal density before an RSV or influenza infection from that during an RSV or influenza infection.

[c]Self-reported history of asthma, lung disease, heart disease, stroke, spinal epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease, or diabetes.

[d]We defined BMI categories as follows. Underweight: age <18 years, weight for age or BMI <–2 SD of the WHO child growth standards; age ≥18 years, BMI <18.5 kg/m^2. Overweight: age <18 years, BMI >+1 and ≤+2 SD of the WHO growth standards; age ≥18 years, BMI ≥25 and <30 kg/m^2. Obese: age <18 years, BMI >+2 SD of the WHO growth standards; age ≥18 years, BMI ≥30 kg/m^2.

[e]Symptoms included at least 1 of the following: fever (self-reported or measured tympanic temperature ≥38 °C), cough, difficulty breathing, sore throat, nasal congestion, chest pain, muscle aches, headache, vomiting, or diarrhea.

Notes

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J. M., F. W., C. C., A. v. G., and N. W. collected data and performed laboratory experiments. M. C., J. K., S. T., C. C., and N. W. accessed and verified the underlying data. M. C., J. K., C. C., A. v. G., and N. W. analyzed the data and interpreted results. All authors critically reviewed the article. All authors had access to all the data reported in the study.

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