Clinical Infectious Diseases

MAJOR ARTICLE

Influenza-like Illness Exacerbates Pneumococcal Carriage in Older Adults

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Background. In older adults, pneumococcal disease is strongly associated with respiratory viral infections, but the impact of viruses on *Streptococcus pneumoniae* carriage prevalence and load remains poorly understood. Here, we investigated the effects of influenza-like illness (ILI) on pneumococcal carriage in community-dwelling older adults.

Methods. We investigated the presence of pneumococcal DNA in saliva samples collected in the 2014/2015 influenza season from 232 individuals aged ≥60 years at ILI onset, followed by sampling 2–3 weeks and 7–9 weeks after the first sample. We also sampled 194 age-matched controls twice 2–3 weeks apart. Pneumococcal DNA was detected with quantitative polymerase chain reaction assays targeting the *piaB* and *lytA* genes in raw and in culture-enriched saliva. Bacterial and pneumococcal abundances were determined in raw saliva with *16S* and *piaB* quantification.

Results. The prevalence of pneumococcus-positive samples was highest at onset of ILI (42/232 [18%]) and lowest among controls (26/194 [13%] and 22/194 [11%] at the first and second samplings, respectively), though these differences were not significant. Pneumococcal carriage was associated with exposure to young children (odds ratio [OR], 2.71 [95% confidence interval {CI}, 1.51–5.02]; $P < .001$), and among asymptomatic controls with presence of rhinovirus infection (OR, 4.23 [95% CI, 1.16–14.22]; $P < .05$). When compared with carriers among controls, pneumococcal absolute abundances were significantly higher at onset of ILI ($P < .01$), and remained elevated beyond recovery from ILI ($P < .05$). Finally, pneumococcal abundances were highest in carriage events newly detected after ILI onset (estimated geometric mean, 1.21×10^{-5} [95% CI, 2.48×10^{-7} to 2.41×10^{-5}], compared with preexisting carriage).

Conclusions. ILI exacerbates pneumococcal colonization of the airways in older adults, and this effect persists beyond recovery from ILI.

Keywords. *Streptococcus pneumonia*; carriage; influenza-like illness; older adults.

Despite the availability of pneumococcal vaccines, *Streptococcus pneumoniae* (pneumococcus) remains a leading cause of invasive bacterial disease and is the primary bacterial cause of community-acquired pneumonia (CAP), particularly in older adults [1]. Pneumococcus is also a frequent commensal of the upper respiratory tract (URT), and carriage of pneumococcus is a prerequisite for subsequent disease [2]. The high incidence of pneumococcal disease in the very young coincides with high carriage prevalence in children, who are also considered to be the main transmitters of pneumococcus [3]. However, the incidence and burden of pneumococcal pneumonia and invasive pneumococcal disease is largest in older adults [1, 4], though in this age group pneumococcal carriage detection is often <5% or virtually absent [5, 6]. We and others have attributed these low pneumococcal carriage detection rates in adults to the poor sensitivity of the currently advised detection methodology [6–10].

The World Health Organization gold-standard method for pneumococcal carriage detection is the isolation of live pneumococci from nasopharyngeal swab cultures [11]. While this approach is effective in young children (infants and toddlers) with high pneumococcal carriage density, the culture of nasopharyngeal swab proves inadequate for adults due to low carriage density of pneumococci in the URT [6–10]. To improve carriage detection, the nasopharyngeal swabs can be complemented with other respiratory samples such as oropharyngeal swabs, nasal or oral washes, or saliva [5–9, 11]. However, the high density and diversity of competing nonpneumococcal bacteria in the oral niche hamper the detection of pneumococcal presence by conventional culture [6–9, 12]. Molecular

Received 7 July 2020; editorial decision 1 October 2020; published online 30 October 2020.

Presented in part: European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, The Netherlands, 2019; and Europneumo, Greifswald, Germany, 2019.

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Clinical Infectious Diseases® 2021;73(9):e2680–9

DOI: 10.1093/cid/ciaa1551

diagnostics can overcome this limitation. We have previously shown that when pneumococcal DNA was detected using quantitative polymerase chain reaction (qPCR) targeting both *piaB* and *lytA* genes, saliva samples were universally superior to nasopharyngeal or oropharyngeal swabs for carriage detection in adults [7, 8]. Subsequent isolation of live pneumococci from samples identified as positive with qPCR further supports this approach to carriage detection [7, 8, 12]. Saliva as the preferred sample for pneumococcal isolation was already described in century-old studies reporting on high rates of pneumococcal carriage across all ages when saliva or oral washes were tested by animal inoculation [5, 6, 13–18].

Epidemiological studies suggest a strong link between pneumococcal disease and viral respiratory infections [19]. It is assumed that perturbations in the URT by viruses and the subsequent host responses may disrupt the balanced host-commensal relationship, allowing outgrowth of pneumococci [20]. Similar to pneumococcal disease, the burden of influenza disease is highest in older adults [21, 22]. Due to the low reported rates of pneumococcal carriage, the impact of respiratory viruses on pneumococcal colonization in older adults remains poorly understood.

To investigate the dynamics of pneumococcal carriage during respiratory viral infections in older adults, we applied molecular diagnostics in saliva collected from individuals with acute influenza-like illness (ILI), after they recovered from ILI, and from age-matched controls without ILI complaints. Here, we show that pneumococcal carriage density in older adults with ILI is elevated compared with controls, and this effect persists in the first months after recovery from ILI. Our findings not only unveil the dynamics of pneumococcal colonization in older adults during ILI, but also emphasize the importance of using molecular methods and saliva in studies on pneumococcal carriage in older age cohorts.

MATERIALS AND METHODS

Study Design

Pneumococcal carriage was investigated in a prospective observational study conducted in community-dwelling adults aged ≥60 years [23]. The study was approved by the Medical Ethics Committee Noord Holland in the Netherlands (NTR4818 on http://www.trialregister.nl) and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. Individuals were monitored for ILI during the entire 2014/2015 influenza season (October 2014–April 2015) and were instructed to contact the study center in case of ILI symptoms described by the Dutch "Pel criteria": fever (>37.8°C) and a sudden onset of ≥1 of the following symptoms: cough, rhinorrhea, sore throat, headache, chest pain, or myalgia [24]. Presence of respiratory viruses was detected in nasopharyngeal and oropharyngeal samples with a real-time PCR–based multiplex ligation-dependent probe amplification assay as previously described [23]. Asymptomatic control participants were equally distributed over the different age groups and the influenza season, and were sampled and questioned. Controls who developed ILI during the study were excluded from all analyses (Figure 1). At the first visit, participants were asked to report medication in the past 3 months and at each visit changes in medications

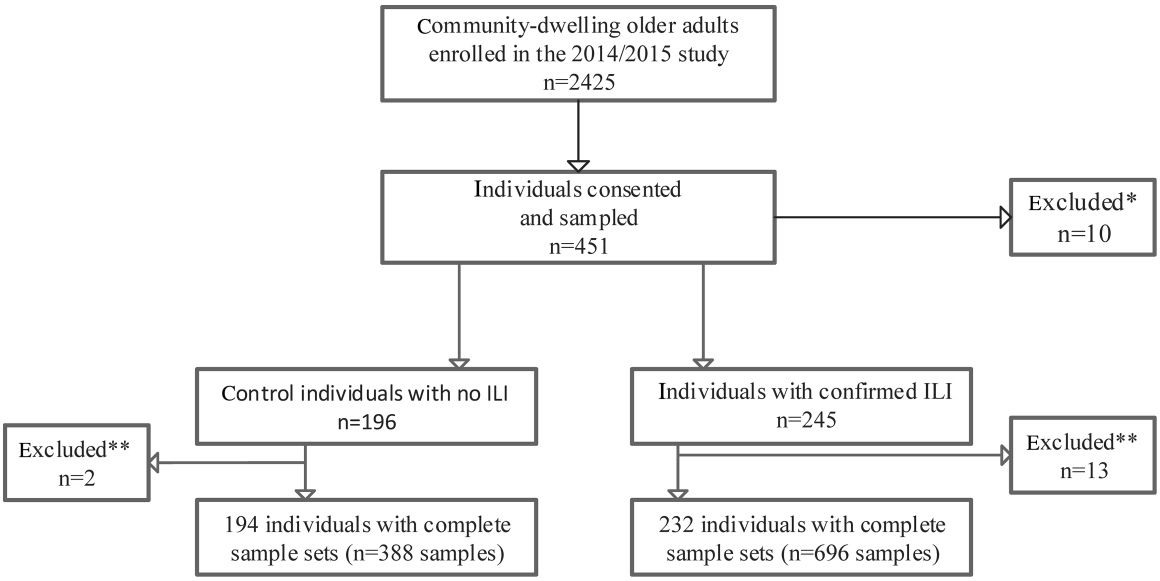

Figure 1. Flow diagram depicting enrollment into the study and processing of saliva samples. From 2424 enrolled individuals, 451 were sampled during the influenza season of 2014/2015. We excluded from the analyses 10 individuals who were first sampled as control and again after influenza-like illness (ILI) onset (*), and 15 individuals for having incomplete saliva samples sets (**).

were recorded; this includes antimicrobial drugs. In the current study, we included all individuals of whom complete sets of saliva samples were available.

Collection of Saliva Samples

From individuals who reported ILI, saliva samples were collected thrice: within 72 hours of symptom onset, 2–3 weeks later, and finally 7–9 weeks after the first sampling event. The control group consisted of individuals without ILI prior to sample collection, and these participants were sampled twice, 2–3 weeks apart. Saliva collection, freezing, transport to the diagnostic facility, and storage at −80°C were conducted as described previously [8].

Processing of Saliva in the Diagnostic Laboratory

Two hundred microliters of saliva was diluted in an equal volume of phosphate-buffered saline. Half of it was used for DNA extraction with the DNeasy Blood and Tissue Kit (Qiagen) and considered to represent unprocessed (raw) saliva. The second half was used to inoculate SB7-GENT culture plates (Oxoid) as previously described [8]. Harvests of these cultures were considered as samples culture-enriched for pneumococci and were also processed for DNA extraction (Supplementary Data).

Detection of Pneumococcal DNA and Carriage Density Analysis

Pneumococcal DNA was quantified using PCR with primers and probes targeting sequences within *piaB* and *lytA* genes [9, 25]. A sample was considered positive for pneumococcus when both signals were cycle threshold <40; this combination is considered to be highly specific for pneumococcus. Overall bacterial load was quantified in raw saliva using *16S* qPCR targeting bacterial DNA [26]. To compare overall bacterial abundances and the absolute pneumococcal abundances, we normalized bacterial concentrations in raw samples for human DNA quantified with a qPCR targeting *CRP* [27, 28], and relative abundance of pneumococcus was determined as the proportion of pneumococcal DNA in overall bacterial DNA. Taking into account reports on nonpneumococcal streptococci to generate positive results in *lytA*-specific qPCR [29, 30], analysis of pneumococcal abundances was based on *piaB* data of raw saliva samples.

Statistical Analysis

Data analysis was performed in GraphPad Prism software version 8.4.1 and RStudio software version 4.0.0. Carriage prevalences were compared with a permutation test for χ^2 and McNemar tests, whereas carriage densities were compared with a permutation test for Wilcoxon-Mann-Whitney tests, all included in the "coin" R package. In addition to the per protocol, comparisons with viral presence were limited to participants with data available for individual respiratory viruses. Stratified tests were corrected for multiple comparisons with Sidak tests [31]. Linear mixed-effects modeling was employed to assess the effects of ILI and pneumococcal carriage acquisition on longitudinal pneumococcal abundances (Supplementary Data). A P value < .05 was considered significant.

RESULTS

During the 2014/2015 influenza season, 2425 individuals were enrolled into the study and 451 were sampled (Figure 1), including 245 with confirmed ILI and 196 controls. In addition, 10 control individuals who developed ILI later in the season were excluded from the analyses. Fifteen individuals were excluded as the sets of saliva samples were incomplete. As such, data from 426 individuals, including 232 individuals with ILI and 194 controls, were analyzed to assess the impact of ILI on pneumococcal carriage. Sampling dates differed between study groups, with controls sampled earlier in the season ($P < .0001$) and a lower median age in individuals with ILI ($P < .01$). These differences in age and the timing of sample collection were due to the study design [23]. Sex, the prevalence of comorbidities, antibiotic therapy, and exposure to young children did not significantly differ between study groups (Table 1).

Rates of Pneumococcal Carriage

Overall, 150 of 1084 tested samples (14%) were classified as positive for pneumococcus either in raw (97/1084 [9%]) or culture-enriched (133/1084 [12%]) saliva (Supplementary Table 1). Of all 426 individuals, 92 were tested positive for pneumococcus at least once in the study (period prevalence of 22%). This corresponded to a period prevalence of 25% (57/232) and 18% (35/194) among individuals with ILI and controls, respectively. For samples positive for pneumococcus, there was a significant correlation between *piaB* and *lytA* DNA concentrations, indicative of high specificity of the molecular detection (Supplementary Figure 1).

The carriage rate at a given study time-point (point prevalence) was highest at ILI onset (18%) and lowest at the second sampling event in controls (11%) (Table 1). To account for differences in timing of sampling between study groups, results were stratified by sampling month. It did not have an impact on the outcome of comparisons between pneumococcal carriage presence and study groups. A significant reduction in carriage prevalence was observed between the first and second and also between the first and third sampling events in the ILI group (18.1% vs 13.3% and vs 12.5%, respectively; $P < .05$ for both). Longitudinal patterns of pneumococcal carriage acquisition and clearance are depicted in Figure 2.

Demographic features and respiratory virus data were compared to pneumococcal point prevalence data to identify potential risk factors of pneumococcal carriage. Overall, individuals with exposure to young children (daily to monthly) were more likely to be positive for pneumococcal carriage at any of the sampling events (odds ratio [OR], 2.71 [95% confidence interval

Table 1. Characteristics of Individuals Sampled in the Study and in the Context of Pneumococcal Carriage Detected at the Time of Sampling

	Controls (n = 194)					ILI (n = 232)						
Characteristic		Day 0		Day 14			Onset		Day 14		Recovery	
Group	All (n = 194 [100%])	Noncarriers (n = 168 [86.6%])	Carriers (n = 26 [13.4%])	Noncarriers (n = 172 [88.7%])	Carriers (n = 22 [11.3%])	All (n = 232 [100%])	Noncarriers (n = 190 [81.9%])	Carriers (n = 42 [18.1%])	Noncarriers (n = 201 [86.6%])	Carriers (n = 31 [13.4%])	Noncarriers (n = 203 [87.5%])	Carriers (n = 29 [12.5%])
Sampling period	Oct–May	Oct–Apr	Oct–Apr	Oct–May	Oct–Apr	Oct–June	Oct–Apr	Oct–Apr	Oct–May	Nov–May	Nov–Jun	Dec–Jun
Median age, y	71	71	68	71	70	68	68	68	68	68	68	68
Sex, female	100 (51.5)	85 (50.6)	15 (57.7)	88 (51.2)	12 (54.5)	110 (47.4)	87 (45.8)	23 (54.8)	94 (46.8)	16 (51.6)	92 (45.3)	18 (62.1)
Comorbidities[a]	76 (39.2)	67 (39.9)	9 (34.6)	67 (39.0)	9 (40.9)	98 (42.2)	80 (42.1)	18 (42.9)	89 (44.3)	9 (29.0)	89 (43.8)	9 (31.0)
Antibiotic therapy	20 (10.3)	20 (11.9)	0	18 (10.5)	2 (9.1)	38 (16.4)	33 (17.4)	5 (11.9)	36 (17.9)	2 (6.5)	37 (18.2)	1 (3.4)
Contact with children		*					**		***		***	
Daily	7 (3.6)	4 (2.4)	3 (11.5)	5 (2.9)	2 (9.1)	8 (3.4)	5 (2.6)	3 (7.1)	5 (2.5)	3 (9.7)	6 (3.0)	2 (6.9)
Weekly	54 (27.8)	44 (26.2)	10 (38.5)	44 (25.6)	10 (45.5)	87 (37.5)	62 (32.6)	25 (59.5)	67 (33.3)	20 (64.5)	66 (32.5)	21 (72.4)
Monthly	34 (17.5)	30 (17.9)	4 (15.4)	31 (18.0)	3 (13.6)	26 (11.2)	23 (12.1)	3 (7.1)	24 (11.9)	2 (6.5)	25 (12.3)	1 (3.4)
Rarer than monthly	99 (51.0)	90 (53.6)	9 (34.6)	92 (53.5)	7 (31.8)	111 (47.8)	100 (52.6)	11 (26.2)	105 (52.2)	6 (19.4)	106 (52.2)	5 (17.2)

Data are presented as no. (%) unless otherwise indicated.

Abbreviation: ILI, influenza-like illness.

[a]For reported comorbidities, we refer to Kaaijk et al (unpublished data) and van Beek et al [23]. In brief, reported comorbidities included respiratory disease, cardiovascular disease, chronic kidney disease, cancer, diabetes, asplenia, organ or bone marrow transplantation, and human immunodeficiency virus infection. The permutation test for χ^2 was used to test for significant differences between groups. Odds ratios are reported in Supplementary Tables 2 and 3. Only significant differences are marked, as $^{*}P < .05$, $^{**}P < .01$, or $^{***}P < .001$.

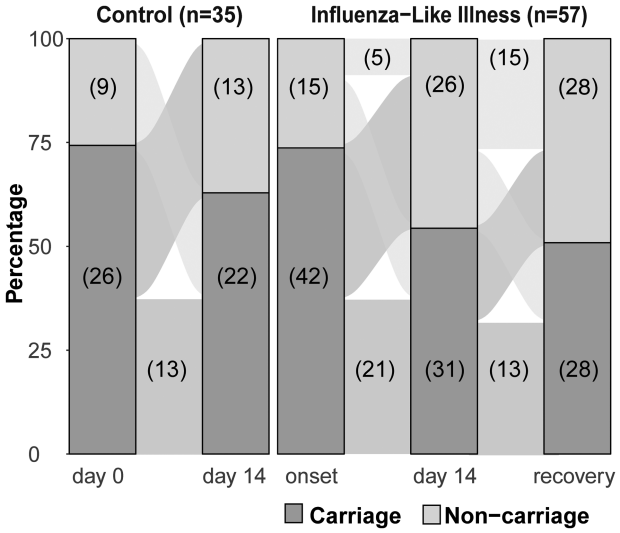

Figure 2. The longitudinal pattern of pneumococcal carriage acquisition and clearance among carriers identified in the control group and among individuals with influenza-like illness (ILI). Sankey diagram depicting the longitudinal pattern of carriage acquisition and clearance among individuals who were positive for pneumococcus in either raw or culture-enriched saliva at 1 or more sampling events, including 35 carriers among controls (left) and 57 carriers in the ILI group (right). The numbers in brackets represent the number of individuals of a given status. The numbers on the bars represent carriers and noncarriers, and numbers between bars represent individuals in whom the status did not change between sampling events.

{CI}, 1.51–5.02]; $P < .001$). This association remained significant after stratifying for antibiotic therapy ($P < .05$) (Table 1). The risk of pneumococcal carriage due to exposure to young children has also been identified for the first sampling of ILI group and controls separately (ORs, 3.12 [95% CI, 1.42–7.29], $P < .05$ and 2.18 [95% CI, .86–5.86], $P < .05$, respectively).

The incidence of respiratory viruses was highest at ILI onset, and influenza virus, rhinovirus, coronavirus, and respiratory syncytial virus were the most frequently detected (incidence of 7% or more) among viruses tested (Table 2) (Kaaijk et al, unpublished data). Pneumococcal carriage was not associated with individual viruses during ILI. In the control group, risk of pneumococcal carriage was significantly higher at the first of 2 sampling events (OR, 4.23 [95% CI, 1.16–14.22]; $P < .01$) and was associated with individuals positive for rhinovirus.

Bacterial and Pneumococcal Abundances in Saliva

Bacterial and pneumococcal abundances were calculated and normalized in raw saliva to determine whether ILI has an impact on pneumococcal presence in upper airways. No significant differences were observed in the overall bacterial abundance in raw saliva of carriers between and within study groups (Figure 3A). However, absolute abundances of pneumococcus were uniformly elevated in individuals with ILI compared to controls (Figure 3B). This higher abundance tended to persist

Table 2. Respiratory Viruses Detected in Pneumococcal Carriers and Noncarriers per Study Group and Sampling Event

	Controls				ILI					
Virus	Day 0		Day 14		Onset		Day 14		Recovery	
Status	Noncarriers (n = 168)	Carriers (n = 26)	Noncarriers (n = 172)	Carriers (n = 22)	Noncarriers (n = 187)	Carriers (n = 42)	Non carriers (n = 195)	Carriers (n = 31)	Noncarriers (n = 191)	Carriers (n = 29)
Any respiratory virus[a]	23 (13.7)	7 (26.9)	27 (15.7)	1 (4.5)	147 (64.2)	31 (13.5)	62 (27.4)	7 (3.1)	34 (15.5)	6 (2.7)
Influenza virus	2 (1.2)	1 (3.8)	3 (1.7)	0	71 (31.0)	18 (7.9)	15 (6.6)	1 (0.4)	6 (2.7)	1 (0.5)
Rhinovirus	11 (6.5)	6** (23.1)	10 (5.8)	1 (4.5)	31 (13.5)	6 (2.6)	19 (8.4)	3 (1.3)	12 (5.5)	3 (1.4)
Coronavirus	8 (4.8)	0	4 (2.3)	0	17 (7.4)	5 (2.2)	13 (5.8)	1 (0.4)	13 (5.9)	1 (0.9)
RSV	0	0	3 (1.7)	0	14 (6.1)	3 (1.3)	2 (0.9)	2 (0.9)	0	0

Data are presented as no. (%). The permutation test for χ^2 was used to test for significant differences between groups. The number of individuals included this analysis are limited to individuals who have data of the described respiratory virus type available. Odds ratios are reported in Supplementary Table 3.

Abbreviations: ILI, influenza-like illness; RSV, respiratory syncytial virus.

[a]Tested respiratory viruses are influenza virus (influenza virus A and B), rhinovirus, coronavirus (OC43, 229E, NL63, HKU-1), RSV-A and -B, human parainfluenza virus (1–4), human metapneumovirus (A and B), adenovirus, and bocavirus.

**$P < .01$, for a difference between fraction of samples positive for a virus among collected from noncarriers vs carriers.

in the first weeks after ILI onset (second sampling in the ILI group compared with first sampling of controls; $P = .05$) or became significant (all remaining comparisons between ILI group vs controls; $P < .05$). After exclusion of virus-infected controls, all differences in pneumococcal absolute abundances between groups became significant ($P < .05$; Supplementary Figure 2A).

Pneumococcal relative abundances were calculated to determine whether ILI was associated with an outgrowth of pneumococci within the URT microbiota of carriers. Relative abundance of pneumococci was indeed significantly elevated at ILI onset compared with controls ($P < .05$; Figure 3C). After exclusion of controls positive for respiratory viruses, pneumococcal relative abundances were significantly elevated also at recovery from ILI (Supplementary Figure 2B).

Longitudinal Impact of ILI on Pneumococcal Abundances

In persistent carriers the relative and absolute abundances were 3-fold higher in individuals in the ILI group compared with controls. New carriage events 2–3 weeks after acute ILI displayed a 10-fold higher absolute pneumococcal abundance (Figure 4A) and 8-fold higher pneumococcal relative abundance (Figure 4B) when compared to new carriage events among controls ($P < .01$ and $P < .05$ for difference in absolute and relative abundance, respectively). Modeling the effects of pneumococcal carriage acquisition and ILI sampling events on pneumococcal abundances in a linear mixed-effects model showed that carriage acquisition after ILI onset was indeed significantly associated with elevated pneumococcal relative abundances 2–3 weeks after ILI onset (Figure 4C and 4D and Supplementary Table 4; geometric mean, 1.21×10^{-5} [95% CI, 2.48×10^{-7} to 2.41×10^{-5}]), amounting to an 8-fold increase when compared to carriage acquisition prior to ILI onset (geometric mean, 1.55×10^{-6} [95% CI, -3.81×10^{-1} to 3.81×10^{-1}] after ILI onset). Compared with controls, new carriage acquisitions after ILI onset were associated with significantly elevated pneumococcal relative abundances (Supplementary Figure 3 and Supplementary Table 5). Taken together, carriage densities remained elevated after ILI onset irrespective of prior colonization, yet both relative and absolute abundances of pneumococci were highest in newly identified carriers 2–3 weeks after ILI onset.

Associations Between Viruses and Pneumococcal Carriage Density

Pneumococcal absolute and relative abundances at ILI onset were compared to virus-free controls to determine the contribution of individual respiratory viruses on pneumococcal carriage density. Acute ILI individuals infected with rhinovirus displayed elevated absolute and relative abundances of pneumococcus ($P < .01$ and $P < .05$, respectively) when compared to virus-free controls (Supplementary Figure 4A and 4B). A similar observation was noted for pneumococcal absolute and relative abundance of ILI-onset individuals with influenza virus infection when compared with virus-free controls of second sampling event ($P < .05$) and as a trend for the first sampling event ($P = .06$; Supplementary Figure 4C and 4D). When the effect of individual respiratory viruses on pneumococcal abundance was assessed among carriers in the ILI group and within a given sampling time-point, none of the tested respiratory viruses significantly contributed to pneumococcal abundances.

DISCUSSION

In this prospective observational study, we showed that ILI had a significant impact on pneumococcal abundances of concurrent and subsequent pneumococcal colonization in older

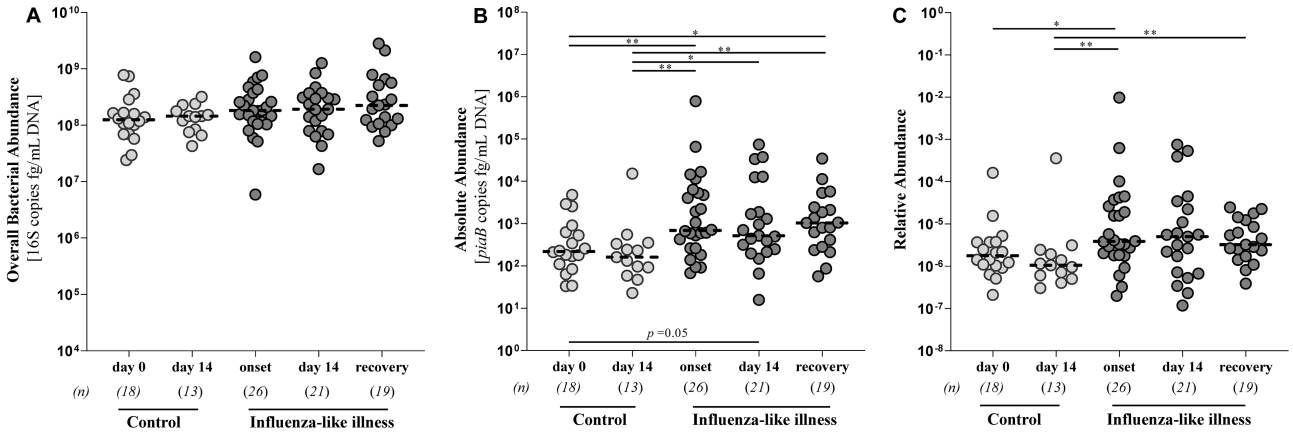

Figure 3. Bacterial absolute and relative abundances in raw saliva samples positive for pneumococcus. *A*, Concentrations of overall bacterial DNA in femtograms of bacterial DNA per mL (fg/mL) quantified by quantitative polymerase chain reaction (qPCR) of bacterial ribosomal *16S* and normalized for human *CRP*, representing overall bacterial abundance. *B*, Concentrations of pneumococcal DNA quantified with *piaB* and normalized for *CRP*, and representing pneumococcal absolute abundance. *C*, Pneumococcal relative abundances reported as a fraction of pneumococcal DNA within overall bacterial DNA. Overall bacterial abundance analysis is based on *16S*-specific qPCR quantification and pneumococcal abundance analysis on *piaB* qPCR quantification. Light gray dots indicate the controls and dark gray the individuals with influenza-like illness. Medians are indicated by dashed lines. The permutation test for Wilcoxon-Mann-Whitney was used to determine statistical significance between medians. *$P < .05$; **$P < .01$.

adults. Pneumococcal abundances were highest when pneumococcal carriage was acquired shortly after onset of ILI and elevated pneumococcal carriage abundances persisted beyond recovery from ILI. To our knowledge, this is first study that unveils the effects of ILI on pneumococcal carriage in older adults (≥60 years). We also showed substantial circulation of

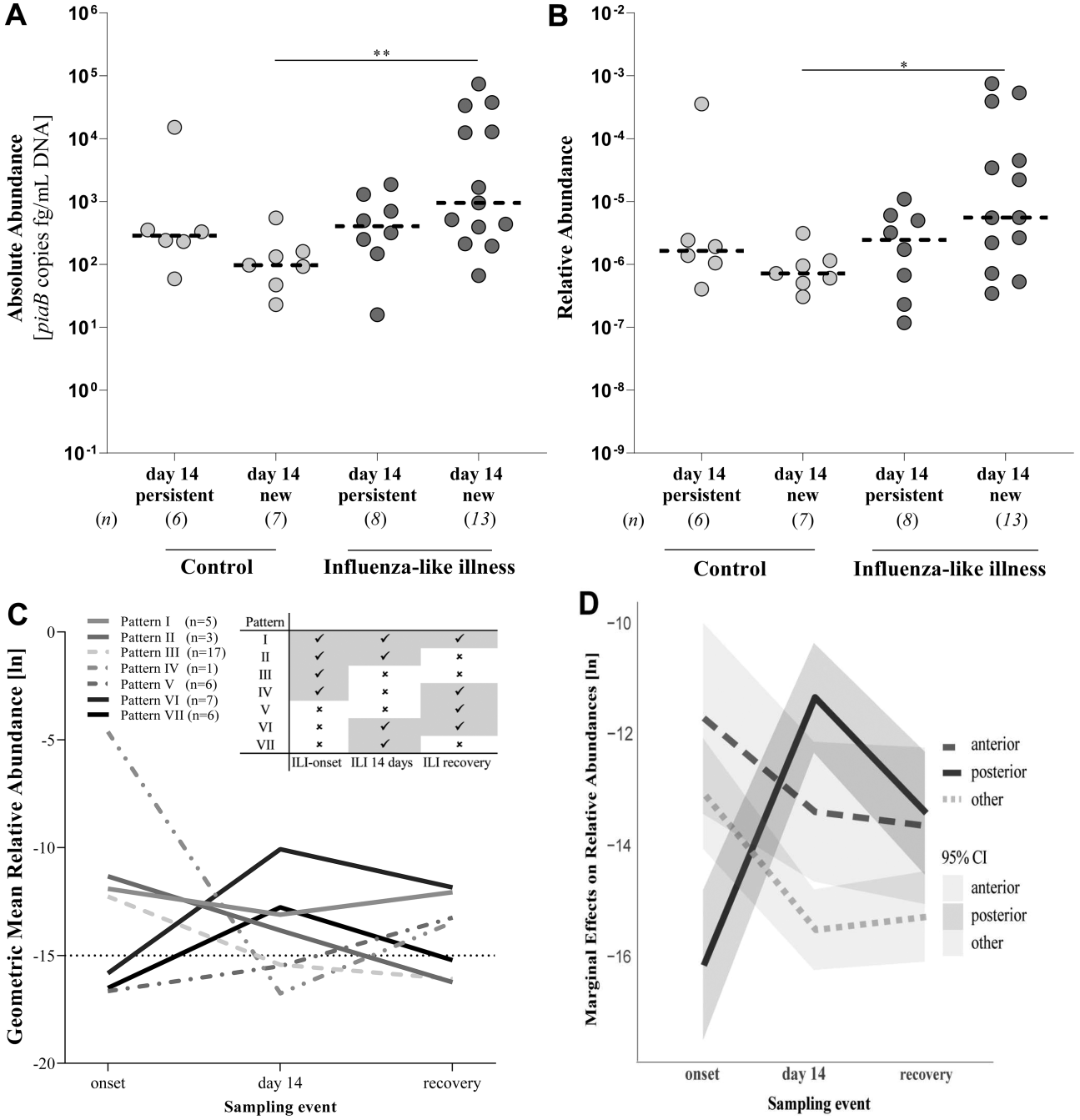

Figure 4. Differences in the pneumococcal abundances between persistent and newly acquired carriage. The normalized pneumococcal absolute abundance (concentration of pneumococcal DNA) (*A*) and pneumococcal relative abundance (*B*) of individuals who were positive for pneumococci at the second sampling event only (day 14 new), and individuals who were positive for pneumococci at both the first and second sampling events (day 14 persistent). Abundance analysis is based on *piaB* quantitative polymerase chain reaction quantification. Light gray dots depict samples from controls; dark gray dots depict samples from individuals with influenza-like illness (ILI). Dashed lines indicate medians. The permutation test for Wilcoxon-Mann-Whitney was used to determine statistical significance between medians: $^{*}P < .05$; $^{**}P < .01$. *C*, Temporal projections of log (natural)–transformed pneumococcal relative abundances of individuals in the ILI group. Inset table shows pneumococcal carriage pattern characteristics in terms of positivity (✓) or negativity (×) for pneumococcal DNA based on *piaB*. Dotted horizontal line separates samples positive (above the line) from negative (below the line) for pneumococcus. *D*, Predicted relative abundances from linear mixed-effects model fit. Relative abundances are presented in natural log scale. Pneumococcal carriage patterns I and II were combined to predict pneumococcal abundances of pneumococcal carriage prior to ILI onset (anterior). Patterns VI and VII were combined to predict pneumococcal abundances of pneumococcal carriage after ILI (posterior). Remaining patterns (III–V) are displayed as "other." Shaded areas represent 95% confidence intervals (CIs).

pneumococcus in carriage among older adults and observed association of pneumococcal carriage and exposure to young children.

With ≥11% carriage prevalence at any study time-point, the carriage of pneumococcus we observed was much higher than contemporary reports by others of community-dwelling older adults [5, 32]. We contribute this result to the application of sensitive diagnostic methods that have allowed for detection of pneumococcus in an age group with a comparatively low density of carriage [6–10, 33].

Young children are considered to be the main reservoir of pneumococci and the primary driver of pneumococcal transmission in the population [7, 32]. This assumption is supported by the risk of all-cause CAP in older adults being elevated by exposure to children [34]. In line with this, exposure of older adults to young children was associated in our study with pneumococcal carriage. A similar finding was previously reported by Hamaluba et al for adults aged ≥65 years in the United Kingdom [35]. However, exposure to children may be also a proxy for intensity of social interaction with other age groups, and older adults on their own may substantially contribute to pneumococcal transmission [36].

Interactions between respiratory viruses and pneumococcus colonizing older adults, and in particular the consequences of such interactions for pathogen transmission across the whole population and for respiratory diseases, remain poorly understood. Studies in children have demonstrated that pneumococcal colonization is positively associated with rhinovirus [37], and increased pneumococcal acquisition during rhinovirus infection results in enhanced transmission of pneumococcus [38]. Similar observations have been reported for influenza virus in animal models [39]. Although we did not observe an increase in pneumococcal carriage prevalence following ILI, as has previously been reported for young children [40], influenza virus and rhinovirus were indeed positively associated with higher pneumococcal abundances in individuals with ILI, supporting the notion that respiratory viruses may enhance carriage acquisition and pneumococcal transmission also in older adults.

Interestingly, while we observed a decline in pneumococcal carriage prevalence after ILI onset, the density (absolute abundance) of pneumococci in raw saliva of individuals with ILI was persistently elevated compared to similarly aged controls. Our data suggests that acute respiratory infections in older adults favor pneumococcal colonization of the airways, a finding previously reported for children [41–44]. Pathobionts such as pneumococcus can thrive during dysbiosis of the URT, facilitating transmission, dissemination, and progression to CAP [45]. Increased pneumococcal relative abundance in adults with ILI suggests such a dysbiosis already in milder forms of respiratory infections and, indeed, in our study rhinovirus was associated with pneumococcal carriage also among controls without ILI.

The risk of secondary bacterial infections including pneumococcal pneumonia is reported to be highest within the first 2–3 weeks after the onset of acute viral respiratory infection [19]. In our study, pneumococcal abundances were more elevated among newly identified carriers after ILI onset compared with new carriers among controls, supporting the notion of carriage acquisition after viral infection as a risk factor for pneumococcal pneumonia. Linear mixed-effects model analysis predicted that pneumococcal carriage acquisition shortly after ILI onset was indeed associated with elevated pneumococcal relative abundances. This observation mirrors the effect of live attenuated influenza vaccine (LAIV) on pneumococcal carriage observed by Rylance et al in a human coinfection model, reporting that administration of LAIV prior to carriage acquisition increased pneumococcal abundances [46]. As we have observed these elevated pneumococcal abundances at the time when viruses may have been already cleared, it seems likely that the synergistic effects between respiratory viruses and pneumococcus may be primarily related to the host response against viral infection rather than to direct interactions between pneumococcus and respiratory viruses. This is in line with reports that viral infection impairs innate immune responses to pneumococcus [20].

Since the study was designed primarily to monitor ILI in adults, it has a number of limitations [23], including differences in age and timing of sample collection between study groups. Due to advanced age and impaired immune function, classical symptoms of viral respiratory infection may have been absent in older adults who participated as asymptomatic controls but were found to be positive for respiratory viruses. Of note, CAP in elderly individuals is more likely to be of atypical nature, with 1 or more of 3 classical symptoms (cough, dyspnea, and fever) missing [47]. Furthermore, effects of respiratory viruses other than influenza virus and rhinovirus on pneumococcal carriage may have been missed due to underpowered analysis. In addition, we cannot ascertain whether either pneumococcal carriage or abundances prior to an ILI episode may already have been elevated and whether such differences impact susceptibility to ILI.

Since old age is a risk factor of pneumococcal disease and pneumococcal vaccination of older adults was introduced to the national immunization program in the Netherlands in 2020, further studies on the importance of pneumococcal reservoirs for disease and transmission in older adults are warranted. Furthermore, determining potential characteristics in the carriage dynamics of serotypes associated with pneumonia could help delineate important interactions between viral respiratory disease and pneumococcus. These studies on pneumococcal pneumonia are also warranted.

To summarize, pneumococcal carriage in this age group was associated with exposure to young children and rhinovirus infection. While the prevalence of pneumococcal carriage was not different

among individuals with and without ILI, pneumococcal abundances were persistently elevated in the ILI group when compared with controls. Furthermore, impact of ILI was largest on pneumococcal abundances of subsequently acquired pneumococcal carriage episodes. These findings demonstrate that acute viral respiratory infections exacerbate pneumococcal colonization.

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. E. A. M. S., J. v. B., and K. T. concepted and initiated the study. J. v. B., H. J. B., and K. T. secured the funding. W. R. M., J. v. V., H. J. B., N. Y. R., E. A. M. S., J. v. B., and K. T. wrote the protocol. W. R. M., J. v. V., R. M., T. B., H. J. B., N. Y. R., J.v. B., and K. T. managed the study and collected the data. W. R. M. and J. v. V. were responsible for and performed the assays. W. R. M., M. A. N., and K. T. provided analytical tools. W. R. M., J. v. V., J. v. B., and K. T. analyzed and interpreted the data. W. R. M. and K. T. drafted the manuscript. All authors amended and commented on the final manuscript.

Acknowledgments. The authors thank the study participants for their time and commitment to the study. They thank all members of the research team at the National Institute for Public Health and the Environment (RIVM); Spaarne Gasthuis Academia in Hoofddorp; and the laboratory staff of the Regional Laboratory of Public Health in Haarlem, who made this project possible. The authors also thank Guy A. M. Berbers for helpful comments on the manuscript.

Financial support. This work was supported by the project PneuMolCare from RIVM, by the Dutch Ministry of Health, Welfare and Sport and by Investigator-Initiated Research (IIR) Award from Pfizer to K. T. (Study ID# W229411). Pfizer had no role in the study design, data collection, statistical analysis, interpretation, or writing of the paper. The corresponding author was responsible for the content of the paper and the decision to submit for publication.

Potential conflicts of interest. K. T. has received consultation and speaking fees and funds for unrestricted research grants from Pfizer, funds for unrestricted research grants from GlaxoSmithKline, and consultation fees from Merck Sharp & Dohme, all paid directly to his home institution. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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