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MAJOR ARTICLE

Decreased Pneumococcal Carriage Among Older Adults in Denmark During the COVID-19 Lockdown

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Background. COVID-19 containment measures reduced the burden of invasive pneumococcal disease. Data on pneumococcal carriage rates among adults during the pandemic are scarce.

Methods. Naso- and oropharyngeal swabs and questionnaires were collected during January 2019 to December 2021 from adults ≥64 years of age. Carriage was determined by *lytA/piaB* PCR.

Results. A total of 1556 participants provided paired naso- and oropharyngeal swabs. Their median age was 74 years (IQR, 70–79). *Streptococcus pneumoniae* DNA was detected in 146 (9.4%) oropharyngeal swabs and 34 (2.2%) nasopharyngeal. The carriage rate decreased from 12.9% (95% CI, 10.1%–16.1%, n = 66/511) prelockdown (January 2019–February 2020) to 4.2% (95% CI, 2.0%–7.5%, n = 10/240) during lockdown (March 2020–February 2021) and increased to 12.1% (95% CI, 9.8%–14.7%, n = 87/719) with the reopening of society (March 2021–December 2021; $P = .0009$).

Conclusions. Pneumococcal carriage prevalence declined significantly during pandemic mitigation measures and rebounded to prepandemic levels as measures were lifted.

Keywords. adults; carriage; COVID-19 pandemic; pneumococcus; surveillance.

A range of containment measures followed the emergence of the COVID-19 pandemic to mitigate the transmission of SARS-CoV-2. These nonpharmacologic interventions led to a decline in several infectious diseases, including cases of invasive pneumococcal disease (IPD) globally as well as in Denmark [1, 2]. Upper respiratory tract carriage of *Streptococcus pneumoniae* is a prerequisite for pneumococcal disease and for respiratory droplet horizontal transmission [3]. Furthermore, close contact and crowding are well-known risk factors associated with the spread of pneumococci [4]. An intuitive explanation for the decline in IPD cases during COVID-19 restrictions would therefore involve the reduced transmission of pneumococci. However, data from Israel, Belgium, and France show that pneumococcal carriage in children remained stable during the pandemic [5–7]. The reduction in pneumococcal disease in children might instead be explained by the reduced transmission of respiratory viruses that would normally increase the risk of IPD [5, 7].

There is limited knowledge on the effect of various COVID-19 mitigation strategies on carriage of pneumococci among the adult population. Children are believed to constitute the main pneumococcal reservoir, but the continuously high pneumococcal disease burden in older adults, even after the introduction of pneumococcal conjugate vaccines (PCVs) in childhood immunization programs, has led to a hypothesis that adults could constitute a pneumococcal reservoir as well [8]. Here, we present data on oropharyngeal (OP) and nasopharyngeal (NP) pneumococcal carriage among older adults in Denmark before and after the initial COVID-19 lockdown on 11 March 2020.

METHODS

Study Design, Setting, and Participants

We performed a cross-sectional pneumococcal carriage study, named P3, among older adults in the capital region of Copenhagen, Denmark. Eligible participants were identified and consecutively enrolled in 19 general practices. Participants had a scheduled appointment for either a routine health checkup or vaccination. As the COVID-19 pandemic arose after study initiation, inclusion of participants at a COVID-19 vaccination center was added. Participants were eligible for study inclusion if they were ≥65 years old, had no symptoms of upper respiratory tract infection, and had not received antibiotics within 1 month of inclusion. Study inclusion began in January 2019 and ended in December 2021.

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Data Collection and Transport

For each patient, NP and OP swabs were collected. All participants were asked to fill a questionnaire regarding demography and comorbidities. Data were entered into REDCap (version 12.0.33; Vanderbilt University). Furthermore, data on prior vaccination with PCV and/or the 23-valent pneumococcal polysaccharide vaccine (PPV-23) were retrieved from the Danish Vaccination Registry and linked to participants by a unique 10-digit identifier provided to Danish residents [9].

General practitioners, nurses, and research staff were trained in the collection of swabs by a study investigator. All samples were collected without restriction on intake of food or beverage prior to enrollment. OP and NP swabs were collected with regular and flexible flocked swabs, respectively, and transferred to the ESwab transport media (Amies liquid; Copan). All samples were stored at room temperature for same-day transport or overnight at 4 °C for transportation to the Statens Serum Institut. Upon arrival, samples were transferred to cryotubes containing 110 µL of 10% glycerol and stored at −80 °C.

DNA Extraction and Polymerase Chain Reaction Analysis

Nucleic acids were extracted from 200 µL of sample material by 20% Chelex solution (Bio-Rad Laboratories AB) according to the manufacturer's instructions and stored at −20 °C. Identification of pneumococcal DNA was initially performed with real-time quantitative polymerase chain reaction (qPCR) analysis targeted to *lytA* [10, 11] in a total volume of 50 µL including the following: 0.4 µL of Accustart II Taq DNA Polymerase (Quantabio), 5 µL of 10× PCR Buffer II, 5 µL of 10mM dUTP, 5 µL of 50mM $MgCl_2$, 1 µL of ROX reference dye (Invitrogen), 10 µL of 50% glycerol, 5 µL of internal control λ-DNA at a 10^{-8} dilution (constructed in-house) [12], and 5 µL of sample DNA. Primers and probes were added to a final concentration of 0.32 µM and 0.075 µM, respectively. Primers were LytA-F373 and LytA-R424, and probes were LytA-Pb400 TaqMan and IK-p (Tamra). The final volume was adjusted with sterile polymerase chain reaction (PCR) water. In every PCR run, 3 positive controls of *S pneumoniae* were included (dilution 10^{-4}, 10^{-6}, 10^{-7}); DNA free water and a purification control served as negative controls. Amplification was carried out on a Q5 Quant Studio (Applied Biosystems) via the following cycling conditions: heating at 95 °C for 2 minutes, followed by 50 cycles of 95 °C for 15 seconds and 60 °C for 1 minute.

Samples with a *lytA* signal were broth enriched by transferring 15 µL of sample solution to 3 mL of serum broth and stored overnight at 35 to 36 °C. The serum broth sample (250 µL) was preincubated with MagNA Pure 96 External Lysis Buffer (250 µL) for 10 minutes at 65 °C. DNA extraction of the preincubated sample (500 µL) was performed with the MagNA Pure 96 DNA and Viral NA Large Volume Kit (Roche Diagnostics) and eluted into 100 µL. Broth-enriched DNA samples positive by *lytA* qPCR were further tested by *piaB* qPCR [13]. The *piaB* assay was modified from Trzciński et al [13] and carried out in 50 µL of reaction volume with 25 µL of PerfeCta qPCR Toughmix Low Rox (Quantabio) and 10 µL of DNA, and primers and probes were added to a final concentration of 0.15 and 0.075 µM, respectively. The final volume was adjusted with sterile PCR water. In every run, 5 positive controls of *S pneumoniae* were included (dilution 10^{-2},10^{-3}, 10^{-4}, 10^{-6}, 10^{-7}); DNA free water and a purification control served as negative controls. Amplification was carried out as described for the initial *lytA* assay.

Samples were considered positive for *S pneumoniae* if cycle threshold (Ct) values were <40 for both target genes. All laboratory analyses were performed at Statens Serum Institut.

Statistical Analysis

Study population characteristics are presented as numbers (percentages) for categorical values and medians with IQR for continuous variables.

The prevalence of *S pneumoniae* carriage was defined as the number of participants with at least 1 naso- or oropharyngeal positive swab by PCR divided by the total study population. Carriage prevalence estimates were further calculated for each of 3 study periods defined from a COVID-19 timeline: period 1 with samples collected prelockdown (January 2019–11 March 2020), period 2 with samples collected postlockdown until the withdrawal of containment measures (12 March 2020–February 2021), and period 3 with samples collected after reopening of society (March 2021–December 2021). The chi-square test was used to test for differences in carriage prevalence among these periods.

Association between *S pneumoniae* carriage prevalence and study period was evaluated with logistic regression models and presented as odds ratio (OR) with 95% CI. In a multivariate model, adjustment was made for the following a priori–selected independent variables: age, comorbidity, and vaccination status. The significance threshold was set at 0.05.

All statistical analyses were performed with R software version 4.1.3 (R Foundation for Statistical Computing).

Patient Consent Statement

The patient's written consent was obtained. The study was approved by the local Ethical Committee of the Capital Region of Copenhagen (H-17030204) and the Data Protection Agency (VD-2018-345).

RESULTS

Study Population Characteristics

A total of 1556 paired OP and NP samples from 1556 participants were analyzed. The study population consisted of 54% females and had a median age of 74 years (IQR, 70–79; Table 1). Thirty-five percent (n = 511) of participants were included before the COVID-19 lockdown (period 1), 16% (n = 240) during the COVID-19 lockdown (period 2), and 49% (n = 719) after

the COVID-19 lockdown (period 3). Figure 1 presents an overview of the number of samples collected during the study periods divided into quarters of the years 2019 to 2021.

Fifty-five percent (n = 817) of participants had ≥1 comorbidities. Forty-three percent (n = 627) had received pneumococcal polysaccharide vaccine PPV-23 prior to study inclusion (mean time, 192 days before sampling), and 8% (n = 120) had been vaccinated with PCV-13 and PPV-23. Of the 747 (50%) participants who had received PPV-23, 156 had been vaccinated within 6 months before sampling; 268, 6 to 12 months before; and 323, >12 months before.

Pneumococcal Carriage Prevalence and Association With Study Period

Thirty-four (2.2%) NP samples and 146 (9.4%) OP samples were positive for pneumococcal DNA, while 9 participants were positive in OP and NP samples (Table 2). Ct values for *lytA* and *piaB* in NP and OP samples, respectively, can be seen in Supplementary Figure 1.

There was a significant difference in the carriage prevalence over the 3 study periods ($P = .0009$): 66 positives of 511 (12.9%; 95% CI, 10.1%–16.1%) prelockdown, 10 of 240 (4.2%; 95% CI, 2.0%–7.5%) during lockdown, and 87 of 719 (12.1%; 95% CI, 9.8%–14.7%) after reopening. Prevalence estimates for study periods in quarters of each study year are shown in Figure 1.

We had complete case data in 1440 of 1482 questionnaires (97.2%) for the multivariate analysis. After adjustment for age, comorbidity, and vaccination status, the OR of carriage was significantly increased among participants swabbed during periods 1 (adjusted OR, 2.9; 95% CI, 1.5–5.9; $P = .0038$) and 3 (adjusted OR, 3.3; 95% CI, 1.6–6.6; $P = .0008$) as compared with period 2.

Table 1. Study Population Characteristics in Total and According to Pneumococcal Carriage Status

	No. (%) or Median [IQR]		
	Total	Carriage	Noncarriage
Participants	1482 (100)	163 (11)	1319 (89)
Age, y	74 [70–79]	73 [69–78]	74 [70–79]
64–69	300 (27)	52 (32)	348 (27)
70–74	419 (29)	50 (31)	369 (28)
75–79	357 (24)	28 (17)	329 (25)
>80	294 (20)	33 (20)	261 (20)
Sex			
Male	685 (46)	75 (46)	610 (46)
Female	797 (54)	88 (54)	709 (54)
Sampling period			
Jan 2019–11 Mar 2020	511 (35)	66 (41)	445 (34)
12 Mar 2020–Feb 2021	240 (16)	10 (6)	230 (18)
Mar 2021–Dec 2021	719 (49)	87 (53)	632 (48)
Comorbidity			
0	656 (45)	78 (48)	578 (44)
≥1	817 (55)	83 (52)	734 (56)
Pneumococcal vaccination status			
None	701 (48)	85 (52)	616 (47)
PCV-13	20 (1)	3 (2)	17 (1)
PPV-23	627 (43)	67 (41)	560 (43)
PCV-13 + PPV-23	120 (8)	8 (5)	112 (9)

Abbreviations: PCV, pneumococcal conjugate vaccine; PPV, pneumococcal polysaccharide vaccine.

DISCUSSION

The containment measures introduced to mitigate the spread of SARS-CoV-2 in the Danish population may have reduced the transmission of pneumococci among older adults as reflected by a lower carriage prevalence during this period in our study. Social distancing, the closing of institutions and schools, and use of facial masks in public have the potential to reduce factors that influence the spread of pneumococci, such as close contact between individuals, crowding, and emission of respiratory droplets. Yet, our finding contrasts with that of persistent carriage among children during the pandemic in other countries [5–7] and among adults in the United States [14], especially among those with continued contact with children during restrictions. Results from the latter study were based on saliva samples from 95 individuals. Contact with children is a known risk factor for pneumococcal carriage among adults [15–17]. Carriage rates in Danish children pre- and postpandemic have not been studied. Assuming that these were stable, we can surmise that the decreased carriage rates among our study population may be due to less contact between older adults and infants or toddlers in the months following lockdown. A report on the behavior of citizens in several countries during the pandemic showed that Danish residents exhibited a large degree of support for the government response to COVID-19 and in general had very few social contacts during the spring of 2020 [18]. The number of contacts increased during the summer of 2020 and decreased again during the fall and winter months.

The date limits for the periods pre- and postlockdown in our study are, of course, fluent; however, we chose to define the period of lockdown as March 2020 to February 2021 because, even though there had been some gradual reopenings during this period, facial mask requirements began in the summer of 2020 and another extensive lockdown was initiated in December 2020. The gradual reopening of society began in March 2021 with retail trade and outdoor sport facilities opening.

The mitigation measures led to a marked decline in viral airway infections, such as rhinovirus and respiratory syncytial virus (RSV) in 2020 [19], followed by a subsequent steep increase in cases of RSV in the final half of 2021 [20]. There seems to be an association between viral coinfections and pneumococcal disease in adults [21], and it has been reported that rhinovirus infection increases the density of pneumococcal carriage in adults [17]. We chose to exclude participants with signs of upper respiratory tract infection, as inclusion of such would have

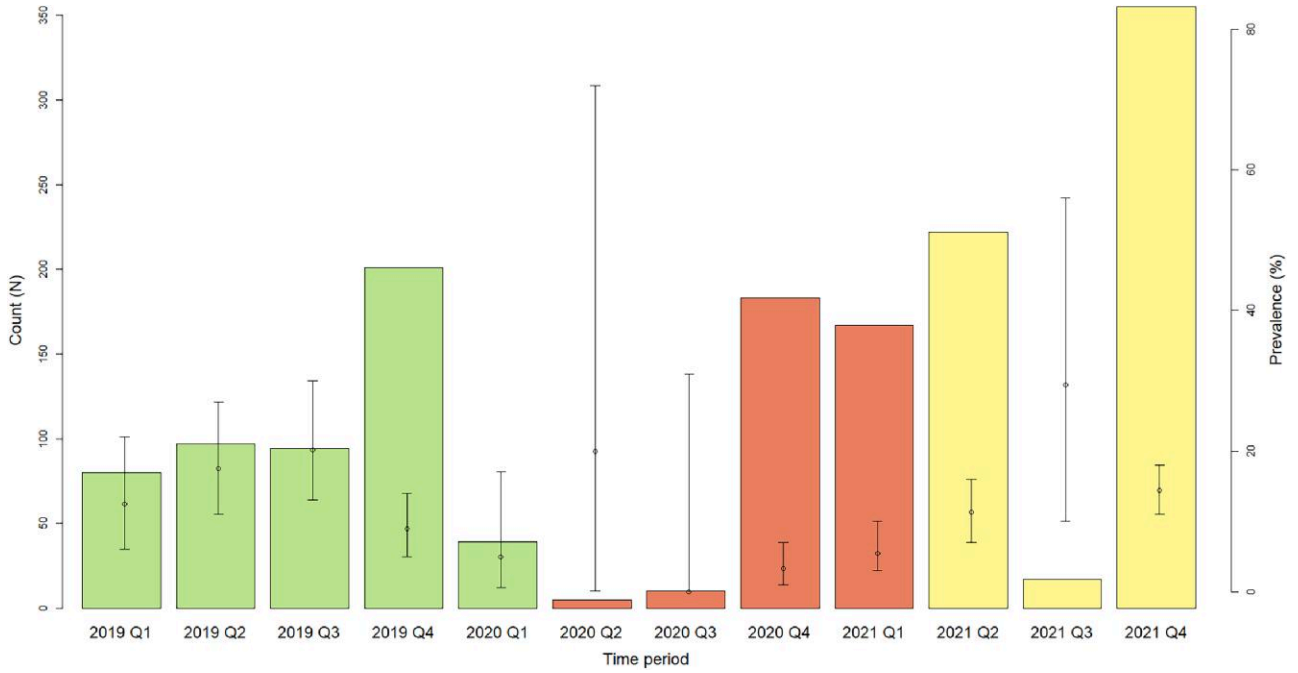

Figure 1. Number of participants sampled (bars) and pneumococcal carriage prevalence (dots with 95% CIs) over time. Q, quarter. Green: samples collected prelockdown (January 2019–March 2020). Red: samples collected during restrictions (March 2020–March 2021). Yellow: samples collected after reopening of society (April 2021—December 2021).

Table 2. Detection of *Streptococcus pneumoniae* in Naso- and Oropharyngeal Samples by *lytA*/*piaB* Quantitative Polymerase Chain Reaction Among 1556 Individuals

	Ct Value ≤40	
Swab	*lytA*	*piaB*
Nasopharyngeal	42	34
Oropharyngeal	195	146
Total	225	171

Abbreviation: Ct, cycle threshold.

had the potential to overestimate pneumococcal carriage due to the association of influenza-like illness and increased pneumococcal density [17]. The decreased pneumococcal carriage prevalence during lockdown found in our study could be mediated by the simultaneous suppression of other respiratory viruses. Likewise, the increase in pneumococcal carriage in quarter 4 of 2021 (Figure 1) may have been a consequence of the increased circulation of RSV and other respiratory viruses.

There are several strengths to our study, including the large number of participants who contributed data over several years. Additionally, participants contributed dual samples to improve detection. Culture of NP samples has been considered the gold standard for detection of pneumococci [22], but this method may be insufficient in adults [23], as evidence suggests that the density of pneumococci in the nasopharynx decreases with age [24]. Analysis of OP or saliva samples by molecular methods has been shown to increase the sensitivity of pneumococcal detection in adults [13, 15, 17, 25–30] as compared with conventional culture [16, 31, 32]. The specificity of molecular methods on oral samples has, however, been debated due to the possibility of false positives from nonpneumococcal streptococci. Hence, we chose to increase specificity by using a dual qPCR targeting 2 pneumococcal-specific sequences, *lytA* and *piaB* [23, 26, 33]. The Ct values of the *piaB* assay were oftentimes higher than those of the *lytA* assay (Supplementary Figure 1). The excess *lytA* signal could stem from *lytA* amplification from other nonpneumococcal streptococci, which highlights the importance of using 2 targets for identification to minimize false positives.

Our study has some limitations. The study participants were predominantly urban community-dwelling adults and did not include nursing home residents, who may have a higher carriage rate of *S pneumoniae* [34]. Furthermore, we did not have information on household contacts or socioeconomic status, which may account for some residual confounding. The quality of specimens may have varied because participants were swabbed at 19 study sites by different study staff despite formalized training. Finally, few samples were collected during the spring and summer of 2020 due to the lockdown, making carriage prevalence estimates uncertain, although there was a high quantity of samples from the fall of 2020 and winter of 2021 while several containment measures remained in place.

CONCLUSION

The prevalence of pneumococcal carriage among Danish older adults declined significantly during pandemic mitigation measures and rebounded to prepandemic levels as measure were lifted. Our study showed that containment measures may have effectively reduced pneumococcal carriage among older adults.

Supplementary Data

Supplementary materials are available at *Open Forum 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

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