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PCR Detection of Respiratory Pathogens in Asymptomatic and Symptomatic Adults

Nicklas Sundell,[a] Lars-Magnus Andersson,[a] Robin Brittain-Long,[b] Pär-Daniel Sundvall,[c,d] Åsa Alsiö,[a,e] Magnus Lindh,[a] Lars Gustavsson,[a] Johan Westin[a]

[a]Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

[b]Department of Infectious Diseases, Aberdeen Royal Infirmary, Aberdeen, Scotland

[c]Närhälsan, Research and Development Primary Health Care, Research and Development Centre Södra Älvsborg, Region Västra Götaland, Sweden

[d]Department of Public Health and Community Medicine/Primary Health Care, Institute of Medicine, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

[e]Department of Infectious Diseases, Skaraborg Hospital, Skövde, Sweden

ABSTRACT The frequency of viral respiratory pathogens in asymptomatic subjects is poorly defined. The aim of this study was to explore the prevalence of respiratory pathogens in the upper airways of asymptomatic adults, compared with a reference population of symptomatic patients sampled in the same centers during the same period. Nasopharyngeal (NP) swab samples were prospectively collected from adults with and without ongoing symptoms of respiratory tract infection (RTI) during 12 consecutive months, in primary care centers and hospital emergency departments, and analyzed for respiratory pathogens by a PCR panel detecting 16 viruses and four bacteria. Altogether, 444 asymptomatic and 75 symptomatic subjects completed sampling and follow-up (FU) at day 7. In the asymptomatic subjects, the detection rate of viruses was low (4.3%), and the most common virus detected was rhinovirus (3.2%). *Streptococcus pneumoniae* was found in 5.6% of the asymptomatic subjects and *Haemophilus influenzae* in 1.4%. The only factor independently associated with low viral detection rate in asymptomatic subjects was age ≥65 years ($P = 0.04$). An increased detection rate of bacteria was seen in asymptomatic subjects who were currently smoking ($P < 0.01$) and who had any chronic condition ($P < 0.01$). We conclude that detection of respiratory viruses in asymptomatic adults is uncommon, suggesting that a positive PCR result from a symptomatic patient likely is relevant for ongoing respiratory symptoms. Age influences the likelihood of virus detection among asymptomatic adults, and smoking and comorbidities may increase the prevalence of bacterial pathogens in the upper airways.

KEYWORDS respiratory pathogens, respiratory viruses, virology

The detection of respiratory viruses in airway samples by real-time PCR enables early and accurate etiologic diagnosis in respiratory tract infections (RTI). However, the results generated by this sensitive technique can be difficult to interpret. Detection may represent prolonged shedding of virus after symptomatic infection or an asymptomatic infection. Thus, it is essential to evaluate the clinical relevance of a positive finding. Early studies on the prevalence of respiratory viruses in asymptomatic individuals had limited sample sizes and focused on children (1–5). The detection rate of respiratory viruses in asymptomatic children exceeds 30% in some reports (6, 7), with even higher rates in infants (8). The prevalence in asymptomatic adults seems to be lower, ranging from 2.1% to 7.1%, but the number of studies in this field is still limited (9, 10). Different definitions of asymptomatic cases can also affect the reported detection rates. Human rhinovirus (RV) has been the predominant finding in samples from the upper airways in

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Address correspondence to Nicklas Sundell, nicklas.sundell@vgregion.se.

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studies of healthy adults. RV detection in asymptomatic persons may occur due to prolonged virus shedding after recovery from a symptomatic illness or to subclinical infection, but reinfection with different serotypes may also explain repeated findings (3, 11–13). *Streptococcus pneumoniae* and *Haemophilus influenzae* are important respiratory pathogens that can be cultured in nasopharyngeal samples in asymptomatic individuals with varied frequencies between age groups, representing asymptomatic carriage (14). Detection of these pathogens in the nasopharynx by PCR probably has higher sensitivity than that of conventional cultures, but further studies in healthy adults are needed to evaluate the clinical relevance of detection with this technique for respiratory tract infection and/or asymptomatic carriage (14–19).

The aims of the present study were to determine the prevalence of respiratory pathogens in the upper airways of asymptomatic adults compared with a reference population of patients with respiratory tract infection, and to investigate risk factors associated with the detection of respiratory pathogens in these populations.

MATERIALS AND METHODS

Study outline. Nasopharyngeal (NP) swab samples were prospectively collected from adults with and without ongoing symptoms of RTI during 12 consecutive months (June 2015 to June 2016). A specially trained study nurse performed sampling and collection of baseline data. The study subjects were recruited from three different primary care centers and three hospital emergency inpatient wards at a 2,000-bed teaching hospital in western Sweden. Inclusion of study subjects was made by the study nurse, who made one or two recruitment visits at each study site (inpatient and primary health care) per week across the entire study period. Asymptomatic subjects were recruited among patients seeking primary health care for, or being admitted to hospital care for, reasons unrelated to respiratory tract infections (e.g., blood pressure controls, annual health check-ups, or lower urinary tract infection in primary care, or minor stroke or ischemic heart disease for hospitalized inpatients). Symptomatic patients in primary health care and inpatient hospital care were recruited as a reference population among patients seeking health care for, or being admitted to hospital for, symptomatic respiratory tract infections. Clinical and laboratory data were recorded in a web-based case report form (CRF), which constituted the study database. The study was approved by the regional ethical review board in Gothenburg, Sweden. All participants provided written informed consent.

Asymptomatic study subjects. Inclusion criteria for asymptomatic subjects were age ≥18 years and absence of symptoms consistent with RTI during the 2 weeks before enrollment. An NP swab sample was collected (FLOQSwabs; Copan Industries, Inc.), and all participants completed a standardized study-specific questionnaire on demographic and medical data. At 1 week postenrollment, asymptomatic study subjects underwent a telephone-based interview regarding the development of any symptoms of RTI within 4 days after sampling. Individuals who were unable to accurately provide a history, developed symptoms of RTI within 4 days after enrollment, had a history of fever, diarrhea, or antibiotic treatment in the preceding 2 weeks, or resided in a health care facility (e.g., nursing home or residential home) were excluded from participating.

Reference population of symptomatic subjects. Inclusion criteria were age ≥18 years and symptoms consistent with RTI, as defined below, for a duration of ≤10 days. To distinguish between upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI), we adapted the definition of URTI from the Wisconsin Upper Respiratory Symptom Survey (WURSS) (20, 21), i.e., at least 1 out of 4 symptoms (nasal discharge, nasal obstruction, sneezing, and/or sore throat) and at least two of the following: sneezing, headache, malaise, chilliness, nasal discharge, nasal obstruction, sore throat, or cough. LRTI was defined according to the Joint Taskforce of the European Respiratory Society (ERS) and European Society for Clinical Microbiology and Infectious Diseases (ESCMID) as an acute illness, usually with cough as the main symptom and at least one of the following symptoms from the lower respiratory tract: sputum production, dyspnea, wheezing, and/or chest discomfort/pain (22). Exclusion criteria for symptomatic subjects were an inability to provide an accurate history, admission to hospital in the last 10 days, or admission from a health care facility, such as a nursing home or residential home. In addition to the standardized study-specific questionnaire, symptomatic subjects also completed a symptom score questionnaire at enrollment and at follow-up (WURSS score for URTI or the Community Acquired Pneumonia Symptom Questionnaire [CAP-Sym] for LRTI [23]).

PCR detection. All NP swab samples were transported to the laboratory without delay. The samples were analyzed for presence of respiratory pathogens with an in-house multiplex PCR panel that targets 16 viruses and four bacteria. The panel included influenza A (IFA) and influenza B (IFB) virus, respiratory syncytial virus (RSV), rhinovirus (RV), enterovirus (EV), coronavirus (CoV) of four different types (NL63, OC43, 229E, and HKU1), metapneumovirus (MPV), adenovirus (AdV), parainfluenza virus (PIV) types 1 to 4, and bocavirus (BoV), as well as the bacteria *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. Briefly, nucleic acid from a 100-μl specimen was extracted into an elution volume of 100 μl by a MagNA Pure LC robot (Roche Molecular Systems, Mannheim, Germany) using the total nucleic acid protocol, and amplified in an ABI 7900 real-time PCR system (Applied Biosystems, Foster City, CA) in 25-μl reaction volumes. After a reverse transcription step, 45 cycles of two-step PCR were performed. Each sample was amplified in 8 parallel reactions, each

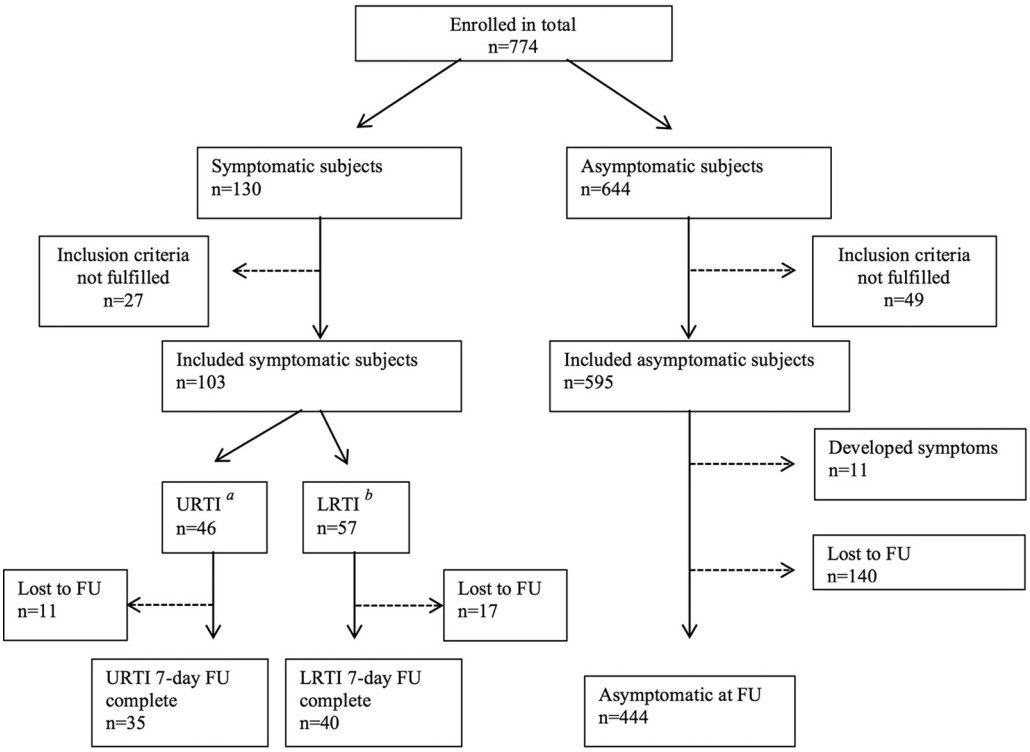

FIG 1 Flowchart depicting the enrollment of asymptomatic and symptomatic subjects in the study and subjects lost to follow-up (FU).

containing specific primers and probes for 2 or 3 targets. The method has previously been described in detail (24, 25). The cycle threshold (C_T) values of positive reactions were recorded, and a positive reaction with C_T value of <40 was considered a detection. In cases with a positive signal for both RV and EV with a cycle difference of <5 cycles, indistinguishable EV/RV was recorded.

Statistical analysis. The frequency of detection was compared with Pearson chi-square or Fisher's exact test, as appropriate. Comparisons of C_T values and clinical parameters were made with simple linear regression and the Pearson correlation coefficient. Factors associated with detection of virus or bacteria with a P value of <0.2 in univariate comparisons were included in multivariate logistic regression models. P values of <0.05 were considered statistically significant (2-sided). All statistical analyses were made using the SPSS software package version 22.0.0.0 (IBM, Armonk, NY).

RESULTS

In total, 774 adults agreed to participate in the study. A flowchart of the study subjects is shown in Fig. 1. Altogether, 595 asymptomatic subjects were included, of whom 444 completed follow-up (FU) at day 7. Of the 103 patients with respiratory tract infection who were enrolled in the reference population (46 with URTI and 57 with LRTI), 35 participants with URTI and 40 participants with LRTI completed FU. Demographic data of the included asymptomatic subjects and symptomatic reference patients are presented in Table 1. Monthly seasonal distribution of samples and overall detection rates of respiratory virus and bacteria included in the panel are presented in Table 2.

Detection of pathogens in asymptomatic subjects. Pathogen detection rates are presented in Table 3. A respiratory pathogen was detected in 49 of 444 (11%) asymptomatic subjects. Overall, the detection rate of viruses was low (4.3%). No one in this group had multiple virus findings. The most common virus was RV, followed by CoV. *Streptococcus pneumoniae* was found in 5.6% of the asymptomatic subjects and *Haemophilus influenzae* in 1.4%. Of the 140 subjects who did not complete FU, a virus was detected in 5% ($n = 3$ IFA virus, 2 RV, 1 IFB virus, and 1 CoV) and a bacterium in 5.7% ($n = 7$ *Streptococcus pneumoniae* and 1 *Haemophilus influenzae*). There were no significant differences in the detection rates between the asymptomatic group and the group that was lost to FU. Among the 11 subjects who were excluded due to the

TABLE 1 Demographic data in adults asymptomatic or symptomatic of respiratory tract infection[a]

Characteristics[b]	Asymptomatic subjects ($n = 444$)	Symptomatic reference subjects ($n = 103$)	*P* value[c]
Age (median [IQR]) (yr)	66 (57–76)	69 (54–77)	0.7
Female sex	242 (55)	65 (63)	0.1
Current smoker	55 (12)	21 (20)	0.03
Chronic lung disease	66 (15)	30 (29)	0.0006
Asthma	39 (9)	18 (18)	0.009
COPD	20 (5)	12 (12)	0.005
Lung cancer	3 (1)	1 (1)	1
Other lung disease	10 (2)	7 (7)	0.03
Any chronic medical condition[d]	286 (64)	62 (60)	0.4
Chronic heart disease	101 (23)	25 (24)	0.7
Diabetes mellitus	96 (22)	11 (11)	0.01
Chronic kidney disease	14 (3)	1 (1)	0.3
Chronic liver disease	4 (1)	0	0.6
Malignancy	21 (5)	11 (11)	0.02
IBD	12 (3)	4 (4)	0.5
Rheumatic disease	33 (7)	8 (8)	0.9
Immunodeficiency	29 (7)	6 (6)	0.8
Other chronic disease	62 (14)	9 (9)	0.2
Children at home	57 (13)	6 (6)	0.04
Children at daycare	16 (4)	3 (3)	1
Influenza vaccination	166 (37)	34 (33)	0.4
Pneumococcal vaccination	30 (7)	7 (7)	1
Antibiotics in last 14 days		50 (49)	
Duration of symptoms (>7 days)		30 (29)	
Included at hospital	97 (22)	58 (56)	<0.0001
Included at primary health care	347 (78)	45 (44)	<0.0001

[a]Data presented as number (%), unless otherwise specified.
[b]IQR, interquartile range; IBD, inflammatory bowel disease.
[c]Pearson chi-square (or Fisher's exact test when appropriate). For age, the Mann-Whitney test was used.
[d]Includes asthma, COPD, lung cancer, and other lung disease.

development of symptoms within 4 days after inclusion, a virus was detected in 18% ($n = 2$, both RV). There were no statistically significant differences regarding detection rates between asymptomatic subjects included in primary health care and those in inpatient hospital care (data not shown).

Detection of pathogens in reference population of symptomatic subjects. The detection rate was significantly higher among symptomatic subjects than among asymptomatic subjects (Table 3). Overall, 51 out of 103 (50%) patients with RTI were positive for any respiratory pathogen, and in 37 cases (36%), a virus was detected. No one in this group had multiple virus detections. RV was the predominant virus, followed by CoV. *Haemophilus influenzae* was significantly more frequent in symptomatic than in asymptomatic subjects, but the frequency of *Streptococcus pneumoniae* was similar in the two groups. There were no statistically significant differences regarding detection rates between symptomatic subjects included in primary health care and those in inpatient hospital care (data not shown).

TABLE 2 Monthly distribution of total sampling, including number of positive samples for a respiratory virus or bacterium

	No. of samples by mo-yr													
Test result by subject group	Jun-15	July-15	Aug-15	Sep-15	Oct-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16	May-16	Jun-16	Total
Asymptomatic subjects	37	2	29	41	39	53	29	30	24	20	39	60	41	444
Positive for respiratory virus[a]	1	0	3	4	1	2	3	0	1	0	1	1	2	19
Positive for bacteria[b]	1	1	5	2	1	4	2	4	1	1	4	2	3	31
Symptomatic subjects	12	0	4	7	10	11	9	8	3	2	12	19	6	103
Positive for respiratory virus[a]	7	0	2	3	2	5	2	4	1	2	3	4	2	37
Positive for bacteria[b]	4	0	2	1	4	4	0	0	0	0	0	1	1	17

[a]All respiratory viruses included in the PCR panel.
[b]Bacteria included in the PCR panel, i.e., *Streptococcus pneumoniae* and *Haemophilus influenzae*.

TABLE 3 Frequency of pathogens detected in the asymptomatic and symptomatic subjects

Pathogen	No. (%) of subjects		*P* value[a]
	Asymptomatic ($n = 444$)	Symptomatic reference subjects ($n = 103$)	
Any pathogen (including bacteria)	49 (11)	51 (50)	<0.0001
Any virus	19 (4.3)	37 (36)	<0.001
>1 virus detected[b]	0	0	
Rhinovirus	14 (3.2)	23 (22)	<0.001
Influenza A virus	0	2 (1.9)	NC
Influenza B virus	0	2 (1.9)	NC
Coronavirus	2 (0.5)	6 (5.8)	0.0008
Enterovirus	1 (0.2)	0	NC
Adenovirus	0	0	NC
Parainfluenzavirus	0	1 (1.0)	NC
Bocavirus	1 (0.2)	0	NC
RS-virus	0	2 (1.9)	NC
Metapneumovirus	1 (0.2)	1 (1.0)	NC
S. pneumoniae	25 (5.6)	7 (6.8)	0.65
H. influenzae	6 (1.4)	10 (9.7)	0.0001
M. pneumoniae	0	1 (1.0)	NC

[a]Pearson chi-square (or Fisher's exact test when appropriate).
[b]NC, not calculated due to small numbers.

Factors associated with detection of virus or bacteria in asymptomatic subjects. In the univariate model, age ≥65 years and previous vaccination against influenza were factors associated with a low probability of viral detection. In the multivariate model, only age ≥65 years remained independently associated with viral detection. For bacteria, current smoking and the presence of any chronic medical condition were associated with a high probability of detection in both the univariate and multivariate models. No other predictive factors associated with detection of virus or bacteria was identified in asymptomatic subjects or in the reference patients with respiratory symptoms (data not shown).

Viral load and symptom scores. For the three most common pathogens (RV, *Streptococcus pneumoniae*, and *Haemophilus influenzae*), comparisons of pathogen load, as estimated by the C_T value, between the asymptomatic and the symptomatic groups were made. A trend toward lower pathogen load was observed in asymptomatic compared with symptomatic subjects, but the difference was not statistically significant for any of the three pathogens (data not shown). Among the 35 symptomatic reference subjects who fulfilled the criteria for URTI according to WURSS score at day 0 and day 7, a virus was detected in 43% ($n = 15$ [9 RV, 3 CoV, 2 IFA virus, 1 and IFB virus]). Another 40 reference patients fulfilled the criteria for LRTI and completed CAP-Sym at day 0 and day 7. In 17 (43%) cases, a virus was detected (11 RV, 1 IFB virus, 1 CoV, 1 MPV, 1 PIV, and 1 RSV). Thirty-one patients (78%) with LRTI reported treatment with antibiotics in the last 2 weeks compared to 3 (8.6%) cases with URTI ($P < 0.01$). Further analysis of the WURSS score and CAP-Sym score did not reveal any relevant significant results (data not shown).

DISCUSSION

In this prospective study, we analyzed the prevalence of respiratory viruses by PCR in nasopharyngeal swab samples in a large group of asymptomatic adults sampled across all seasons. The main finding is that the detection rate of respiratory viruses in asymptomatic adults was low (4.3% positive). Only 1% of the participants were positive for other viruses than rhinovirus, which has previously been reported to cause prolonged shedding postinfection. There were no significant differences between samples from primary health care and inpatient samples. In contrast, the viral detection rate was high in the reference population of patients with symptomatic respiratory tract infection, where viruses were found in more than one-third of samples. The results suggest that asymptomatic infections are rare in immunocompetent adult patients and that detection of respiratory viruses in this group in general is clinically relevant. We detected relatively few cases of IFA virus among the symptomatic

subjects. The duration of the influenza season was relatively short this year, and healthy subjects are generally advised to avoid seeking care for typical symptoms of uncomplicated seasonal influenza. Furthermore, a relatively large proportion (43%) of the symptomatic subjects age ≥65 years stated having previous vaccination against IFA.

Early reports on the prevalence of virus in subjects without respiratory tract symptoms, summarized in a meta-analysis by Jartti et al., indicated that respiratory viruses were rarely (≤5%) present (although persistence might last up to a few weeks) and, accordingly, that positive results likely reflect recently acquired respiratory infections (26). More recent studies have reported a high frequency of respiratory viruses in asymptomatic children, ranging from 28% to 52% or even higher in infants (8, 27–30), often with predominance of RV (6). Available data indicate that detection rates in asymptomatic adults are much lower. For example, a respiratory virus was detected in 2% of asymptomatic controls in two studies of community-acquired pneumonia (10, 31), observations that agree with our results. Higher rates of virus infections were found by Lieberman et al. in 450 asymptomatic adults (7.1%) and in 201 adults with LRTI without pneumonia (54.7%) (9). Collection of both oropharyngeal swabs and nasopharyngeal washings from each participant, in addition to nasopharyngeal swab samples, might have led to higher detection rates in that study. Moreover, no follow-up was performed, and patients sampled in the presymptomatic phase of an upcoming infection might have been included in the asymptomatic group. Self et al. reported virus detection in 24.5% of patients with community-acquired pneumonia (CAP) compared to 43% in our group with LRTI (10). However, only patients with chest imaging suggestive of pneumonia were enrolled in that study. This might have resulted in a high rate of bacterial infections.

As we have reported earlier, RV is the most frequently detected virus in patients with respiratory symptoms, without marked seasonality in a temperate climate (32). In the present study, RV was the major finding in asymptomatic and in symptomatic adults. In immunocompetent subjects, RV infections and concomitant virus shedding commonly resolve within 1 to 2 weeks, although RV may be detected in subjects without respiratory symptoms (3, 11, 12, 30). Based on the large number of asymptomatic subjects, our data suggest that RV infections are less frequent in adults without symptoms of RTI than what was reported by Granados et al., who found RV in about 8% of asymptomatic students (33). Possibly, regional and age differences account for some of the discrepancy. In previous studies, evidence of prolonged shedding has been described, but other studies have also suggested that early reinfection with other RV serotypes is common (13, 34–36). This might imply that RV can cause subclinical infections, with no or mild symptoms, in adults. In the present study, only one sample per individual was obtained, which does not permit a distinction between prolonged shedding and reinfection with a new virus subtype. This issue, as well as to what extent transmission of virus occurs from asymptomatic individuals, warrants further investigation.

Our study is the first, to our knowledge, to explore risk factors for viral infections, as identified by PCR, in a large cohort of asymptomatic adults. Age ≥65 years was significantly associated with a lower viral detection rate in both univariate and multivariate models. In line with our findings, Graat et al. found RV in 2% of asymptomatic elderly (age >60 years) subjects (37). It is possible that the age group <65 years in our study was more exposed to respiratory viruses through close contact with small children, although we did not find any impact of having children at home or in daycare on viral or bacterial detection. Further studies are warranted to explore the association between viral detection in asymptomatic adults and age.

Cigarette smoking increases the risk for bacterial invasion of the airways through several mechanisms, and pneumococcal disease is common in patients with chronic obstructive pulmonary disorder (COPD) (38–41). In line with this, we found an overall carriage rate of *S. pneumoniae* in 15% of smokers compared to only 4.5% of nonsmokers. However, it is noteworthy that the detection of this pathogen in smokers may be unrelated to their symptoms and that detection may simply reflect asymptomatic carriage. Pneumococcal colonization in the elderly has been reported to be low (<5%),

with detection based on culture methods from nasopharyngeal samples. More recent investigations, based on PCR, have presented considerably higher frequencies in this group (15–18). Our PCR-based finding of 4.1% pneumococcal carriage in adult nonsmokers is lower than in these previous reports, although the oropharyngeal tract was not sampled in our study, which may account for the discrepancy. Detection of *H. influenzae* was common among reference subjects with respiratory symptoms, but the clinical relevance of this finding is unclear. Earlier studies of cultures of NP samples in healthy adults have found various frequencies of *H. influenzae*, ranging from 1.1% to 29%. Our findings are in line with those from another Swedish study by Gunnarsson et al. (14). In the study by Rawlings et al. (19), samples were collected through the oral cavity and also excluded healthy subjects who had received antibiotics within 4 weeks before sampling. This could possibly have contributed to the higher detection rates found in their study. Although it may be difficult to compare culture methods and PCR-based techniques, we believe that it is important to present the detection rates of *S. pneumoniae* and *H. influenzae* in our study, since there remains a lack of knowledge on how to interpret PCR-based detection of these pathogens in NP samples in relation to respiratory symptoms.

We used the WURSS score and CAP-Sym, both validated questionnaires, for symptom scoring in symptomatic cases (21, 23). Although antibiotic prescription patterns were beyond the scope of this article, we did find that 78% of patients who fulfilled the inclusion criteria for the CAP-Sym had been administered antibiotics in the last 2 weeks, compared to 8.6% of the patients with URTI. We did not, however, identify any significant difference in the rates of viral or bacterial pathogens between patients with URTI or LRTI.

This study has limitations. Although we included a large number of asymptomatic adults, the number of symptomatic cases was limited. A difficulty of including younger symptomatic subjects in primary health care due to work-related schedules might have introduced a selection bias toward older participants during recruitment. Sampling, especially of symptomatic subjects, was uneven across the study period, and our series may not reflect the true incidence of infection with any agent. A limited number of study subjects were included during the peak season for influenza and RSV activity (February and March), which may have contributed to an underestimation of the incidences of these viruses among symptomatic cases. The low rate of viral detection in the asymptomatic group may affect the possibility to draw strong conclusions regarding risk factors for the detection of virus in healthy adults. Further studies are needed to evaluate if the risk factors identified in our study are reproducible in a larger cohort. It is also important to note that bacterial findings in this study are based on PCR, which may differ from culture methods in terms of sensitivity.

In conclusion, detection of respiratory viruses in asymptomatic adults is uncommon. A positive PCR result from a symptomatic patient is likely to be relevant for ongoing respiratory symptoms.

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