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Characterization of Viral Agents Causing Acute Respiratory Infection in a San Francisco University Medical Center Clinic during the Influenza Season

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Background. With use of polymerase chain reaction (PCR) and a centrifugation-enhanced viral culture method, we characterized the viruses causing acute respiratory infection in adults during an influenza season.

Methods. During January–March 2002, nasopharyngeal wash specimens from previously healthy adults presenting with respiratory symptoms were evaluated for viral pathogens with centrifugation-enhanced viral culture and PCR.

Results The diagnoses in 266 cases included unspecified upper respiratory infection (in 142 [54%] of the cases), acute bronchitis (42 [16%]), sinusitis (23 [9%]), pharyngitis (22 [8%]), and pneumonia (17 [6%]). The use of a shell vial assay and PCR identified a pathogen in 103 (39%) of the patients, including influenza A or B in 54, picornavirus in 28 (including rhinovirus in 24), respiratory syncytial virus (RSV) in 12, human metapneumovirus in 4, human coronavirus OC43 in 2, adenovirus in 2, parainfluenza virus type 1 in 1, and coinfection with influenza and parainfluenza virus type 1 in 2.

Conclusion. Our findings demonstrate that, even during the influenza season, rhinovirus and RSV are prevalent and must be considered in the differential diagnosis of adult acute respiratory infection before prescribing antiviral medication. Human coronavirus and human metapneumovirus did not play a substantial role. PCR was an especially useful tool in the identification of influenza and other viral pathogens not easily detected by traditional testing methods.

Community-acquired respiratory infections in adults contribute substantially to morbidity and mortality. Although often self-limited, these infections can lead to lost days of work, increases in medical costs, severe illness, and even death. Historically, the etiology of acute respiratory illness (ARI) has been difficult to define. Although "no isolated pathogen" is a frequent finding (found in up to 50% of cases), it likely represents viral pathogens that the diagnostic tests were unable to detect [1]. Although there can be frequent overlap, historically, rhinoviruses, human coronaviruses, and adenoviruses have been commonly associated with upper respiratory tract infections (e.g., the common cold, otitis media, and sinusitis), whereas influenza, respiratory syncytial virus (RSV), and parainfluenza viruses are commonly found in cases of lower respiratory tract infection (e.g., acute bronchitis, bronchiolitis, and pneumonia) [2].

Although their use is limited primarily to reference laboratories and research settings, new molecular diagnostic techniques may offer better sensitivity and specificity for detection of respiratory agents than are offered by current standard clinical methods (e.g., antigen detection or isolation). In the past decade, PCR technology has improved our understanding of the burden of respiratory viruses that have traditionally been difficult to detect by routine methods, such as human metapneumovirus, rhinovirus, RSV, and human coronavirus [3–12]. However, many studies employing PCR techniques have tested for only a single or a limited number of respiratory agents [3–9,13–15]. Others have focused on specific populations (e.g., pediatric patients

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or elderly persons) or those with specific underlying illnesses (e.g., adults with chronic lung disease) or have focused on outbreak settings [1, 3, 10–14, 16, 17]. In this study, we used centrifugation-enhanced viral culture (R-Mix Fresh Cells; Diagnostic Hybrids) and PCR techniques to test for respiratory viruses in a previously healthy population of adults, with the goals of, first, better characterizing the spectrum of viral agents causing ARI, and second, comparing the sensitivity of newer molecular techniques with that of conventional methods for diagnosing viral respiratory pathogens.

METHODS

Study design. During January–March 2002, consecutive adults aged ⩾18 years and seeking care at the University of California, San Francisco (UCSF) Acute Ambulatory Care Clinic or Emergency Department with symptoms of an ARI were eligible for participation. UCSF is a tertiary care university hospital with a large associated primary care clinic. The UCSF Acute Ambulatory Care Clinic is the primary referral clinic for patients sent from the UCSF primary care clinic for evaluation of nonurgent medical problems, whereas the Emergency Department evaluates acutely ill patients. Symptoms of ARI were defined as development of a new illness within the past 3 weeks with cough, sinus pain, congestion, sore throat, or fever. Patients with the following noninfectious, self-reported conditions were excluded: pregnancy, systemic inflammatory disorders (e.g., rheumatoid arthritis, systemic lupus erythematosus, or inflammatory bowel disease), coexistent infections, severe tissue damage within the previous 7 days (e.g., trauma, surgery, or burns), myocardial infarction or unstable angina, cancer (metastatic or untreated), and AIDS or other immunosuppressive disorders.

Trained research assistants continuously reviewed triage notes to identify potentially eligible patients. Once the patients were identified, a standardized encounter form was used to ascertain the patient's age, race or ethnicity, underlying medical conditions, and clinical signs and symptoms. Clinical diagnosis was assigned by the evaluating clinician and was recorded in a free text area of the form. Because the diagnoses depended on the individual clinician's judgment, diagnoses may not have been mutually exclusive. Patients were subsequently evaluated and were treated by the clinicians in the usual manner. Medical record review was performed to ascertain vital signs, physical examination findings, diagnoses, and treatments. Study patients received a $10 gift certificate for their time. The study protocol and procedures were reviewed and approved by the UCSF Committee for Human Research.

Laboratory methods. At the time of enrollment, nasopharyngeal wash specimens were obtained using 5 cc of normal saline, placed in viral transport media, aliquoted into multiple sterile tubes, and stored at −70° C. Collection of conventional swab samples and routine testing for bacteria were not performed, because the primary objective of the study was to examine the frequency of pathogens that cause acute bronchitis. Group A *Streptococcus* and encapsulated bacteria (e.g., *Streptococcus pneumoniae, Haemophilus influenza,* and *Moraxella cattarhalis)* are not thought to be common causes of acute bronchitis in adults without underlying lung disease [2].

Specimens were inoculated into duplicate R-Mix Fresh Cell shell vial cultures with coverslips (Diagnostic Hybrids) and were cultured at 37° C for 24 and 48 h. R-Mix Fresh Cells, a combination of mink lung cells and human adenocarcinoma cells, are more sensitive than conventional culture and have a relatively rapid turnaround time [18]. At culture termination, cells were scraped from coverslips and spotted onto multiwell slides, air-dried, and fixed in methanol. Initially, all cell preparations were stained by direct immunofluorescence assay using a polyvalent antibody (Bartels-Trinity Biotechnology) directed against 7 viruses (influenzas A and B, RSV, adenovirus, and parainfluenza virus types 1–3). The duplicates of cultures with positive results were stained using virus-specific monoclonal antibodies.

Total nucleic acid was extracted from respiratory specimens using the MasterPure Complete DNA and RNA Purification Kit (Epicentre Technologies). Conventional 1-step RT-PCR assays were performed according to Erdman et al. [10], with primers for adenovirus, influenza A and B, RSV, and parainfluenza virus types 1–3 [10]; parainfluenza virus type 4 [13]; human metapneumovirus [3]; human coronavirus OC43 and 229E forward primer [4]; human coronavirus 229E reverse primer [7]; and picornavirus (inclusive of rhinovirus and enterovirus) (D. Erdman, personal communication). To differentiate rhinovirus from enterovirus, a 2-step RT-PCR process was modified from Kares et al. [19]. A real-time RT-PCR assay was used to improve sensitivity for RSV [9]. To confirm conventional RT-PCR findings for influenza A, a real-time RT-PCR assay was used (S. Lindstrom, personal communication).

Data analysis. Univariate procedures were used to examine the distribution of illness characteristics, physical examination findings, and laboratory results. Statistical tests of comparison included χ^2 tests for dichotomous variables, *t* tests for normally distributed continuous variables, and Wilcoxon rank sum tests for nonnormally distributed variables. All statistical analysis was performed using SAS software (SAS Institute).

RESULTS

This study was performed during January–March 2002, in the midst of peak influenza activity, as measured by regional trends in outpatient visits for influenza-like illness, influenza-associated hospitalizations, and laboratory detections of influenza in northern California [20].

A total of 408 patients in the UCSF Acute Ambulatory Care

Clinic and Emergency Room who were being evaluated for acute respiratory symptoms were approached to enroll in the study. Of these, 289 patients enrolled, 281 had clinical specimens collected, and 266 had specimens suitable for diagnostic testing (table 1). The mean and median ages of patients were 38.7 and 34 years, respectively. The mean and median durations of illness were 7.0 and 5.0 days, respectively. Most patients were previously healthy; the most common reported underlying medical problems included hayfever or allergies (75 [28%] of 266 cases), asthma (36 [14%] of 266), and diabetes (14 [5%] of 266). Nine patients (4%) were hospitalized.

The following diagnoses were assigned by the evaluating clinician: nonspecific upper respiratory infection (142 [54%] of 266), bronchitis (42 [16%] of 266), sinusitis (23 [9%] of 266), pharyngitis (22 [8%] of 266), and pneumonia (17 [7%] of 266) (table 1). Among the 142 cases with a diagnosis of upper respiratory infection, the most common agent was influenza (found in 34 [24%] of the cases). Among the 42 patients with a diagnosis of bronchitis, the most common agent was picornavirus (found in 10 [24%] of the patients; in 9 of these cases, the agent was further classified as rhinovirus), followed by influenza (in 6 [14%] of the patients). For the small numbers of cases of sinusitis, pharyngitis, and pneumonia, detected agents are listed in table 1.

Using both PCR and the shell vial assay, a viral agent was detected in a total of 103 (39%) of the cases (table 2). A virus

Table 1. Demographic and clinical characteristics and etiology of acute respiratory illness in 266 adults, by pathogen.

Variable	All subjects	Flu A or B[a]	Picorna[b]	RSV	HMPV	HCoV	PIV-1[a]	Adeno	No pathogen identified
No. (%) of total subjects with the specified pathogen	266 (100)	54 (20)	28 (10)	12 (5)	4 (2)	2 (1)	3 (0.1)	1 (0.1)	163 (60)
Age									
Mean years (±SD)	38.7 (15.1)	38.0 (13.9)	39.3 (16.4)	33.0 (9.3)	41.8 (11.5)	26 (0)	36.7 (15.0)	21	39.6 (15.8)
Median years (IQR)	34 (28–44)	35 (27–46)	37 (29.5–43)	30 (27–41)	42 (33–51)	26 (26–26)	29 (27–54)	21	34 (28–45)
Male sex	108 (41)	19 (36)	13 (46)	4 (36)	2 (50)	0 (0)	1 (33)	1 (100)	66 (41)
Ethnicity,[c] no. of subjects									
White	148	28	16	10	2	0	3	0	93
Black	23	6	1	0	0	0	0	1	15
Hispanic	25	9	1	2	1	0	0	0	12
Asian	49	8	8	0	1	0	0	0	30
Other	20	3	2	0	0	2	0	0	11
Smoker	37	7	7	0	1	0	0	0	22
Received influenza vaccine, no. of subjects	40	5	6	3	2	0	0	1	22
Chronic illness									
Allergies	75 (28)	15 (28)	10 (36)	5 (42)	0	1 (50)	0	1 (100)	43 (27)
COPD	2 (1)	0	0	0	0	0	0	0	2 (1)
Heart disease	9 (3)	2 (4)	0	0	0	0	0	0	7 (4)
Liver disease	3 (1)	1 (2)	1 (4)	0	0	0	0	0	1 (1)
Diabetes	14 (5)	2 (4)	0	1 (8)	0	0	0	0	11 (7)
Cancer	5 (2)	2 (4)	0	0	0	0	0	1 (33)	3 (2)
Asthma	36 (14)	4 (7)	7 (25)	3 (25)	1 (25)	0	0	0	21 (13)
Clinician diagnosis[d]									
Bronchitis	42 (16)	6 (12)	10 (38)	0	0	0	0	0	25 (16)
Pharyngitis	22 (8)	2 (4)	3 (12)	0	0	0	0	0	18 (11)
Pneumonia	17 (7)	4 (8)	0	1 (8)	1 (25)	0	0	0	11 (7)
Sinusitis	23 (9)	4 (8)	2 (8)	1 (8)	0	0	0	0	16 (10)
URI	142 (54)	34 (65)[a]	10 (38)	9 (75)	3 (75)	2 (100)	1 (100)	3 (100)	80 (50)
Other	15 (6)	2 (4)	1 (4)	1 (8)	0	0	0	0	11 (7)
Antibiotic treatment	89 (34)	18 (33)	12 (43)	3 (27)	3 (75)	1 (50)	0	0	53 (33)
Hospitalization[e]	9 (4)	1 (2)	1 (4)	1 (11)	0	0	0	0	6 (4)

NOTE. Data are no. (%) of subjects with specified pathogen, unless otherwise specified. Specimens were tested with R-Mix shell vial assay (Diagnostic Hybrids) and PCR. Flu, influenza; Picorna, picornavirus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus, HCoV, human coronavirus; PIV-1, parainfluenza virus type 1; Adeno, adenovirus; IQR, interquartile range; COPD, chronic obstructive pulmonary disease; URI, upper respiratory infection.

[a] There were 2 cases of coinfection, including 1 patient coinfected with influenza A and parainfluenza virus type 1 and 1 patient coinfected with influenza B and parainfluenza virus type 1.

[b] Twenty-four cases were further classified as due to rhinovirus, and 4 cases were unable to be further classified.

[c] Data were available for only 265 cases.

[d] Data were available for only 261 cases.

[e] Data were available for only 223 cases.

Table 2. Comparison of centrifugation-enhanced viral culture and PCR in the diagnosis of viral agents of acute respiratory illness in 266 previously healthy adults.

	No. (%) of subjects with a positive result, by test		
Pathogen	Overall	Enhanced viral culture[a]	PCR
Influenza A	49	26 (10)[b]	47 (18)
Influenza B	5	6 (2)[c]	5 (2)
Influenza (subtype not specified)	…	6 (2)	0 (0)
Respiratory syncytial virus	12	12 (5)	12 (5)
Parainfluenza virus types 1–3	3	2 (1)[d]	1 (1)
Adenovirus	2	2 (1)	1 (1)
Picornavirus	28	…	28 (11)
Human metapneumovirus	4	…	4 (2)
Human coronavirus OC43	2	…	2 (1)
Human coronavirus 229E	0	…	0 (0)
Parainfluenza virus type 4	0	…	0 (0))
Overall	103 (39)	52 (20)	100 (38)

NOTE. Individual rows do not add up to column totals, because patients with coinfection are included in multiple rows.

[a] Centrifugation-enhanced viral culture was performed with R-Mix Fresh Cells (Diagnostic Hybrids).

[b] One patient was coinfected with influenza A and parainfluenza virus type 1.

[c] One patient was coinfected with influenza B and parainfluenza virus type 1.

[d] Both patients had evidence of dual infection; one with influenza A and the other with influenza B.

was detected by the shell vial assay in 52 (20%) of 266 patients, whereas PCR identified a virus in 100 (38%) of the patients. Of note, PCR identified 38% more influenza cases than did the shell vial assay. Specific, targeted PCR identified additional viruses not assessed by the shell vial assay, including picornavirus (28 cases), human metapneumovirus (4), and human coronavirus OC43 (2). Of the 28 cases of picornavirus infection, additional PCR testing differentiated 24 as due to rhinovirus; the other 4 picornaviruses could not be classified further on the basis of the sensitivity of the assay.

Compared with the shell vial assay, PCR identified additional cases of influenza A (17 cases) and parainfluenza virus type 1 (1 case) in patients who had negative results according to the shell vial assay but failed to detect 5 cases in patients who had positive results according to the shell vial assay (influenza A in 2 patients, adenovirus in 1 patient, and the parainfluenza virus type 1 infections in 2 patients with influenza). Disparity in influenza subtype was seen in 6 cases: the shell vial assay could not distinguish between influenzas A and B in 5 cases and identified influenza B in 1 case that was typed as influenza A according to 2 different PCR methods.

Using the shell vial assay as a gold standard, the conventional influenza A RT-PCR assay showed sensitivity, specificity, and positive and negative predictive values of 94%, 93%, 64%, and 99%, respectively. A second RT-PCR assay for influenza A showed concordance with these 17 PCR-positive, shell vial assay–negative samples. When these 17 samples were considered true positives, the values increased to 96%, 100%, 100%, and 99%, respectively.

Clinical signs and symptoms for the 3 most common viral pathogens are listed in table 3. When comparing patients infected with influenza with those infected with rhinovirus or RSV, patients with influenza were more likely to have myalgias ($P < .05$). Patients with influenza were more likely to have fever, compared with patients with rhinovirus ($P < .05$). There was a trend toward an association with fever and RSV ($P = .054$); however, the small number of patients in the RSV group ($n = 12$) limited our power to detect a significant difference. Patients infected with rhinovirus or RSV were more likely to have wheezing at examination than were others ($P < .05$).

DISCUSSION

In this study, applying comprehensive diagnostic testing enabled identification of a viral agent in almost 40% of previously healthy adults who presented for evaluation of ARI. Influenza accounted for more than one-half of the pathogens identified. Other frequently detected pathogens included picornavirus (most of which were confirmed to be rhinovirus), which comprised one-quarter of identified etiologies, and RSV.

Our detection rate of almost 40% by PCR is somewhat greater than that found in similar studies that have attempted to determine the etiology of viral ARI in healthy adults using nonmolecular methods. The Tecumseh study, a large longitudinal prospective study of community ARI that was begun in 1965, detected a viral agent by isolation in <20% of adults >20 years old [21]. Another early study of upper respiratory infection involving 221 adults identified a viral etiology with use of isolation methods in 34% of the patients [22]. With use of a combination of isolation and serological testing, a study involving 278 college students with respiratory symptoms found an etiology in 32% of the patients [23]. A later study of adult ARI that used a combination of culture, ELISA, and immunostaining techniques yielded a viral etiology in 25% of patients aged 15–24 years and in 35% of patients aged 25–65 years [24]. The improved detection rate of PCR for specific individual respiratory agents (e.g., rhinovirus, enterovirus, human coronavirus, and parainfluenza virus) has been shown by others, but our study is one of the few to compare PCR with nonmolecular methods for a broad array of pathogens [1, 3, 4, 6, 13, 16].

In our study, PCR was substantially more sensitive than isolation or shell vial techniques for detecting influenza and identified almost 40% more cases than did the shell vial assay. PCR gave no advantage over the shell vial assay for detection of RSV, influenza B, adenovirus, or parainfluenza virus type 1 in our population. However, PCR was especially useful in identifying pathogens that frequently are not detected with conventional

Table 3. Comparison of clinical characteristics among adult patients with acute respiratory illness infected with influenza, picornavirus, and respiratory syncytial virus.

Clinical characteristic	No. (%) of patients, by pathogen			
	Flu A or B (n = 54)	Picornavirus (n = 28)	RSV (n = 12)	P
Temperature >38.0°C	14 (26)	0	0	<.05[a,b]
Headache	43 (80)	19 (68)	9 (75)	
Myalgias	45 (83)	13 (46)	6 (50)	<.05[a,b]
Sore throat	38 (70)	18 (64)	11 (92)	
Runny nose	45 (83)	24 (86)	11 (92)	
Abnormal tympanic membrane	9 (17)	4 (14)	2 (18)	
Sinus tenderness	8 (15)	4 (14)	1 (9)	
Tonsillar swelling	5 (9)	4 (14)	2 (17)	
Tonsillar exudates	3 (6)	2 (7)	0	
Cough	52 (96)	26 (93)	12 (100)	
Lymphadenopathy	13 (25)	5 (18)	4 (33)	
Wheezing at examination	7 (13)	12 (43)	5 (42)	<.05[a,b]
Shortness of breath	32 (59)	15 (54)	4 (33)	
Chest pain	19 (35)	12 (43)	5 (42)	
Nausea/vomiting	6 (11)	5 (18)	1 (8)	
Diarrhea	8 (15)	3 (11)	0	
Underlying medical condition(s)				
Hayfever/allergy	15 (28)	10 (36)	5 (42)	
Asthma	4 (7)	7 (25)	3 (25)	<.05[a]
Other	6 (11)	1 (4)	1 (8)	
Clinical course				
Received antibiotics	18 (33)	12 (43)	3 (27)	
Duration of illness, days				
Mean (SD)	5.0 (4.7)	6.6 (5.5)	3.7 (1.2)	
Median (25th–75th percentile)	4 (3–5)	5 (3–8)	3.5 (3–4.5)	

NOTE. There was a trend toward an association with fever and RSV when compared to influenza (P = .054); however, the small number of patients in the RSV group (n = 12) limited our power to detect a significant difference. Flu, influenza; RSV, respiratory syncytial virus; TM, tympanic membrane.

[a] Influenza A or B versus picornavirus.
[b] Influenza A or B versus RSV.

diagnostic techniques, including rhinovirus, human metapneumovirus, and human coronavirus.

For example, rhinovirus has not traditionally been considered an important "winter" pathogen. Early epidemiologic studies demonstrated a distinct seasonality to rhinovirus infection, with high incidences occurring in the fall and spring in temperate regions; the major peaks in the fall were presumed to be a result of children returning to school [25–27]. Studies conducted in the winter months and specifically focusing on adults have also found a low prevalence of ARI caused by rhinovirus [22–24]. These studies relied on culture-based methods and may have underestimated true rates of infection. However, more recent studies using PCR have also identified high incidences of rhinovirus infection in the spring and fall, when rhinovirus can account for as much as 80% of ARI in healthy adults, and much lower prevalences of rhinovirus infection (up to 4%) in the winter months [1, 23, 28–30]. In contrast, 10% of the patients in our study were infected with rhinovirus, suggesting that rhinoviruses cause more cases of ARI during the influenza season than has previously been suspected.

Likewise, RSV was a frequent finding in this population. Historically, RSV has been considered a respiratory pathogen primarily found in children and responsible for increased rates of bronchiolitis and pneumonia during the winter months [5]. This perception may, in part, be due to higher viral shedding in the nasal secretions of children, compared with those of adults, thus facilitating detection in children. Recent studies have recognized the importance of RSV in the elderly population, in adults with underlying malignancy and cardiopulmonary disease, and as a cause of nosocomial infections [31].

Our results concur with those of other studies identifying RSV as an important cause of ARI in previously healthy adults, including a PCR-based study that identified RSV as a cause of up to 20% of cases of influenza-like illness [5, 32].

The negative findings in our study—which occurred despite our use of sensitive, targeted PCR testing—are worth noting. The infrequent detection of human coronavirus in our study is somewhat surprising. Older studies, based mostly on serologic testing, estimated that human coronaviruses account for up to 35% of cases of upper respiratory illness, with infrequent case reports describing severe disease in infants, in the elderly population, and in adults with chronic pulmonary disease [33]. Recent studies utilizing PCR have confirmed that human coronavirus can play an important role in asthma exacerbations in adults and in respiratory outbreaks; however, we were unable to confirm that human coronavirus contributes substantially to clinically significant ARI in healthy adults [16, 17].

Human metapneumovirus was also infrequently identified. Although human metapneumovirus was only recently discovered, evidence suggests that the virus has been circulating in humans for several decades and has epidemiologic and clinical characteristics similar to those of RSV [34]. To date, little is known about the contribution of human metapneumovirus to ARI in adults; limited data estimate human metapneumovirus prevalence among nonhospitalized individuals at 1%–9% [3, 15, 34]. Employing the same primers as other investigators, we identified human metapneumovirus in only 1% of cases. Our results agree with existing data suggesting that human metapneumovirus can cocirculate at the same time as influenza and that it is an infrequent cause of ARI in healthy young adults.

The limitations of PCR testing should be noted. In this study, the specimens tested by PCR underwent multiple freeze/thaw cycles, which may have adversely affected assay sensitivity. Unlike standard culture methods, PCR limits detection to the targeted agents, and the sensitivity and specificity of individual PCR tests may vary depending on the target pathogen and the specific primers and probes used. It is possible that samples with positive PCR results but negative shell vial assay results may represent false positives; however, in this study, the PCR test results were corroborated with concurrent conventional testing or with a second RT-PCR assay. In addition, previous non–culture confirmed positive PCR results in our laboratory have been confirmed by product sequencing [35].

Our findings have important clinical implications. Early studies established a correlation between circulation of influenza virus in the community and concurrent increases in the incidence of severe ARI, hospitalizations for pneumonia, and influenza-associated mortality [36]. This led some to conclude that, when influenza is circulating within the community, patients with specific clinical signs and symptoms (e.g., cough and fever within 48 h after onset of symptoms) are likely to have influenza and may benefit from empiric antiviral therapy to reduce the duration of illness and the number of influenza-associated hospitalizations [37–39]. However, these recommendations are based on retrospective pooled analysis of antiviral clinical trials, in which the inclusion criteria required presence of fever and/or symptom of fever, which may have conferred a selection bias. In our study, the major clinical differences among the most common agents—influenza, rhinovirus, and RSV—were the presence of myalgias with influenza and fever with influenza and RSV. A finding of wheezing on examination was associated with rhinovirus or RSV infection. The association of wheezing with both rhinovirus and RSV infection has been described and has been postulated to be due to increased bronchial reactivity caused by airway injury, diminished β-adrenergic function, IgE production, and enhanced leukocyte histamine release [40]. When assessing patients with nonspecific symptoms of influenza-like illness, these additional clinical clues may be of use to the clinician considering the benefits and drawbacks of prescribing antiviral therapy, which can be expensive or may have associated adverse effects. For example, the adamantane drugs are associated with CNS-related adverse events, particularly in the elderly population, and oseltamivir can cause adverse gastrointestinal effects [34].

In conclusion, our findings demonstrate that, even during the influenza season, rhinovirus and RSV are prevalent and must be considered in the differential diagnosis of adult ARI before prescribing antiviral medication. Human coronavirus and human metapneumovirus did not appear to play a substantial role. Finally, PCR was an especially useful tool in the identification of influenza and other viral pathogens not easily detected by traditional testing methods.

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