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Frequency of viral etiology in symptomatic adult upper respiratory tract infections

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ABSTRACT

Aims: To determine the frequency of viral pathogens causing upper respiratory tract infections in non-hospitalized, symptomatic adults in the city of Rio de Janeiro.
Methods: Respiratory samples (nasal/throat swabs) were collected between August 2010 and November 2012 and real time PCR was used to detect different viral pathogens.
Results: Viruses were detected in 32.1% (43/134) of samples from 101 patients. Specifically, 9% (12/134) were positive for HBoV, 8.2% (11/134) were positive for HAdV, 5.2% (7/134) were positive for HRV, and 1.5% (2/134) were positive for FLUBV or HMPV, as single infections. HRSV-A, HPIV-3, and HCoV-HKU1 were detected in one (0.75%) sample each. Co-infections were detected in 4.8% (6/134) of the samples. Peaks of viral infections were observed in March, April, May, August, and October. However, positive samples were detected all year round. Only 23.3% (10/43) of the positive samples were collected from patients with febrile illness.
Conclusion: Results presented in this report suggest that respiratory viral infections are largely under diagnosed in immunocompetent adults. Although the majority of young adult infections are not life-threatening they may impose a significant burden, especially in developing countries since these individuals represent a large fraction of the working force.
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Introduction

Acute respiratory infections represent a significant morbidity and mortality burden worldwide and are caused primarily by viral infections.[1–3] The high morbidity and mortality rates due to respiratory viruses have made these infections a global health concern. Since 1977, the World Health Organization has advocated the surveillance of acute viral respiratory disease programs worldwide.[4] However, the

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majority of the data collected regarding infections in children and the epidemiology of community-acquired viral respiratory infections among adults is insufficient, perhaps with the exception of infections caused by the influenza virus. Most data available focuses on specific populations such as immunosuppressed,[5–9] homeless,[10,11] or elderly individuals.[5,12,13]

Viruses are the leading causes of acute respiratory disease throughout the world. Causative agents of respiratory disease in humans include human respiratory syncytial virus (HRSV), human parainfluenza virus (HPIV), influenza A virus (FLUAV) and influenza B virus (FLUBV), human adenovirus (HAdV), human coronavirus (HCoV), human rhinovirus (HRV), human metapneumovirus (HMPV), and human bocavirus (HBoV).[1–3] In addition, two human polyomaviruses (HPyV), KIPyV and WUPyV, have been detected in patients with respiratory infections.[1,2]

In 2000, the Brazilian Ministry of Health established a countrywide surveillance system for respiratory viral infections. This system comprises a network of sentinel units that include outpatient clinics, emergency care departments, and general hospitals that issue a weekly report (using an online system) informing the total number of visits and the number of visits associated with influenza-like illness (ILI) (defined as a case of fever accompanied by cough or sore throat with no other diagnosis).[14,15] The system is designed to detect only a small number of virus species including FLUAV, FLUBV, HRSV, HAdV, and HPIV-1, -2, and -3.[14] Infections caused by other important viral pathogens such as HPMV, HRV, HBoV, and HCoV are not monitored.

The aim of this study was to determine the frequency and type of upper respiratory viral infections in non-hospitalized adults in the city of Rio de Janeiro, Brazil over a period of two years using molecular diagnostic methods.

Patients and methods

Immunocompetent patients ($n = 134$) who attended the Immunology Service of the Hospital Universitário Clementino Fraga Filho (HUCFF)/Federal University of Rio de Janeiro (UFRJ) between August 2010 and November 2012 (median age 50.5 years; ranging from 19 to 80 years) were enrolled in the present study. The age distribution of the subjects was as follows: six were 19–24 years old, 21 were 25–35 years old, 17 were 36–45 years old, 33 were 46–55 years old, 37 were 56–65 years old, and 20 were >65 years old (Table 1).

Respiratory samples (nasal/throat swabs) were obtained from all participants. Following collection swabs were placed into viral transport media and stored at −70 °C until processed. Relevant clinical information including age, sex, and clinical symptoms were collected during the medical visit using a standardized questionnaire. Respiratory illness was defined by the presence of rhinorrhea and/or cough and/or respiratory distress and/or sore throat, with or without fever.

Nucleic acid was extracted from 200 μL of the collected samples using the Wizard Genomic DNA Purification KIT (Promega, Madison, WI) and the Totally RNA® Kit (Applied Biosystems/Ambion, Grand Island, NY) according to the manufacturer's instructions. Specimens were tested for the presence of FLUAV and FLUBV,[16] HRSV (variant A and B),[17] HPIV species 1–4,[18] HRV species A and B (HRV-A/B),[19] HMPV,[20] HAdV,[21] HBoV species 1–4,[22,23] WUPyV and KIPyV,[24] and HCoV species OC43, 229E, NL63, and HKU1[25] by real time PCR assays. Genomic RNAs of FLUV, HRSV, HPIV, HRV, HMPV and HCoV were subjected to one cycle of reverse transcription (5 min at 25 °C followed by 45 min at 42 °C) using 50 pmol random primer (hexamer pd[N]$_6$, Life Technologies, Carlsbad, CA, USA) in a Veriti 96 well (Applied Biosystems, Foster City, CA, USA) thermocycler, prior to PCR amplification. Real-time PCR amplifications were performed for each virus separately, in an ABI StepOne Real-time PCR System (Applied Biosystems). The amplification conditions consisted of 10 min at 95 °C, followed by 45 cycles of 10 s at 95 °C and 60 s at 60 °C. Amplification was carried out in 24 μL reaction volumes, including 5 μL of DNA, specific primer for each virus and 12 μL of Maxima® SYBR Green qPCR Master Mix (Fermentas/Thermo Fischer Scientific, Canada). Each PCR assay for HMPV, HRV-A/B, HAdV, KIPyV or WUPyV used a single pair of primers. Multiplex assays were used to detect and discriminate the distinct species of FLUV, HRSV, HBoV, HIPV and HCoV.

A conventional RT-PCR protocol was used for detection of HRV species C (HRV-C).[26] After the reverse transcription step, the viral cDNA was subjected to one step of 8 min at 94 °C followed by 35 cycles of PCR each consisting of 45 s at 94 °C, 45 s at 60 °C and 45 s at 72 °C, and a final extension step of 8 min at 72 °C in a Veriti 96 well thermocycler. The PCR products were analyzed by 1.2% (w/v) agarose gel electrophoresis and visualized by staining with ethidium bromide.

Positive and negative controls were included in each run. Infected cell cultures were used as positive controls for HRSV (HEp-2 cells), HPIV (Vero cells), HMPV (LLC-MK2 cells), HRV (MRC-5 cells) and HAdV (A549 cells). Positive controls of FLUAV and FLUBV consisted of allantoic fluid from FLUV infected embryonic eggs. Clinical samples of HRV-C, HCoV, KIPyV, WUPyV and HBoV, confirmed by PCR amplification and sequencing analysis, were obtained from patients with respiratory illness and used as positive controls. Negative controls consisted of viral transport medium.

Results

Patients ($n = 101$) (16 male and 85 female) presenting with respiratory illness symptoms were enrolled in the present study. Respiratory samples ($n = 134$) were collected and analyzed for the presence of viruses. The most common symptoms observed were cough (88.4%), sneezing (67.4%), and nasal congestion (58.1%). Fever was reported in 22.4% (30/134) of respiratory infection episodes (Fig. 1).

Patients were stratified into six age groups (Table 1). The overall detection frequency of any viral respiratory infections was 33.3% (2/6) in individuals <24 years old, 33.3% (7/21) among those 25–35 years old, 41.2 (7/17) among patients 36–45 years old, 24.2 (8/33) among patients 46–55 years old, 35.1 (13/37) among patients 56–65 years old, and 30.0% among patients >65 (6/20) years old. The identification rates of respiratory viruses did not differ significantly between age groups.

Co-infections occurred in six (4.5%) specimens, of which five were double infections (HAdV + HBoV1; HAdV + HBoV2;

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