

Establishing the Epidemiology of Respiratory Virus Infections Using Molecular Technology



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ABSTRACT

With the discovery of five new respiratory viruses since the year 2000 and advances in molecular technology allowing the detection of up to 20 different respiratory viruses in a single test (Mahony et al. 2007), we have investigated the cause of viral respiratory tract infections in both children and adults over a 12 consecutive month period. A total of 1,060 nasopharyngeal specimens were collected from symptomatic patients and used in the study. Approximately 100 specimens per month, 50 pediatric (<20 yr) and 50 adult (>20 yr), were selected by randomized stratified sampling from specimens submitted to the Regional Virology Laboratory from November 2005 to October 2006. One half of the specimens were from pediatric patients (N=526) and one half were from adults (N=534). Specimens were collected blindly without knowledge of DFA and culture results. Total nucleic acid (DNA and RNA) was extracted using the MiniMag extractor (Biomerieux) and an aliquot was tested by the ID-Tag™ RVP Test from TmBioscience Corporation. The RVP assay is a multiplex PCR coupled to a fluid microarray that detects and identifies 20 respiratory virus types and subtypes including Influenza A subtypes H1, H3, and H5, Influenza B, Parainfluenza types 1-4, RSV types A and B, Adenovirus, Metapneumovirus, Rhino/Enterovirus, Coronaviruses OC43, 229E, NL63, HKU1, and SARS-CoV. A second aliquot was tested for Bocavirus using a separate PCR. Clinical information was collected by chart review. Results were analysed by month and by age group. Of the 1,060 specimens tested, 424 (40%) were positive for at least one respiratory virus including: 205 (19.3%) Rhino/Enterovirus, 58 (5.5%) Influenza type A or B, 45 (4.1%) Parainfluenza (types 1-4), 41 (3.9%) RSV (type A or B), 41 (3.9%) Metapneumovirus, 39 (39/947, 4.1%) Bocavirus, 20 Adenovirus (1.9%), and 14 Coronavirus (1.3%) (OC43, HKU1, NL63). Twenty five of the 39 (64.1%) Bocavirus infections represented dual infections and were positive for a second respiratory virus. Only Rhino/Entero, RSV and Bocavirus were more prevalent in the <20 age group (p<0.05). Influenza, Metapneumovirus and Coronavirus were only present in winter/spring months while Parainfluenza type 4 was present only in summer months; all others were distributed across the majority of months. The average rate of positives diagnosed per month for the pediatric group was 63% compared to 23% for adults (p<0.05). The epidemiology of respiratory virus infection in symptomatic pediatric and adult patients was studied using the new ID-Tag™ RVP Test from TmBioscience. The following conclusions could be made from this study: 1) Rhino/Enterovirus was the most prevalent respiratory virus infection of symptomatic children and adults, 2) only RSV, Rhino/Enterovirus, and Bocavirus showed higher infection rates in children compared to adults, 3) almost two thirds of Bocavirus infections were dual respiratory virus infections, 4) 90% of dual infections (mostly Boca and Rhino/Enterovirus) occurred in children in the summer months, and 5) Influenza, Metapneumovirus and Coronavirus displayed a winter seasonality. The RVP test should assist hospital and public health laboratories in diagnosing the etiology of respiratory tract infections in individuals and in outbreak situations.

INTRODUCTION

The epidemiology of conventional respiratory viruses was first studied in the mid 1960s. In the following four decades the most important infections in pediatric populations worldwide have been RSV, Influenza, Parainfluenza types 1-3 and Adenovirus. Historically these infections have been diagnosed by culture and DFA but over the past decade nucleic acid amplification tests (NAAT) have been introduced for many respiratory viruses providing an enhanced sensitivity over that of DFA and culture (1). Multiplex PCR and real time PCR assays have more recently been introduced for the detection of emerging respiratory viruses in respiratory tract specimens and collectively have increased our knowledge of the epidemiology of respiratory virus infections (1-4).

The emergence of five new respiratory viruses since 2000 including Metapneumovirus, SARS-CoV, Avian influenza H5N1, Coronavirus NL63 and HKU1 and human Bocavirus has presented challenges for the virology laboratory. The absence of commercially available tests often leaves laboratories without the ability to diagnose these important virus infections or the ability to conduct epidemiological studies on respiratory virus infections in selected patient populations. Therefore, there is a need for new and improved diagnostic tests to diagnose conventional and emerging respiratory virus infections with improved sensitivity.

Recently a multiplex PCR test called the ID-Tag™ Assay has been introduced by TmBioscience Corporation (now called Luminex Molecular Diagnostics). This multiplex assay can detect and identify up to 20 different respiratory viruses in a single five hour test (2,3).

OBJECTIVE

The objective of this study was to employ the ID-Tag™ Assay to study the epidemiology of respiratory virus infections and in particular to determine the virus-specific positivity rates, seasonality and age distribution (pediatric and adult) for respiratory virus infections over a 12 month period in Hamilton Ontario.

METHODS

1. Specimens – A total of 1,060 nasopharyngeal specimens were collected from symptomatic patients and used in the study. Approximately 100 specimens per month, 50 pediatric and 50 adult, were selected by randomized stratified sampling from specimens submitted to the Regional Virology Laboratory between November 2005 and October 2006. One half of the specimens were from pediatric patients (N=526) and one half were from adults (N=534). Specimens were collected blindly without knowledge of DFA and culture results. Whole specimens were divided into aliquots and one aliquot was processed in the routine laboratory by DFA and shell vial culture (DHI) and a second aliquot was processed for testing by the ID-Tag RVP Test from Luminex Molecular Diagnostics, Toronto, Ontario (formerly TmBioscience Corporation) a new multiplex PCR test that can detect 20 different respiratory viruses in a single five hour test.

2. DFA and Shell Vial Culture – DFA was performed using standard methods. Slides were stained using virus-specific monoclonal antibodies (Diagnostic Hybrids Inc.) and read by experienced virology technologists. DFA negative specimens were set up in shell vial cultures and stained with a panel of 8 monoclonal antibodies at 48 hr. Shell vial cultures containing R-Mix cells were purchased from DHI.

3. Nucleic acid extraction– Total nucleic acid (DNA+RNA) was extracted using the Biomerieux MiniMag or EasyMag extractor.

4. ID-Tag™ RVP Test – This is a new multiplex PCR test for detection of respiratory viruses developed by TmBioscience Corporation. The test involved the following steps: one step RT-PCR using 14 pairs of primers, interrogation of amplicons with a multiplex target specific primer extension (TSPE) reaction using 21 oligonucleotide probes specific for 21 different respiratory virus types and subtypes, hybridization of TSPE products to a microfluidic array of 21 spectrofluorometrically-labeled microspheres containing virus-specific probe sequences, and of microbeads on a bench-top Luminex-100 flowcell (Luminex Corporation). The entire RVP assay takes approximately 5 hours for 96 specimens from sample input to result.

5. Bocavirus PCR – A subset of 947 or the 1,060 specimens was tested for Bocavirus. Bocavirus DNA was amplified with primers targeting the NS-1 gene and the NP-1 genes as described previously by Lu et al. (2006) and Sloots et al. (2006). Only specimens positive for both gene targets were considered positive for this study.

RESULTS

We have used the new ID-Tag™ RVP Test to determine the epidemiology of respiratory virus infections in pediatric and adult patients presenting with signs/symptoms of upper or lower tract infection during a 12 month period from November 2005 to October 2006. We tested a total of 1,060 nasopharyngeal specimens collected from symptomatic patients. Up to 100 specimens per month, 50 pediatric and 50 adult, were selected by randomized stratified sampling from specimens submitted to the Regional Virology Laboratory between November 2005 and October 2006. One half of the specimens were from pediatric patients (N=526) and one half were from adults (N=534).

In the present study all patients presented with signs or symptoms of either upper or lower respiratory tract infection. Chart review of 80% of the cases revealed clinical presentations with at least one of the following: cough, fever, rhinorrhea, SOB, apnea, nasal flaring, tracheal tug, grunting, indrawing, wheezing or crackles. Admitting diagnoses included either URTI, bronchiolitis, croup, pneumonia, fever, asthma exacerbation or otitis media.

Of the 1,060 specimens tested, 424 (40%) were positive for at least one respiratory virus. These included the following: 205 (19.3%) Rhino/Enterovirus, 58 (5.5%) Influenza type A or B, 45 (4.1%) Parainfluenza (types 1-4), 41 (3.9%) RSV (type A or B), 41 (3.9%) Metapneumovirus, 39 (39/947, 4.1%) Bocavirus, 20 Adenovirus (1.9%), and 14 Coronavirus (1.3%) (OC43, HKU1, NL63).

Only Rhino/Entero, RSV and Bocavirus were more prevalent in the pediatric group (p<0.05). The positivity rates for Rhino/Enterovirus was 28.9% for pediatric patients (152/526) and 9.9% (53/534) for adults (p<0.05). The positivity rates for RSV was 6.7% (35/526) for pediatric patients and 1.1% (6/534) for adults (p<0.05). Of interest was the difference seen for RSV type A and B. For RSV type A, there was no significant difference between the positivity rates for pediatric and adult patients (1.1% vs. 0.4% respectively, p=0.175) whereas for RSV type B, the positivity rates for pediatric cases (7.5%) was significantly higher than for adult cases (0.8%) p<0.001. For Bocavirus the positivity rate in pediatric patients, was 7.5% (35/467) compared with 0.8% (4/482) for adults (p<0.05).

In terms of seasonal distribution most of the viruses were distributed across the majority of months. Rhino/Enterovirus peaks were between April and May and September and December with no activity from January to April or June to August. Influenza A infections were distributed from January and April while Influenza B showed a peak activity in children in March. Parainfluenza types 1-3 peaked between mid November and the end of February and again in July and August. Only three viruses viz. Influenza, Metapneumovirus and Coronavirus were present only in the winter and spring months. Parainfluenza type 4 was present only in summer months.

Using the ID-Tag™ RVP Test we have consistently seen a dual respiratory virus infection rate of 5-8% for symptomatic patients and even some triple virus infections. In the current study 90% of the dual infections occurred in children in summer months. Twenty five of the 39 (64.1%) Bocavirus infections represented dual infections as the specimens were positive for a second respiratory virus. In the current study we found patients positives for RSV and influenza A, RSV and Parainfluenza 3, RSV and MPV, combinations which have previously been reported in the literature plus new combinations of viruses not previously reported including Influenza A with Metapneumovirus, Parainfluenza 3 with Rhino/Enterovirus, Metapneumovirus with Rhino/Enterovirus, and Metapneumovirus with Coronavirus OC43.

RESULTS

Of interest was the rate of positivity for the detection of any virus in pediatric and adult specimens across the entire year. The positivity rates for either pediatric or adult cases for any month ranged from a low of 8% to a high of 83%. The average rate of positives diagnosed per month was 63% for pediatrics compared to 23% for adults (p<0.05) indicating that a respiratory virus is detected more often in pediatric specimens compared with adult specimens. The monthly positivity rates for pediatric cases ranged from 36.4% to 82.7% with the highest in March 2006. In contrast the monthly positivity rates for adults ranged from 8.7% to 44.2% with the highest month in September 2006.

In the month of January 2007 up to 12 different respiratory viruses were co-circulating in the community in Hamilton. By comparison, one year earlier in January 2006, up to 6 different viruses were detected in the community at any one time using a combination of DFA and culture.

The low overall number of RSV infections during the twelve month study period (N=41) suggests that 2006-2007 may have been an "atypical" year for respiratory virus infections. The high number of RSV infections seen in the month of January of 2007 (N=197) confirms that huge differences can occur from year to year. For this reason we are extending the study another year.

CONCLUSIONS

- The RVP test has increased our understanding of the epidemiology of respiratory viral infections. In the present study of respiratory tract infections from November 2005 to October 2006, most of the viruses were distributed across the majority of months. Only three viruses viz. Influenza, Metapneumovirus and Coronavirus were present only in the winter and spring months. Parainfluenza type 4 on the other hand was present only in summer months.
- Most respiratory viruses were present in both pediatric and adult populations. Only Rhino/Entero, RSV and Bocavirus were more prevalent in the pediatric group (p<0.05). The average rate of positives diagnosed per month for the pediatric group was 63% compared to 23% for adults (p<0.05).
- During January of 2005 there were up to 12 different respiratory viruses co-circulating in the pediatric population in Hamilton. In previous years using DFA and culture we have only seen 6 viruses co-circulating in the month of January.
- We saw a predominance of Rhinovirus and Enterovirus infections representing approximately 48% of all respiratory viruses detected during the 12 month study period. This 12 month period may have represented an "atypical" respiratory year due to the unusually low number of RSV positives detected. In the last six months we have seen a much higher prevalence of RSV infections with nearly 200 in January alone and it will be interesting to see how the other viruses stack up during this time frame.
- Twenty five of the 39 (64.1%) Bocavirus infections represented dual infections. In this and other studies we have seen dual respiratory tract infections in up to 8% of symptomatic patients. We have also seen a small number of triple infections. In the current study we found patients positive for RSV and influenza A, RSV and parainfluenza 3, RSV and MPV, combinations which have been reported in the literature plus new combinations of viruses not previously reported including Influenza A with Metapneumovirus, Parainfluenza 3 with Rhino/Enterovirus, Metapneumovirus with Rhino/Enterovirus, and Metapneumovirus with Coronavirus OC43. Clinical studies are presently being conducted to determine whether dual respiratory virus infections carry an increased risk for adverse outcomes or increased hospital stays for pediatric and adult patients.

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