Research Article

The Epidemiology of Upper Respiratory Infections at a Tertiary Care Center: Prevalence, Seasonality, and Clinical Symptoms

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Improved multiplex PCR detection methods are facilitating the correlation of the etiology of respiratory tract infections with specific symptoms or clinical manifestations. We conducted a retrospective analysis of the incidence of respiratory pathogens and initial symptoms in 1,286 patients at a tertiary care center tested by multiplex respiratory pathogen PCR from July 1, 2012, to June 30, 2013. Rhinovirus/enterovirus (Rhino/Entero) infections were the most prevalent (25.4%) followed by respiratory syncytial virus (RSV) (13.6%) and influenza A (6.2%). Eleven percent of patients were positive for multiple analytes with Rhino/Entero and RSV being the most common combination. Asthma or asthma exacerbation was the most common presenting symptom in patients positive for Rhino/Entero (38.4%) or positive for Rhino/Entero along with RSV or hMPV (34.8%). Of the patients positive for Rhino/Entero and presenting with asthma, 97% were \( \leq 17 \) years of age. RSV positive patients most commonly presented with respiratory distress (40.3%) followed by asthma (18%) or pneumonia (18%). The most prevalent initial clinical manifestation for influenza was fever (27.4%) followed by respiratory distress (13%) or pneumonia (11.9%). The significant percentage of patients positive for Rhino/Entero virus presenting with asthma supports the role of rhinovirus as an important trigger for asthma exacerbation.

1. Introduction

Acute respiratory tract illnesses are the most frequent illnesses of humans and are an important cause of disability and days lost from school or work [1, 2]. Respiratory infections are an important cause of mortality and hospitalization, particularly in the winter in temperate regions. In children less than 5 years of age, they are the leading cause of death [3]. Most acute respiratory infections are caused by viruses and bacteria, including rhinoviruses, respiratory syncytial viruses, adenoviruses, influenza viruses, and parainfluenza viruses.

Many viruses have characteristic seasonal patterns. Influenza virus and respiratory syncytial virus (RSV) often contribute to the winter peak, but other respiratory viruses such as human metapneumovirus (hMPV), parainfluenza viruses (Para), and coronaviruses (CoronaV) also circulate in the fall and winter [4, 5]. Respiratory viruses such as adenovirus (Adeno) and rhinovirus cause illness year-round. An association between these specific respiratory agents and certain clinical syndromes has been shown to some degree [2]. Knowledge of the clinical signs and symptoms that are most predictive of the etiologic agent could help physicians more accurately diagnose and treat patients in a timely manner.

The recent increasing use of respiratory pathogen PCR amplification methods in studies of upper respiratory tract infections has provided new information on the epidemiology of respiratory tract infections and has contributed to a better understanding of the seasonality of these etiologic agents and their association with certain clinical manifestations [3, 6, 7]. This study explores the etiology, prevalence, seasonality, and clinical manifestations of upper respiratory tract infections at a tertiary care medical center using a rapid multiplex PCR respiratory pathogen panel.

2. Methods

Study Participants. Testing took place from July 1, 2012, to June 30, 2013, on nasopharyngeal specimens (NPS) from
1,286 patients that were originally sent to Medical College of
Georgia (MCG) Clinical Microbiology Laboratory (Augusta, GA) from the 500-bed adult and 154-bed pediatric hospital at MCG for respiratory pathogen PCR assay by the FilmArray Respiratory Panel (RP) (BioFire Diagnostics, Inc., Salt Lake City, UT). Nasopharyngeal specimens were obtained from patients with symptoms of a respiratory infection and collected from the patients according to standard technique and placed in viral transport media (Remel MicroTest M4RT Viral Transport tube). Specimens were tested as soon as possible after collection with an average time of 30 minutes. During high volume respiratory season the longest time between collection and assay run could be as long as 24 hours. The project was approved by the institutional review board of our institution; informed consent for the project was waived. Demographic data, such as chief complaint, age, and gender, was obtained for each specimen tested.

FilmArray RP Assay. The FilmArray assay was performed according to the manufacturer’s instructions. In brief, 1 mL of purified water included in the kit was injected into the FilmArray pouch to rehydrate the reagents. Then 300 μL of the viral transport media that had contained the NPS specimen was mixed with 500 μL of sample buffer and then injected into the sample port of the pouch. The pouch was then placed into the FilmArray instrument and a respiratory PCR Panel program was started. The first stage of the program consists of a multiplexed PCR, followed by an individual nested second-stage real-time PCR contained within a microarray chip. The FilmArray RP includes two internal controls: an RNA process control and controls for every step inside the pouch. Results are analyzed using melting curve data.

The organism/viruses detected by the FilmArray included adenovirus, influenza A (FluA), influenza B (FluB), parainfluenza virus 1 (Para 1), parainfluenza virus 2 (Para 2), parainfluenza virus 3 (Para 3), parainfluenza virus 4 (Para 4), respiratory syncytial virus (RSV), coronavirus 229E (CoronaV 229E), CoronaV NL63, CoronaV HKU1, CoronaV OC43, human metapneumovirus (hMPV), Bordetella pertussis, Chlamydia pneumoniae, and Mycoplasma pneumoniae. Due to genetic similarity between the human rhinoviruses and enteroviruses, a positive result with PCR primers to these viruses was listed as Rhino/Entero. The FluA viruses could also be subtyped as far as FluA/H1, FluA/H3, or FluA-2009 if present.

2.1. Statistics. A two-sample Student’s t-test between proportions was performed to determine whether there was a significant difference between the viruses with respect to the percentage of initial clinical symptoms in both single virus infections and mixed infections [8]. Statistical analysis was performed using the software package StatPac for Windows (Pepin, WI).

3. Results

3.1. Analysis of Positivity Rates and Prevalence within Age Groups. Specimens from a total of 1,286 patients were analyzed by PCR. The age range of the patients was 3 days to 95 years of age, average 14.6 years, median 4 years. The male:female ratio was 1.30. Rhino/Entero infections were the most prevalent (25.4%) followed by RSV (13.6%) and influenza A (6.2%) (Table 1).

The data was divided according to age groups. The largest age group in our study was that of children ≤2 years old with 558 patients (43%) followed by 7–21 age group with 257 patients (20%) and 3–6 age group with 201 patients (16%) (Figure 1). The older age groups, 22–49, and ≥50 years were smaller compared to the first two groups with 101 (8%) and 169 (13%) patients in each group, respectively.

The highest total rate of PCR positivity was observed in children 3–6 years of age with a rate of 72.1% (Figure 2) followed closely by 69.9% for the youngest age group, ages ≤2. The positivity rate for the older age groups 7–21, 22–49, and ≥50 decreased progressively with age, with positivity rates of 51.0%, 29.7%, and 29.6%, respectively. The overall positivity rate was 58.0% for one or more viruses.

The highest absolute number of positive cases for the different viruses and bacteria occurred in the ≤2 age group, except for FluB, in which the highest number of cases occurred in the 7–21 age group. Ninety-eight percent of RSV and 93% of Rhino/Entero cases were detected in patients 21 years old or less in age. Nine RSV cases were detected in the 22–49-year-old group. Three of the nine patients were coinfected with one other virus, namely, Rhino/Entero, Para 1, and CoronaV OC43. One RSV infection occurred in a 49 year old patient. Three infections occurred in the ≥50 age group. Eighty-three percent of FluA cases and 81% percent of hMPV cases were detected in patients 21 years old or less. Four cases of FluA were detected in the 22–49 age group and 10 in the ≥50 age group. Five cases of hMPV were detected in each of the 22–49 and ≥50 age groups. Seventy-five percent of CoronaV infections were detected in patients 21 years old or less with 21% of cases (11 patients) occurring in the ≥50 age group.

Figure 1: Age ranges of study subjects from July 1, 2012, to June 30, 2013, MCG.
Table 1: Prevalence of respiratory pathogens tested in different age groups: July 1, 2012, to June 30, 2013. Results of 1,286 nasopharyngeal samples analyzed by multiplex real-time PCR in relation to age group.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>≤2 years</th>
<th>3–6 years</th>
<th>7–21 years</th>
<th>22–49 years</th>
<th>≥50 years</th>
<th>All ages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positivity rate</td>
<td>Prevalence (n = 558)</td>
<td>Positivity rate</td>
<td>Prevalence (n = 201)</td>
<td>Positivity rate</td>
<td>Prevalence (n = 257)</td>
</tr>
<tr>
<td>Rhino/Entero</td>
<td>152</td>
<td>27.2%</td>
<td>75</td>
<td>37.3%</td>
<td>78</td>
<td>30.4%</td>
</tr>
<tr>
<td>RSV</td>
<td>141</td>
<td>25.3%</td>
<td>21</td>
<td>10.4%</td>
<td>9</td>
<td>3.5%</td>
</tr>
<tr>
<td>hMPV</td>
<td>24</td>
<td>4.3%</td>
<td>10</td>
<td>5.0%</td>
<td>8</td>
<td>3.1%</td>
</tr>
<tr>
<td>Para 1</td>
<td>9</td>
<td>1.6%</td>
<td>3</td>
<td>1.5%</td>
<td>3</td>
<td>1.2%</td>
</tr>
<tr>
<td>Para 2</td>
<td>7</td>
<td>1.3%</td>
<td>6</td>
<td>3.0%</td>
<td>1</td>
<td>0.4%</td>
</tr>
<tr>
<td>Para 3</td>
<td>30</td>
<td>5.4%</td>
<td>2</td>
<td>1.0%</td>
<td>6</td>
<td>2.3%</td>
</tr>
<tr>
<td>Para 4</td>
<td>7</td>
<td>1.3%</td>
<td>2</td>
<td>1.0%</td>
<td>1</td>
<td>0.4%</td>
</tr>
<tr>
<td>Parainfluenza total</td>
<td>53</td>
<td>9.5%</td>
<td>13</td>
<td>6.5%</td>
<td>11</td>
<td>4.3%</td>
</tr>
<tr>
<td>CoronaV 229E</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>CoronaV HKU1</td>
<td>1</td>
<td>0.2%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>CoronaV NL63</td>
<td>4</td>
<td>0.7%</td>
<td>2</td>
<td>1.0%</td>
<td>5</td>
<td>1.9%</td>
</tr>
<tr>
<td>CoronaV OC43</td>
<td>22</td>
<td>3.9%</td>
<td>3</td>
<td>1.5%</td>
<td>2</td>
<td>0.8%</td>
</tr>
<tr>
<td>Coronavirus total</td>
<td>27</td>
<td>4.8%</td>
<td>5</td>
<td>2.5%</td>
<td>7</td>
<td>2.7%</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>16</td>
<td>2.9%</td>
<td>5</td>
<td>2.5%</td>
<td>3</td>
<td>1.2%</td>
</tr>
<tr>
<td>Influenza A total</td>
<td>28</td>
<td>5.0%</td>
<td>22</td>
<td>10.9%</td>
<td>16</td>
<td>6.2%</td>
</tr>
<tr>
<td>FluA/H3</td>
<td>28</td>
<td>5.0%</td>
<td>19</td>
<td>9.5%</td>
<td>15</td>
<td>5.8%</td>
</tr>
<tr>
<td>FluA/2009 H1</td>
<td>0</td>
<td>0%</td>
<td>3</td>
<td>1.5%</td>
<td>1</td>
<td>0.4%</td>
</tr>
<tr>
<td>Influenza B</td>
<td>3</td>
<td>0.5%</td>
<td>6</td>
<td>3.0%</td>
<td>7</td>
<td>2.7%</td>
</tr>
<tr>
<td>C. pneumoniae</td>
<td>1</td>
<td>0.2%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>M. pneumoniae</td>
<td>4</td>
<td>0.7%</td>
<td>2</td>
<td>1.0%</td>
<td>1</td>
<td>0.4%</td>
</tr>
<tr>
<td>B. pertussis</td>
<td>1</td>
<td>0.2%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total positive: one or more analytes</td>
<td>390</td>
<td>69.9%</td>
<td>145</td>
<td>72.1%</td>
<td>131</td>
<td>51.0%</td>
</tr>
</tbody>
</table>
Figure 2: Positivity rate in different age groups from July 1, 2012, to June 30, 2013, MCG.

Figure 3: Prevalence of viruses detected in multianalyte positive specimens from July 1, 2012, to June 30, 2013, MCG. Percent of multianalyte positive samples positive for the specific analyte.

The percent positivity rate of the specific viruses within each age group, however, differed between the age groups (Figure 2). RSV, Para, and Adeno showed the highest prevalence in the ≤2 age group. Rhino/Entero and FluA virus showed the highest prevalence in 3–6 age group. Human metapneumovirus showed the highest prevalence in both 3–6 age group and 22–49 age group. Influenza B showed the highest prevalence in 22–49 age group. And CoronaV showed the highest prevalence in the ≥50 age group.

3.2. Analysis of Multianalyte Positive Samples. Fifty-eight percent of all specimens were positive for at least one viral or bacterial organism. Of the 746 positive specimens, 11% (83/746) had more than one analyte (Table 2). Nearly all (82/83) of the multianalyte positive samples were detected in patients ≤21 years of age. One patient, 68-year-old patient, described above, was positive for both RSV and M. pneumoniae. The most common viruses detected in multianalyte positive specimens were Rhino/Entero and RSV. Individually, Rhino/Entero and RSV were detected in all multianalyte positive samples, 57.8% and 50.6% of the time, respectively (Figure 3). There was one triple positive sample (Table 2).

Additional viruses and bacteria found in multianalyte positive samples included Para, hMPV, Adeno, FluA, CoronaV (NL63, OC43), and M. pneumoniae. The majority of the FluA/H3 and hMPV multianalyte positive samples were in conjunction with Rhino/Entero. Also the majority of the Para 3 multianalyte positive samples were in conjunction with Rhino/Entero. All M. pneumoniae infections were found
high throughout the year with several peak incidences, the highest occurring in August/September, with three minor peaks in December, February, and May. Two distinct peaks for Para 3 cases were observed in December and May (Figure 5). Para 1 cases peaked in October and November, and Para 2 cases peaked in November. Para 4 cases peaked in May simultaneously with Para 3. The 25 cases of Adeno occurred sporadically from September to June. Six cases of FluA H1 occurred in March and April.

### 3.4. Comparison of Initial Symptoms with PCR Diagnosis

The major initial clinical symptoms of the patients included asthma/asthma exacerbation, respiratory distress, fever, or bronchiolitis. The percentages of these initial symptoms for the more prevalent respiratory pathogens are presented in Figure 6.

For FluA or B, the most prevalent initial symptom was fever with 27.4% of patients presenting with this symptom. CoronaV positive patients also presented most often with fever (21.6%) followed by respiratory distress. Patients positive for Para presented with respiratory distress most commonly, followed by asthma symptoms.

RSV positive patients most commonly presented with respiratory distress (40.3%), which was significantly higher than FluA or B (13%), CoronaV (16.2%), and Para (23.2%) \((P < 0.05)\). The second most common presenting symptom was either asthma (18%) or pneumonia (18%).

For hMPV, the most prevalent initial symptom was pneumonia, which was a significantly higher incidence than CoronaV and Para (27.3%, \(P < 0.05\)). The second most prevalent initial symptom was either asthma (22.7%) or respiratory distress (22.7%).
Rhino/Entero positive patients initially presented most often with asthma symptoms (38.4%) followed by respiratory distress (31.9%). The percentage of Rhino/Entero positive patients with asthma was statistically significant when compared to FluA or B, CoronaV, Para, and RSV (P < 0.001). Mixed Rhino/Entero positive cases that were coinfected with RSV or hMPV also showed a significantly increased incidence of asthma when compared to FluA or B and CoronaV (34.8%, P < 0.05). Mixed Rhino/Entero positive with other respiratory pathogens, however, initially presented more often with respiratory distress (28%) followed by fever (20%) or asthma (20%) symptoms. Of the patients positive for Rhino/Entero with symptoms of asthma or asthma exacerbation, 97% (104/107) were ≤17 years of age and 58.9% were ≤6 years of age.

4. Discussion

Over the course of one year, 1,286 nasopharyngeal swab specimens were analyzed using a respiratory pathogen multiplex PCR. We observed characteristic seasonality with RSV, hMPV, FluA and B, CoronaV, and Para, typical for these viruses. As the age of the patient increased, the positivity rate for the PCR decreased proportionately, starting with a positivity rate of 69.9–72.1% for up to 6 years of age and ending with a 30% positivity rate for patients of 22 years or older. Other studies using multiplex PCR have reported that positivity rates decrease proportionately with the age of the patient [9, 10]. One study showed that older adult patients shed lower titers of viruses. The lower titers demand the use of a highly sensitive methodology such as RT-PCR over conventional culture or DFA [11]. The multiplex PCR assays now available, however, may still not be sensitive enough to detect the lower titers of virus shed by older adult patients.

Though Rhino/Entero was present throughout the year, there were peaks in the number of cases in September, January, March and May. The peaks in September, January, and March appear to correspond to the start of school sessions after school breaks. Peaks of rhinovirus illness have been well documented to occur after school starts in the fall and again in the spring [1, 7, 12].

RSV was the second most prevalent virus, with a very large number of cases in January, followed two months later by an increased number of cases of hMPV in March and May. This follows the pattern often predicted for hMPV, where the peak number of cases usually occurs after the RSV peak [13–17]. The majority of the RSV infections in our study occurred in children. However, hMPV infections were equally distributed among all age groups. We detected a positivity rate of 4.3–5.0% in children up to 6 years of age. This is consistent with other studies, in which hMPV was detected in 6% of hospitalized children and 7% of children in both outpatient clinics and emergency departments [18]. Human metapneumovirus has been known to cause severe respiratory infections in the elderly [19, 20].

The parainfluenza viruses have different patterns of seasonal variation based on type. Para 3, which can be predominant in spring in temperate climates, increased in both December and May in our study [21]. Both Para 1 and Para 2 cases were seen only in the fall months in our study, the typical season for these viruses [1]. The epidemiology of Para 4 is largely unknown but appeared in May alongside Para 3. The seasonality of the coronaviruses is often dependent on geographic location [22]. The number of coronavirus cases peaked in February in our study, indicating winter seasonality for the southeast region. Adenovirus caused illness year-round without any seasonality.

Influenza A started early in October, ended in March, and peaked early in December, compared to the overall peak for the US in late December. According to the CDC, the influenza A season was moderately severe, with influenza A (H3N2) viruses predominating [23]. From the week ending February 23, 2013, through the end of the season, the FluB was the most commonly reported influenza virus, similar to what was observed for the US overall.

Multiple respiratory pathogens were detected in 11% of the specimens with 57.8% of the multianalyte samples being positive for Rhino/Entero, 50.6% positive for RSV, and 20.5% positive for both. Rates of multiple-analyte positive specimens in other studies have also averaged around 8.7–10% [6, 10]. It has been hypothesized that many of the dual positive RSV and Rhino/Entero samples are due to viral shedding from a previous Rhino/Entero infection [24]. The positivity for Rhino/Entero may also be due to presence of the virus before symptoms appear. In one study, asymptomatic children were often found to be positive for rhinovirus by PCR and thought to be asymptomatic carriers [25].

We, however, observed that patients that were Rhino/Entero positive or Rhino/Entero positive along with RSV or hMPV positive were statistically much more likely to present with asthma. Rhino/Entero virus infections that were positive for other viruses or bacteria were more likely to present with respiratory distress rather than asthma. In one recent epidemiologic study, rhinovirus was the only virus type significantly associated with asthma exacerbations in children aged 2–17 years [26]. The type of respiratory virus linked to asthma often depends on the age of the patients. Many studies [27] indicate that, in children ≤2 years of age, RSV predominates, with parainfluenza and rhinovirus as lesser culprits. However, in older children and in adults, rhinovirus accounts for over 50% of viral triggered asthma exacerbations [28].

Our study found fever symptoms to be significantly higher in patients positive for influenza than any other virus. It is not surprising that fever is the single most common presenting symptom for influenza. Numerous studies have shown that when influenza is circulating within the community, patients with fever and cough within 48 hours of onset of symptoms are likely to have influenza [29–33].

Possible limitations of the study include PCR identification of an agent that is not necessarily the agent responsible for the illness. Rhinovirus is often found at a higher frequency than any other virus in asymptomatic patients [25]. A control group in which random samples were collected from asymptomatic patients was not included in the study. Also since the study was based at a tertiary care facility, the results will be skewed toward the detection of pathogens more often
associated with severe illness and possibly with the presence of other chronic conditions, especially in adults.

5. Conclusions

In summary, we observed characteristic seasonality with RSV, hMPV, FluA and B, CoronaV, and Para, typical for these viruses. Cases of FluA and RSV peaked in December, followed by CoronaV in February, hMPV in March, and FluB in April. Parainfluenza viruses peaked in fall, winter, and spring, with Para 1 and Para 2 seen in fall and winter and Para 3 and Para 4 in spring. Rhino/Enterovirus was present throughout the year, demonstrating peaks in September, January, March, and May.

Asthma was the most common presenting symptom in patients positive for Rhino/Enterovirus or positive for Rhino/Enterovirus along with RSV or hMPV. This observation supports the role of rhinovirus as an important trigger for asthma exacerbation. RSV positive patients most commonly presented with respiratory distress followed by asthma or pneumonia. The most common initial clinical manifestation for influenza was fever followed by respiratory distress or pneumonia.

Multiplex PCR has the distinct advantage of allowing for the ability to quickly and specifically diagnose and treat upper respiratory tract infections. Rapid and accurate molecular diagnostic testing methods enhance clinical decision making and promote the implementation of cost-effective treatment strategies, including limiting the use of antibiotics. Active PCR surveillance can also help predict when seasonal viral epidemics will occur, facilitating the rapid institution of seasonal variations of virus infections, Expert Review of Anti-Infective Therapy, vol. 9, no. 8, pp. 615–626, 2011.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References


