Epidemiology and phylogenetic analysis of respiratory viruses from 2012 to 2015 – A sentinel surveillance report from union territory of Puducherry, India

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A R T I C L E   I N F O

Keywords:
Influenza virus
Epidemiology
Surveillance
Phylogenetic analysis

A B S T R A C T

Background: Acute respiratory infections (ARI) are the most common illnesses affecting people of all ages worldwide. Viruses contribute to 30–70% of acute respiratory infections. Identification of these respiratory viruses is not given high priority except influenza; however, the knowledge about prevalence of non-influenza viruses, their seasonal pattern and genetic evolution have significant epidemiological value.

Methods: As a part of National Influenza-like illness surveillance programme, respiratory specimens were collected from children and adults with symptoms of ILI or ARI, between January 2012 and March 2015 (including SARI cases). Real-time PCR was done to identify 13 respiratory viruses. Sequencing was done for representative isolates of each virus using ABI 3730 Genetic Analyzer.

Results: During the study period between January 2012 and March 2015, a total of 648 patients with symptoms of ARI were included in this study. The mean age of the patients was 20.2 years (SD = 19.13, median = 18); 292 (45.1%) were children (≤13 years) and 356 (54.9%) were adults. Respiratory viruses were identified in 44% (287/648) of all patients. Influenza accounted for the maximum number of cases- 179/648 (27.6%). Among the non-influenza viruses, RSV predominated with 34 cases (5.2%), followed by HMPV 24 (3.7%) and PIV-3 20 (3%). Four patients died due to INF A/H1N1 (2012-2, 2015-2) as a result of acute respiratory distress syndrome (ARDS) (CFR 3.7%). Among the non-influenza viruses, no particular seasonality pattern was observed over the different months of the study period.

Conclusion: Antibiotic usage in treating acute respiratory infections empirically is not justified as nearly half of ARI are due to viruses; nearly 28% of them were due to influenza viruses. Among the non-influenza viruses, RSV predominated, followed by HMPV. This study is based on an active influenza surveillance initiated after 2009 pandemic influenza outbreak, in the Union territory of Puducherry which has contributed significantly to the knowledge of the burden of influenza and non-influenza viruses among children and adults. Such surveillance network has paved the way for better diagnosis and timely therapeutic interventions.

1. Introduction

Acute respiratory infections (ARI) are the most common illnesses affecting people of all ages worldwide. Viruses contribute to 30–70% of acute respiratory infections. Influenza viruses are one of the leading causes of respiratory infections, with an annual global attack rate of about 5–10% in adults and 20–30% in children and it is known to cause severe respiratory illness and death in high-risk individuals. Although anti-influenza drugs and vaccines are available, due to the continuous antigenic variations (in the form of drifts and shifts), this virus can lead to yearly epidemics and occasional pandemics; hence influenza remains a global threat. The next common virus associated with significant hospitalization and respiratory distress, particularly in children, is the respiratory syncytial virus (RSV). Nearly 70% of children are infected with RSV by the age of 1 year. Other common viruses are parainfluenza viruses, coronaviruses, human metapneumovirus, measles, adenoviruses and bocavirus. Identification of these respiratory viruses is not given high priority as they mostly cause self-limiting disease and no specific antiviral drugs are available (except for influenza and RSV). However, the knowledge about prevalence of non-influenza viruses, their seasonal pattern and genetic evolution have significant epidemiological value.

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As a part of National Influenza surveillance programme, influenza surveillance was initiated in the Union territory of Puducherry/Pondicherry in 2008, under National center for disease control (NCDC) and Integrated Disease Surveillance Programme (IDSP). In 2009, Regional Influenza Laboratory was set up in JIPMER hospital and till date surveillance activity is continued for identifying influenza viruses in patients with ARI. This study discusses the burden of respiratory viruses in patients with ARI from Union territory of Puducherry as a part of a hospital-based surveillance program between 2012 and 2015, the seasonal pattern of respiratory viruses over these three years and the phylogenetic analysis of representative viral strains.

2. Materials and methods

2.1. Geographic details of union territory of Puducherry

The Union Territory of Puducherry/Pondicherry is in Southern India and lies at a latitude of 11°46′ to 12°30′ North and a longitude of 79°36′ to 79°52′ East. The territory lies on the Coromandel Coast of Bay of Bengal extending over 479 Sq. km. The total population is 12, 44, 464 (2011 census). It has a hot and humid climate with an average maximum temperature of 31.5 °C and average minimum temperature of 23.9 °C. March to July - summer, December to February - winter and September to December - monsoon.

2.2. Study design

Apart from routine influenza testing, according to the guidelines of National Influenza-like illness surveillance programme, respiratory specimens (nasal/throat/nasopharyngeal swabs/tracheal aspirates) were collected from the first 5 patients (children and adults) with symptoms of ILI or ARI attending the outpatient care facilities in the three sentinel centres; Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) Hospital, Puducherry, Indira Gandhi Government General Hospital (IGGH), Puducherry and Government General Hospital (GGH), Karaikal (on designated days of the week, weekly once for each of the 3 centres). In addition, respiratory samples were collected from hospitalized patients with SARI. This descriptive study was conducted in Union territory of Puducherry between January 2012 and March 2015 for the period of three years. Patients (both children and adults) who are residents of Union territory of Puducherry attending the outpatient care facilities of our hospital with symptoms of ILI or ARI and inpatients admitted for severe acute respiratory illness (SARI) were included in the study. Patients with a history of ARI/ILI in the preceding 30 days were excluded. Sample size was calculated using OpenEpi version 3.03a (Frequency of outcome factor to be 30% & absolute precision of 5% and CI of 99.9%) and 648 patients were included in the study. Patient information including age, gender, clinical presentation, underlying conditions, outcome, travel and treatment details were recorded using a structured proforma. The study protocol was approved by the Institutional Human Ethics Committee. Written, informed consent was obtained from patients/guardians before specimen collection.

2.3. Viral nucleic acid extraction

Samples were collected using flocked nylon swabs in Hi-viral transport medium (HiMedia, Mumbai, India) and immediately transported to the laboratory on ice. Samples were stored at −80 °C until further use. QIAamp viral RNA/DNA extraction method (Qiagen, Germany) was used as per manufacturer’s instructions and the viral nucleic acid was stored at −80 °C.

2.4. Real-time PCR

The Real-time PCR reactions were done in Step one real-time system (Applied Biosystems, USA) as one-step reverse transcriptase PCR for RNA or DNA PCR using AgPath-IDTM One Step RT-PCR kit. The primers and probes for influenza A(H1N1)pdm09 were designed based on the CDC protocol of real-time RTPCR for influenza A(H1N1). The primers and probes for other respiratory viruses were designed based on published literature: Influenza A(H3N2) & Influenza B, Respiratory syncytial virus (RSV), Human parainfluenza virus (HPIV) type 1, 2, 3, human coronavirus (hCoV) - 229E & OC-43, Measles virus, Adenovirus, Human bocavirus (HBoV) & Human metapneumovirus (HMPV). The quality of specimen collection was checked by testing all the samples for internal control, RNase P and positive, negative controls were included for all the tests.

2.5. Seasonality and phylogenetic analysis

Seasonality of respiratory viruses was plotted using the meteorological (temperature and rainfall) data of Puducherry for the period between November 2011 and March 2015 obtained from the Regional Meteorological Centre, Chennai [No. 8043/CS-(ER)-032 dated 30-07-2015]. Sequencing was done for representative isolates of each virus using ABI 3730 Genetic Analyzer (Applied Biosystems, USA) employing gene-specific forward and reverse primers of different genes of respiratory viruses. Most identical nucleotide sequences available in the sequence database were identified through NCBI BLAST program (http://blast.ncbi.nlm.nih.gov/blast.cgi). Sequences submitted from various countries were retrieved from the GenBank database and aligned; the evolutionary history was estimated using the Neighbor-Joining method. Evolutionary analyses were conducted in MEGA6. Genbank accession numbers of the study isolates are the following: KM281807, KM406324, KR347116, KR376140, KR608301, KR902754, KT002006-2008, KR704265, KX255651, KX268229, KX446979-6985, KX426263-6266.

2.6. Statistical analysis

Data was represented as proportions or percentages. Statistical analysis was performed using GraphPad InStat software. All the categorical variables were tested using Fisher’s exact t-test or Chi-square test. P-value of < 0.05 was considered statistically significant.

3. Results

3.1. Study population

During the study period between January 2012 and March 2015, a total of 648 patients with symptoms of ARI were included in this study (Table 1). The mean age of the patients was 20.2 years (SD = 19.13, median = 18); 292 (45.1%) were children (≤ 13 years) and 356 (54.9%) were adults. Male: female ratio was 1:1.1. About 32.5% (211) developed a severe respiratory disease and were hospitalized and 67.4% (437) had mild respiratory symptoms.

3.2. Viral etiology of ARI

A total of 648 respiratory samples from an equal number of patients were collected during the study period (November 2011–March 2015) and real-time PCR identified respiratory viruses in 44% (287/648) of all patients (Table 2). Influenza accounted for the maximum number of cases- 179/648 (27.6%); 109 (16.8%) cases of INF A/H1N1, 42 (6.5%) cases of INF B and 28 (4.3%) cases of INF A/H3N2. Among the non-influenza viruses, RSV predominated with 34 cases (5.2%), followed by HMPV 24 (3.7%) and PIV-3 20 (3%). Measles, adenovirus, parainfluenza virus type 1 & 2, human coronavirus OC43, human coronavirus 229E and human bocavirus were aggregated into a single group (OTHER) due to small numbers and to enable comparison statistically. Burden of respiratory viruses is given in (Table 3).
3.3. Co-infections

Thirteen patients had co-infections with more than one virus; there were two cases with RSV-HMPV co-infections. A single case each of the following co-infections were also found: (RSV-INF A/H1N1), (RSV-INF B), (HMPV-INF B), (HMPV-PIV-3), (HMPV-adenovirus), (measles-INF B), (PIV-1-PIV-2), (PIV-1-adenovirus), (PIV-2-INF A/H3N2), (PIV-3-INF A/H1N1), and (PIV-3-hCoV-OC43). Five patients with co-infections had severe respiratory symptoms requiring hospitalization (Highlighted in Bold).

3.4. Respiratory virus distribution according to age

Respiratory virus infections were common among adults (171/356, 48%) compared to children (129/292, 44%), but the proportion difference was not statistically significant (P = 0.34) (Fig. 1). INF A/H1N1, INF B, HMPV and PIV-3 were more common in adults, while RSV was common in children, but the proportional differences were only significant for INF A/H1N1 and RSV (P = 0.0001). Children were more commonly infected with OTHER viruses (PIV-1&2, adenovirus, measles, measles-INF B, (PIV-1-PIV-2), (PIV-1-adenovirus), (PIV-2-INF A/H3N2), (PIV-3-INF A/H1N1) and (PIV-3-hCoV-OC43). Five patients with co-infections had severe respiratory symptoms requiring hospitalization (Highlighted in Bold).

3.5. Burden of respiratory viruses in patients with ARI (n = 648).

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Viruses</th>
<th>Terms used in study</th>
<th>Total positive cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INF A/H1N1pdm09</td>
<td>INF A/H1N1</td>
<td>109 (16.8%)</td>
</tr>
<tr>
<td>2.</td>
<td>INF B</td>
<td>INF B</td>
<td>42 (6.5%)</td>
</tr>
<tr>
<td>3.</td>
<td>Respiratory syncytial virus</td>
<td>RSV</td>
<td>34 (5.2%)</td>
</tr>
<tr>
<td>4.</td>
<td>Seasonal Infuenza A(H3N2)</td>
<td>INF A/H3N2</td>
<td>28 (4.3%)</td>
</tr>
<tr>
<td>5.</td>
<td>Human metapneumovirus</td>
<td>HMPV</td>
<td>24 (3.7%)</td>
</tr>
<tr>
<td>6.</td>
<td>Parainfluenza virus type 3</td>
<td>PIV-3</td>
<td>20 (3%)</td>
</tr>
<tr>
<td>7.</td>
<td>Measles virus</td>
<td>9 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Adenovirus</td>
<td>8 (1.2%)</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Parainfluenza virus type 1</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Human coronavirus OC-43</td>
<td>OTHER</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>11.</td>
<td>Parainfluenza virus type 2</td>
<td>5 (0.8%)</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Human coronavirus 229E</td>
<td>4 (0.6%)</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Bocavirus</td>
<td>3 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>Total number of viruses/no. of patients with viral ARI</td>
<td>300/287</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients negative for all viruses tested</td>
<td>361/648 (56%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#mo.-months, yr-year.
corona virus or bocavirus) compared to adults (8.2% vs. 5.3%). Maximum positivity of INF A/H1N1 was found in age group of 21–50 years (Fig. 2). Of the 109 patients with INF A/H1N1 infection, 76% (83/109) had their residence in Puducherry town and 23% (26/109) in Karaikal (Fig. 3).

3.4.1. Clinical features and mortality

Of the 648 cases, 211 patients were diagnosed with lower respiratory tract infections (LRI) while the remaining 437 patients had upper respiratory tract infections (URI). Clinical symptoms were not distinct in influenza and non-influenza infections, however, in influenza ARI cases, fever, myalgia and respiratory distress were found to be significantly higher than non-influenza viral ARI cases whereas runny nose and nasal congestion was more often associated with non-influenza cases (Table 4).

INF A/H1N1 was identified in 19% (41/211) of the patients
hospitalized due to SARI (Fig. 4); RSV in 8.5% (18/211) (figure). Of 292 children with ARI, 34% (100/292) required hospitalization; 6% (17/292) were hospitalized due to RSV infection which presented as single infection or co-infection. In adults, 31% (111/356) required hospitalization; 6% (17/356) were admitted due to single or mixed INF A/H1N1 infection. In this study, total of 12 pregnant females with ARI (11-2012, 1-2015) were tested and 3 were positive for INF A/H1N1 virus or co-infection. In this study, total of 12 pregnant females with ARI (11-2012, 1-2015) were tested and 3 were positive for INF A/H1N1 infection. Among these, 3 were from Karaikal.

Myalgia 97 (54) 35 (29) 2.9 (1.2)
Sore throat 121 (68) 72 (60) 1.4 (0.8)
Cough 131 (73) 79 (65) 4.7 (2.0)
Nasal congestion 38 (21) 39 (32) 0.5 (0.3)
Wheezing 23 (13) 11 (9) 1.4 (0.8)
Runny nose 40 (22) 43 (36) 0.5 (0.3)
Dyspnea 64 (36) 33 (27) 1.4 (0.8)
Respiratory distress 39 (22) 6 (5) 5.3 (2.1–13.0) 0.001

* chi-square test.

3.4.2. Timing of sample collection and viral positivity

Of the total 648 samples collected from patients with ARI, 299 (46%), 314 (48%), 35 (5%) were collected during the first 3 days, 4–7 days and >7 days of symptoms onset respectively. About 52% (157) of the samples obtained during the first 3 days of symptoms onset were positive for one or more viruses (Fig. 5). The viral positivity rate reduced by 9% if the samples were collected between 4 and 7 days of symptoms onset (P = 0.03) and 35%, if samples were collected after 7 days (P = 0.0002). Timing of sample collection and individual viral positivity is given in Fig. 6.

3.5. Phylogenetic analyses

A few representative samples positive for respiratory viruses (Influenza B, Influenza A(H3N2), RSV, HMPV, HPV-3, coronaviruses, measles, bocavirus) were sequenced and phylogenetic trees were constructed to compare with other Indian and foreign strains. Genetic sequences of Influenza A(H1N1)pdm09 isolates are described in chapter 3.

Influenza B strains isolated from Union territory of Puducherry during 2011, 2012 and 2015 belonged to Victoria lineage and clustered with the current vaccine strain, Influenza B/Brisbane/60/2008, along with strains from India (Mumbai, Jammu Kashmir, Rajasthan and Manipur), Riyadh, and North Carolina (Fig. 7). One of the influenza B isolate, KX446983, collected in 2013, belonged to Yamagata lineage and within that lineage; it formed a separate cluster along with other Indian strains from Haryana, Goa, Delhi and Chhattisgarh. One of the two influenza A(H3N2) strains (KX446985), sequenced, grouped separately along with an isolate reported from Assam (Fig. 8).

All the RSV strains sequenced belonged to type A and they formed a separate subgroup along with strains isolated from other parts of India like Chennai and Maharashtra (Fig. 9). Two HMPV strains isolated in the same year, 2011, were entirely distinct from each other, forming two separate subgroups (Fig. 10). One of the HPV-3 strains, KR704265, isolated in 2013 was closely related to the strain from the neighboring region Chennai which was isolated in the same year, while the other strain KR902754 had a molecular composition similar to isolates from Lithuania, Australia and Seattle (Fig. 11). No Indian study has analyzed HCoV-229E virus and the strains isolated from this study were genetically similar to strains reported from Germany, Malaysia, Japan and Netherlands (Fig. 12). Similarly, no study from India has analyzed the genetic sequence HCoV-OC43 and single isolate sequenced in our study showed a close relationship with a strain from the United Kingdom and the United States (Fig. 13). The measles strains were of genotype D8 and closed circulation of measles strains between Pondicherry and other parts of the neighboring state, Tamil Nadu was observed. These strains were distinct from measles strains of Spain, Moscow, South Africa and it formed a separate subgroup along with other Indian

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Influenza ARI cases (179), n (%)</th>
<th>Non-influenza viral ARI cases (121), n (%)</th>
<th>OR (95% CI)</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>176 (98)</td>
<td>112 (93)</td>
<td>4.7 (1.2–17.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cough</td>
<td>131 (73)</td>
<td>79 (65)</td>
<td>1.4 (0.8–2.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Sore throat</td>
<td>121 (68)</td>
<td>72 (60)</td>
<td>1.4 (0.8–2.2)</td>
<td>0.18</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>64 (36)</td>
<td>33 (27)</td>
<td>1.4 (0.8–2.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>Runny nose</td>
<td>40 (22)</td>
<td>43 (36)</td>
<td>0.5 (0.3–0.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Myalgia</td>
<td>97 (54)</td>
<td>35 (29)</td>
<td>2.9 (1.7–4.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Wheezing</td>
<td>23 (13)</td>
<td>11 (9)</td>
<td>1.4 (0.6–3.1)</td>
<td>0.41</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>38 (21)</td>
<td>39 (32)</td>
<td>0.5 (0.3–0.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Headache</td>
<td>14 (8)</td>
<td>15 (12)</td>
<td>0.5 (0.2–1.2)</td>
<td>0.26</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>9 (5)</td>
<td>9 (7)</td>
<td>0.6 (0.2–1.7)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

* chi-square test.
strains (Fig. 14). Human bocavirus strains of this study were not closely related to other Indian strains, particularly one isolate, KR376140, isolated in 2012 showed a distinct lineage (Fig. 15).

3.6. Seasonality of viral respiratory infection

In Union territory of Puducherry, summer is from March through July and winter from December through February. September to December is the rainy months in this area. During this study, two outbreaks of influenza A(H1N1)pdm09 occurred; first one in 2012 (58 cases) and the next in 2015 (50 cases). Seasonal influenza A(H3N2) and influenza B cases were found in all years, with a slight increase in 2013 and 2015 respectively. The detailed seasonality of influenza viruses in this region has been described in our earlier study on “Epidemiology of influenza viruses from 2009 to 2013.” Among the non-influenza viruses, no particular seasonality pattern was observed over the different months of the study period; however, more number of RSV cases was observed during the rainy and winter season between August and January, while HMPV and PIV-3 were found more during two summer months; April and May (Fig. 16).

4. Discussion

Respiratory viral infections are under-diagnosed due to non-specific clinical symptoms, self-limiting nature of the disease and expensive cost of testing. Literature indicates that clinicians prescribe antibiotics for ARI 40–50% of the time. It is a well-known fact that inappropriate use and abuse of antibiotics for the treatment of ARI has played a significant role in worsening the global problem of antimicrobial resistance. Through this study, we reiterate the fact that nearly half the
ARI cases (44%) could be viral, as reported in many previous studies\textsuperscript{1,13–17} and all the cases do not require antibiotic therapy.

The most common virus detected in this study was influenza A(H1N1)pdm09, with a prevalence of 17%. Globally, influenza A(H1N1)pdm09 virus has been reported to cause 10–30% of ARI.\textsuperscript{13,17–19} In India, over 20,000 people were affected by the pandemic and over 1700 people died by the end of 2010 influenza A(H1N1) pdm09 outbreak.\textsuperscript{20} Although WHO announced the end of the pandemic in August 2010, influenza viruses cause significant morbidity during the annual outbreaks. During this prospective study, we observed two influenza A(H1N1)pdm09 outbreaks, one each in 2012 and 2015 with laboratory-confirmed infection in more than 100 patients altogether. During this study, all the 109 cases with influenza A(H1N1)pdm09 infection, were treated with oseltamivir, despite this, four patients died due to ARDS (Acute respiratory disease syndrome).

The case fatality rate of influenza A(H1N1)pdm09 (3.7%) reported in this study (2011–2015) was much lower when compared to 2009–2010.\textsuperscript{11} Generally, the impact of pandemic influenza in this region was relatively lower when compared to many Indian and foreign states.\textsuperscript{21–24} This could be due to the continuing influenza surveillance activities in this region from 2008, ensuring rapid detection of influenza, timely admission and prompt initiation of prophylaxis in the suspected cases. Patients affected by influenza A(H1N1)pdm09 were mostly of age group 20–40 years; hence this study yet again corroborates this well-known observation made worldwide. This might be due to their active lifestyle environment, where they are at a higher risk of getting infected with this novel influenza A(H1N1) virus, to which they have never been exposed previously.\textsuperscript{25}
The second most common virus identified in this study population was Influenza B virus (6.5%); however, the proportion was much lower compared to a recent survey from East India, where Influenza B was reported as the leading cause of ARI with 12% prevalence.13 A recent report from India attributed 40% hospitalizations in children to influenza B infections,19 however, in our study population, influenza B infection was predominantly found in patients aged 30–50 years and was mild. Seasonal influenza A(H3N2) virus also caused mild respiratory infection in < 5% of the study population; mostly in children aged 0–5 years due to their non-exposure to this virus previously.

In children, viruses are the most common cause of respiratory infections and they are considered a significant health threat in this group. In the present study, of the 292 children with ARI, 44% (129) had one or more viruses and RSV was the most common cause of infection particularly in 0–5 year-old children. Worldwide, RSV predominance in children has been reported26 and the same scenario exists.
in India. RSV is known to co-infect with HMPV and influenza viruses; we observed 3 mixed RSV infections, but no clinical correlation could be made due to the low frequency of the same. After the first Indian report of HMPV appeared in 2004,27 many studies from Lucknow, Vellore and New Delhi have identified it as the second most common cause of ILI in children, next only to RSV.1,28,29 In this study, the prevalence of human metapneumovirus (HMPV) was 3.7%; the predominant group affected was age 14–30 years. This adult preponderance to HMPV is no more a rare phenomenon as recent reports indicate the severity of HMPV in adults’ particularly in elderly leading to hospitalization.30,31

The proportion of other respiratory viruses like measles, adenovirus, coronaviruses and bocavirus was low in this study population. This is the first study to report HCoV OC43 from India, and this is only the second Indian study to document HCoV229E. These two coronaviruses have been shown to affect children causing pneumonia and croup.
leading to hospitalization.\textsuperscript{32} Coronaviruses are more frequently reported from East and South East Asian countries like China, Hong Kong, Singapore,\textsuperscript{11} but in India, the frequency remains low\textsuperscript{13}; this was confirmed in this study by their prevalence of < 1%. Even during the 2012 outbreak of Middle East Respiratory Syndrome-CoV (MERS-CoV), which caused nearly 487 deaths worldwide,\textsuperscript{35} there were no cases reported from India, although one Mumbai resident, returning from the Middle East in August 2013, was suspected but tested negative.\textsuperscript{36}

Clinical features of both influenza and non-influenza viruses were similar and non-specific except for a few symptoms like fever, myalgia and respiratory distress, which were more often associated with influenza;\textsuperscript{37} however, this may not be useful for clinical diagnosis as > 25% respiratory infections are associated with febrile illness.\textsuperscript{37,38} Viral ARI is initiated with mild respiratory symptoms which are likely to be neglected in the first three to five days; during when the maximum virus shedding occurs. This has been well demonstrated in the pathogenesis of influenza and RSV infection\textsuperscript{39,40} and it is emphasized to collect samples in the first 3 days of symptoms onset. This study finding also suggests the importance of sample collection within 3 days of symptoms onset to achieve the maximum viral detection rate. Apart from the timing, the other important aspect of sample collection is the type of sample collected. In this study, we used nasopharyngeal sample collected using flocked nylon swabs, which have been shown to have excellent sensitivity when compared to rayon swabs, and is considered as an alternative to nasopharyngeal aspirate sampling.\textsuperscript{41,42}

A specific pattern in the seasonality of respiratory viruses (other than influenza) was not appreciated in this study as their prevalence was low in this population. In tropical countries, the seasonal pattern of respiratory viruses is reported to be less evident due to the local variations in precipitation, temperature and humidity. This can be demonstrated from the varied seasonality data reported from different parts of India by Chadha et al.\textsuperscript{43} In our study from the southern part of India, the maximum ARI cases were recorded during the months between August and December, except in 2012 and 2015. In both these years, a surge of suspected ARI cases was observed during the early months of these years, due to the outbreak of influenza A(H1N1) pdm09. As described in our earlier studies, both these outbreaks occurred during high mean maximum temperature period of these years and we found no correlation with rainfall.\textsuperscript{11}

This is the first Indian study to submit gene sequences of HCoV OC43 and 229E in the Genbank database, and hence, no intra-national evolutionary comparisons could be made. A few studies have reported human bocavirus (HBoV), but the strains isolated from Union territory of Puducherry, were not closely associated with other Indian strains. However, conclusive information could not be made with single gene sequencing. Co-circulation of RSV-A and RSV-B has been observed in many countries including India\textsuperscript{44,45} but RSV-B is considered as the predominant type in Western part of India.\textsuperscript{46} However, in this study, all the RSV isolates sequenced, belonged to type A. Similar observation of exclusive RSV-A circulation have been reported from North East India.\textsuperscript{49}

In our study, both Yamagata and Victoria lineage strains of influenza B were found in this region, similar to other reports of co-circulation of both the lineages from India, China and Singapore.\textsuperscript{50–52} Globally, Victoria lineage of influenza B viruses is considered the predominant type of influenza B viruses\textsuperscript{43} and the current influenza vaccine contains only the Victoria-like lineage virus (B/Brisbane/60/2008). However, previous studies conducted inhumans have proved that this vaccine could not provide immunity against the Yamagata lineage viruses\textsuperscript{43} and hence recently, CDC has approved the usage of Quadrivalent vaccine with both the types of influenza B viruses.\textsuperscript{53}

5. Conclusion

To conclude, we reconfirm that, antibiotic usage in treating acute respiratory infections empirically is not justified as nearly half of ARI are due to viruses; nearly 28% of them were due to influenza viruses. Among the non-influenza viruses, RSV predominated, followed by HMPV. This study is based on an active influenza surveillance initiated after 2009 pandemic influenza outbreak, in the Union territory of Puducherry which has contributed significantly to the knowledge of the burden of influenza and non-influenza viruses among children and adults. Such surveillance network has paved the way for better diagnosis and therapeutic interventions.

Sample credit author statement

Nandhini: Conceptualization, Acquisition of data, Methodology, analysis and interpretation, Original draft preparation, Writing- Reviewing and Editing, Sujatha: Data curation, Investigation, Supervision.

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