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*Coronavirus Pandemic*

# Paradigm shift of respiratory viruses causing lower respiratory tract infection in children during COVID-19 pandemic in India

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## Abstract

Introduction: Acute lower respiratory tract infections (ALRTIs) are the commonest cause of mortality in children mostly attributed to respiratory viruses. During the coronavirus disease 2019 (COVID-19) pandemic, the dynamics and transmission of infections changed worldwide due to widespread public health measures. This study aimed to understand the pattern of respiratory viruses associated with ALRTIs in children pre and during COVID-19 pandemic in India.

Methodology: Respiratory samples were collected from ALRTI patients during pre-pandemic period (October 2019 to February 2020; n = 166), Delta (July 2021 to December 2021; n = 78) and Omicron wave (January 2022 to July 2022; n = 111). Samples were screened for Influenza (Inf) A pdmH1N1, InfA H3N2, InfB, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), human bocavirus (hBoV), human rhinovirus (hRV), and parainfluenza virus (PIV-2 and PIV-3) by nucleic acid amplification techniques (NAATs).

Results: Significantly higher proportion of children with ALRTIs had virus/es isolated during pre-pandemic period than during mid-pandemic period [78.9% (131/166) vs. 52.9% (100/189); $p < 0.001$). RSV positivity was significantly higher (51.2%) in pre-pandemic period than 10.3% and 0.9% during the Delta and Omicron waves respectively. No significant difference in positivity rate of Inf A pdmH1N1, Inf A H3N2 and Inf B was seen. The increase in positivity of hRV (39.2% vs 42.3% vs 56.8%) and hBOV (1.2% vs 5.1% vs 9%) was documented in pre-pandemic, delta wave and omicron wave respectively.

Conclusions: The COVID-19 pandemic significantly impacted the frequency and pattern of respiratory viruses among hospitalized children with ALRTIs in India.

**Key words:** SARS-CoV2; pneumonia; RSV; hMPV; hBOV.

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## Introduction

Acute respiratory infections (ARIs) are one of the most frequent causes of pediatric consultations [1]. Majority of the infections with mild illness belong to the upper respiratory tract, whereas acute lower respiratory tract infections (ALRTIs) frequently lead to severe illness [acute viral bronchiolitis (AVB) and pneumonia] requiring hospitalization. ALRTIs are estimated to be responsible for nearly 2.3 million deaths worldwide, ranking as the sixth top cause of death across all age groups and the leading cause of death of children under the age of five [2]. Viruses are the predominant etiological agents for ALRTI [3]. Studies on community acquired pneumonia (CAP) attributed respiratory syncytial virus (RSV), influenza virus, parainfluenza virus (PIV), human metapneumovirus (hMPV), and human rhinoviruses (hRV) as the cause of nearly 80% of infections in children below 2 years of age [4].

Infections due to RSV and influenza viruses commonly occur during the winter months. In Europe, RSV accounts for nearly 40% of hospital admissions due to ARIs in children < 2 years of age [5]. In India, RSV associated ALRTI rates in the community for 0-11 months of age was 22.4 (18.6-27.0)/1000 children per year and amongst the hospitalized patients, the rate was 14.1 (11.1-17.8)/1000 children [6]. In India there are nearly 16 million influenza cases among children less than 5 years of age [7] with estimated annual influenza-associated respiratory mortality rates in < 5

years age group at 9.8 (95% CI = 0-21.8) deaths/100,000 population [8]. PIV (types 1 to 4) are known to cause ARIs in the pediatric population. PIV-3 infections are often associated with severe manifestations including AVB and pneumonia [9]. A study by Sumit Bhardwaj *et al*. screened PIV1-4 in 9613 symptomatic patients in pre-pandemic years between January 2017 and March 2020 and revealed a positivity rate of PIV1-4 in 3.2% of the samples [10]. hMPV causes ALRTIs in young children with a disproportionately higher mortality in infants 0-5 months of age and accounts for approximately 58% of hospital admissions and 71% of in-hospital deaths in children under 5 years of age [11]. A prospective study from India detected hMPV in 3% of the respiratory samples from children with ARI and 33% hMPV infected children required hospital admission for management of pneumonia or bronchiolitis [12]. A study in North India identified hBOV positivity in 7.2% of ARI patients [13], however, in South India hBOV was detected in 2/300 children with ARI [14].

In March 2020, the World Health Organization (WHO) declared the coronavirus disease 2019 (COVID-19) as a pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [15]. To prevent the spread of infection throughout the world, numerous infection control and public health initiatives were put in place, including social segregation, stay-at-home directives, school and office closures, travel bans, border closures, and numerous other non-pharmaceutical interventions (NPIs), such as the use of masks and good hand hygiene. These interventions impacted the pattern and spread of other viruses as well. Sharma *et al*. described a drastic reduction in dengue cases in 2021 by 55–65% with the advent of COVID-19 wave across the globe and associated the same with the alteration of human behavioral practices [16]. In addition, there was an 80% reduction in tuberculosis incidence in India as a result of COVID-19 lockdown [17]. Jesus *et al*. noted a reduction > 70% in hospital admissions of infants with AVB [18]. Yeoh *et al*. have demonstrated a substantial reduction of RSV and influenza positivity during the pandemic period in comparison to pre-pandemic period. RSV and influenza positivity dropped from 23%-30% and 3.6%-16% in pre-pandemic period to 0.28% and 0.03% in the post-pandemic period, respectively [19]. According to Van Brusselen *et al.* there was 99% reduction in RSV cases and 92.5% less AVB related hospitalisations in Belgium since the pandemic onset [20].

There is paucity of literature on the disease burden of respiratory viruses among children hospitalised due to ALRTIs in India during the pandemic. Therefore, the current study was planned to determine the impact of the COVID-19 pandemic on the epidemiology, frequency, and pattern of respiratory viruses in hospitalized children with ALRTIs.

India reported the first case of COVID-19 on January 30, 2020 in Kerala. However, during the second wave of SARS-CoV-2 in India, the emergence of the SARS-COV-2 variant of concern (VOC) triggered intensive genomic surveillance across the country [21]. India witnessed the deadly second wave of COVID-19 as well as break through infections due to the Delta variant from April 8, 2021 onwards [22] and the predominance of Delta and Delta AY.1 variants in 92.05% of the sequenced samples in north Indian states post Delta wave emergence were reported [21]. South Africa reported the emergence of a new SARS-CoV2 variant that was named Omicron (B.1.1.529) on November 24, 2021 and this was designated by the WHO as the sixth VOC [23]. India was hit by a third wave due to SARS-CoV2 Omicron variant in January 2022 with sudden upsurge of cases [23]. Hence, the period of July 2021 to December 2021 in north India was categorized as the period of Delta wave and January 2022-July 2022 as the period of Omicron wave.

## Methodology

This study involved three time periods: pre-pandemic period (October 2019 to February 2020), Delta wave pandemic period (July 2021 to December 2021) and Omicron wave pandemic period (January 2022 to July 2022). Children aged between 1 month and 12 years admitted in pediatric emergency, pediatric intensive care unit (PICU) and the wards of the Department of Pediatrics at Postgraduate Institute of Medical Education & Research, Chandigarh with the clinical diagnosis of ALRTIs [AVB (Acute Viral Bronchiolitis), pneumonia, acute respiratory distress syndrome (ARDS)] and negative for SARS-CoV2 during the pandemic period were enrolled for the study. Nasopharyngeal swabs (NPS)/aspirates (NPA) or endotracheal aspirates (ETA) were sent to the Regional Viral Research and Diagnostic Laboratory (RVRDL), Department of Virology for screening of respiratory viruses. Children with acute bronchial asthma exacerbations and pneumonia of non-infectious causes were excluded. The institute Ethics Committee (INT/IEC/2022/Study-579) approved the study.

The NPS or NPA or ETA were collected by trained health personnel within 12 hours of admission in viral

transport medium (VTM) vial and transported under cold chain to the virology laboratory for molecular investigation of respiratory viruses, i.e., RSV, Influenza A pdmH1N1, influenza A (H3N2), influenza B, hMPV, hBoV, PIV-2, PIV-3, and hRV. The samples were subjected to nucleic acid extraction using QIAamp Viral RNA Mini Kit (Qiagen, Heidelberg, Germany) and the extracted RNAs were reverse transcribed utilizing high-capacity cDNA reverse transcription kits (Life Technologies, Massachusetts, USA). Amplifications of seasonal influenza A, pandemic influenza A H1N1, and influenza B were screened by one step Real Time Polymerase chain reaction RT-PCR method using commercially available TRUPCR H1N1/H3N2 with Inf B kit following manufacturer's instructions (BlackBio Biotech, Bhopal, India). Quality of the extracted nucleic acid was assessed through amplification of *Ribonuclease P* gene, which is an integral component of influenza screening. The nucleocapsid genes of RSV, PIV-2 and PIV-3 were targeted to screen for presence of genomes in complementary DNA by the method reported by Bharaj *et al* [24]. Amplification of RSV was done in monoplex single tube format whereas for the amplification of PIV-2 and PIV-3, multiplex PCR was used. The viral genome of hMPV was detected in clinical samples by using primers as described by Bouscambert-Duchamp *et al*. [25]. In order to detect human rhinovirus, a highly conserved 5' un-translated region of the genome was amplified with primers as described by Wisdom *et al*. [26]. The extracted DNA was subjected to conventional PCR using Hotstart Hi-Fidelity Taq polymerase targeting hBOV NP1 gene for screening of human bocavirus DNA in clinical samples [27]. The primer sequences and expected amplified products are mentioned in Supplementary Table 1. The amplified DNA fragments were identified on a 2% agarose gel with ethidium bromide and visualized under a UV transilluminator.

*Statistical analysis*

Data entry and statistical analysis were done by using Statistical Package for the Social Sciences SPSS 25.0 software. Categorical variables were expressed as numbers and percentages. Comparison of categorical variables was performed using the Fisher exact test and $\chi^2$-test. The comparison was done over three time periods regarding the percentage positivity for viruses; positivity for influenza and other non-influenza viruses (RSV, hMPV, hBoV, PIV, and hRV). All the tests were two-tailed, and a $p$ value of $< 0.05$ was considered statistically significant.

**Table 1.** Virus positivity results during pre-pandemic and pandemic periods (Delta and Omicron waves) among children with acute lower respiratory tract infections.

| Viruses screened | Pre-pandemic period | Pandemic period | | *p* value | | |
|---|---|---|---|---|---|---|
| | October 2019-February 2020 [A] (n = 166) | Delta wave July-December 2021 [B] (n = 78) | Omicron wave January-July 2022 [C] (n = 111) | A vs. B | A vs. C | B vs. C |
| **Individual virus positivity rates** | | | | | | |
| Inf A H3N2 | 7.2% (12) | 5.1% (4) | 2.7% (3) | 0.782 | 0.174 | 0.450 |
| Inf A H1N1 | 0 | 0 | 0.9% (1) | 1 | 0.401 | 1 |
| Inf B | 1.2% (2) | 3.8% (3) | 0 | 0.331 | 0.518 | 0.069 |
| RSV | 51.2% (85) | 10.3% (8) | 0.9% (1) | 0.0001*** | 0.0001*** | 0.004** |
| hMPV | 0.6% (1) | 2.6% (2) | 7.2% (8) | 0.240 | 0.003** | 0.202 |
| hBoV | 1.2% (2) | 5.1% (4) | 9% (10) | 0.085 | 0.004** | 0.404 |
| hRV | 39.2% (65) | 42.3% (33) | 56.8% (63) | 0.676 | 0.005** | .056 |
| PIV-2 | 0.6% (1) | 0 | 0.9% (1) | 1 | 1 | 1 |
| PIV-3 | 4.2% (7) | 3.8% (3) | 2.7% (3) | 1 | 0.745 | 0.692 |
| **Virus positivity rate** | | | | | | |
| Any virus isolated | 78.9% (131) | 48.7% (38) | 55.8% (62) | 0.0001*** | 0.0003*** | 0.376 |
| Viral mono-infection | 54.2% (90) | 32% (25) | 44.1% (49) | 0.0015** | 0.111 | 0.098 |
| Viral co-infection | 24.6% (41) | 16.6% (13) | 11.7% (13) | 0.187 | 0.008** | 0.392 |
| **Age-wise distribution and age specific virus positivity rate** | | | | | | |
| < 2 months | 90% (18/20) | 55% (5/9) | 53.8% (7/13) | 0.055 | 0.035* | 1.00 |
| 2-12 months | 78.9% (101/128) | 45.2% (24/53) | 52% (26/50) | 0.0001*** | 0.0008*** | 0.556 |
| 1-5 years | 66.6% (12/18) | 66.6% (8/12) | 57.14% (16/28) | 1.00 | 0.553 | 0.729 |
| > 5 years | 0 | 25% (1/4) | 65% (13/20) | - | - | 0.272 |

Inf A H3N2: influenza A H3N2 virus; Inf A H1N1: influenza A H1N1 virus; Inf B: influenza B virus; RSV: respiratory syncytial virus; hMPV: human metapneumovirus; hBOV: human bocavirus, hRV: human rhinovirus; PIV-2: parainfluenza-2 virus; PIV-3: parainfluenza-3 virus. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$].

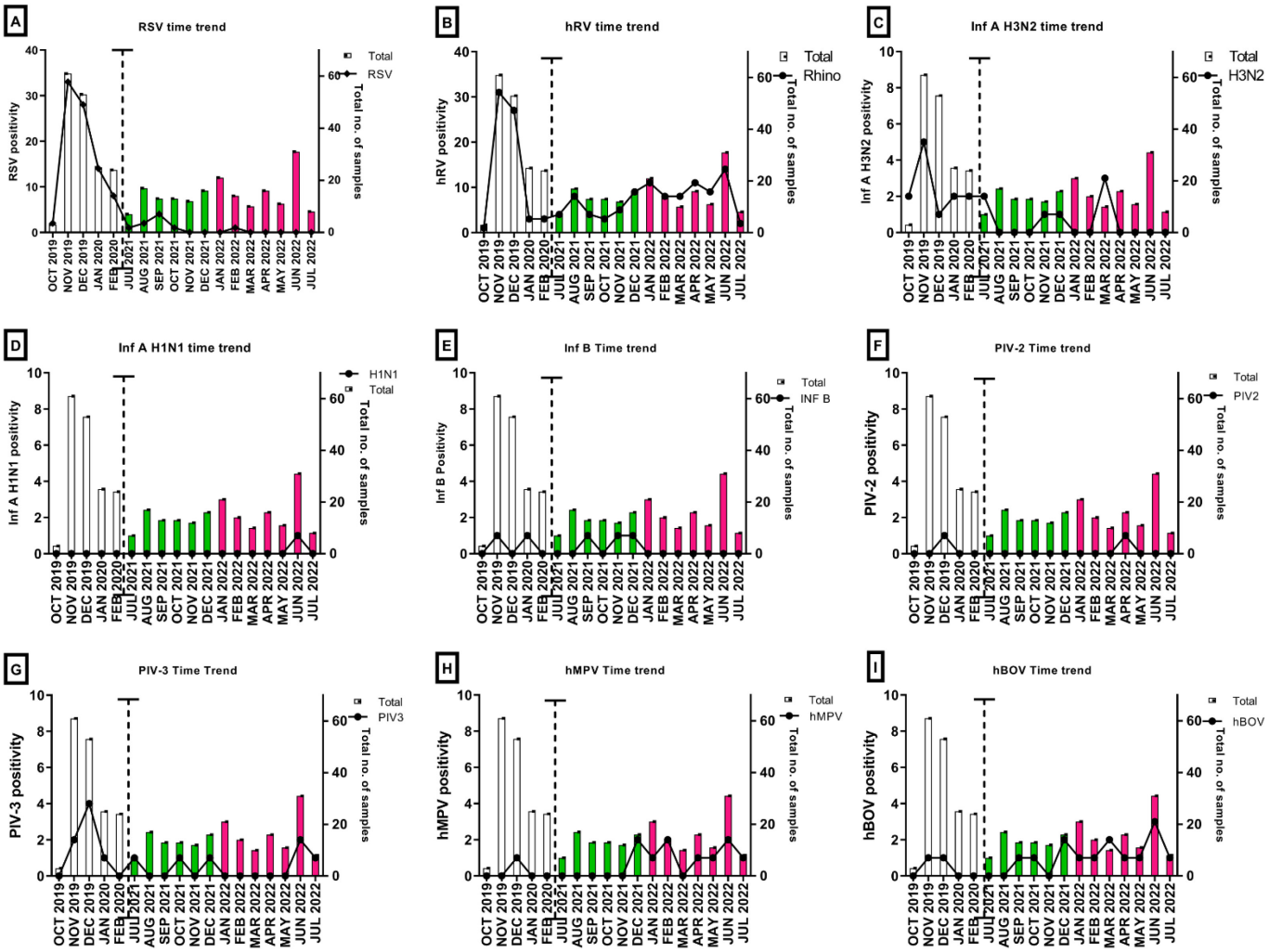
## Results

A total of 355 samples from children with ALRTIs were tested for respiratory viruses; 166 during pre-pandemic period (October 2019 to February 2020), 78 during Delta variant wave (July-December 2021) and 111 during Omicron variant wave (January-July 2022).

There was statistically significant reduction in RSV positivity from 51.2% (85/166) during pre-pandemic to 10.3% (8/78) during Delta and 0.9% (1/111) during Omicron waves ($p < 0.001$ for both) (Table 1). There was a significant increase in rates of hMPV 0.6% to 7.2% ($p = 0.003$); hBoV from 1.2% to 9% ($p = 0.004$); and hRV from 39.2% to 56.8% ($p = 0.005$) from pre-pandemic period to that during Omicron wave, respectively. There was no difference in positivity rate of influenza A H3N2, influenza A pdmH1N1, influenza B, PIV-2, PIV-3 during pre-pandemic and pandemic periods. During pre-pandemic period, the commonest virus isolated was RSV (51.2%); whereas, during pandemic period the most common virus isolated was hRV (42.3% during Delta wave and 56.8% during Omicron wave) in comparison to pre-pandemic period with 39.2% positivity. As compared to the pre-pandemic period, positivity rate for viral etiology was significantly lower during the Delta and Omicron waves [78.9% vs. 48.7% (38/78), ($p < 0.001$; and 55.9% (62/111), $p < 0.001$, respectively) (Table 1).

As rhinovirus is an ubiquitous pathogen and rate of asymptomatic infection in infants less than 4 years of age could range from 12-32%, hence hRV positivity from patient samples leads to a diagnostic dilemma [28]. To delineate the impact of SARS-CoV2

**Figure 1.** Month-wise time trend of respiratory virus positivity before SARS-CoV2 pandemic and during pandemic.




Pre-pandemic period (October 2019 to February 2020) denoted as white, pandemic period Delta variant wave (July-December 2021) as green and Omicron variant wave (January-July 2022) as magenta. Inf A H3N2: Influenza A H3N2 virus, Inf A H1N1: Influenza A H1N1 virus; Inf B: Influenza B virus; RSV: Respiratory Syncytial Virus; hMPV: Human Metapneumovirus, hBOV: human Bocavirus, hRV: human Rhinovirus; PIV-2: Parainfluenza-2 virus; PIV-3: Parainfluenza-3 virus.

emergence and associated public health restrictions on influenza and other pathogenic respiratory viruses, the data of the present study was also analysed following exclusion of human rhinovirus. Respiratory virus positivity except hRV was 21.8% (17/78) and 20.7% (23/111) during Delta ($p$ = 0.0002) and Omicron wave ($p$ = 0.0003) respectively in comparison to pre-pandemic period 59% (98/166). Significant reduction of viral mono-infection pattern was also observed upon analysing the data excluding the hRV during the delta wave 12.8% ($p$ = 0.0001) and omicron wave 17.1% ($p$ = 0.0001) than pre-pandemic period i.e., 51.2%.

During the pre-pandemic phase, infants aged < 2 months had the highest positivity rates (90%, 18/20), followed by 2-12 months (78.9%) and 1-5 years (66.6%) age groups for virus positivity. However, during the Delta wave, maximum positivity was noted among children aged 1-5 years (66.6%) followed by < 2 months (55%), and 2-12 months (45.2%). During Omicron wave, the highest viral positivity was noted among children aged > 5 years (65%) followed by 1-5 years (57.1%) (Table 1).

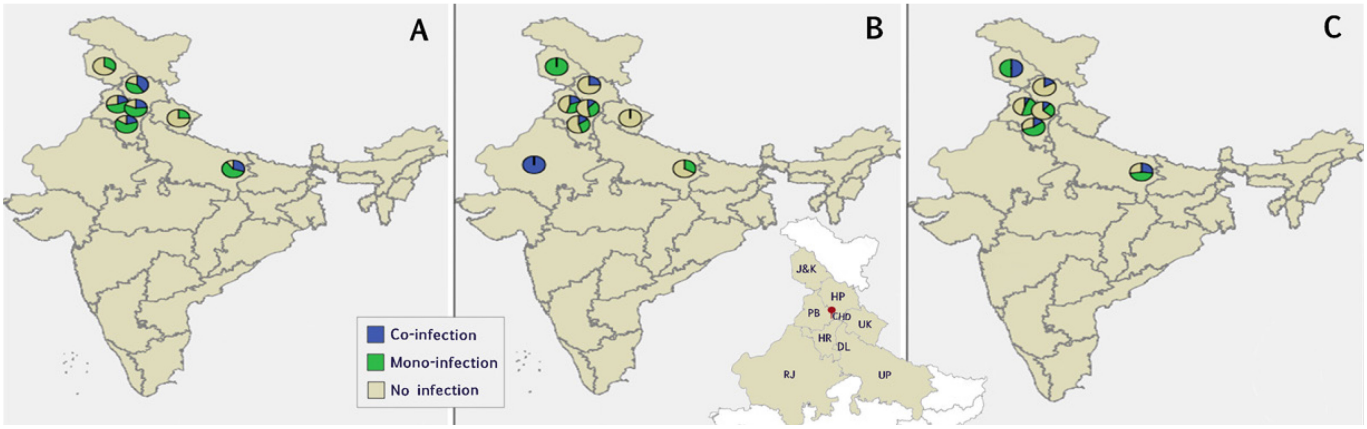
During the pre-pandemic period, the highest proportion of RSV positivity was observed in November 2019 [54.1% (33/61)] followed by December 2019 [52.8% (28/53)]. During the Delta wave, maximum RSV positivity was observed in the month of Sept 2021 [30% (4/13)] followed by July 2021 [14.3% (1/7)] suggestive of significant reduction compared to pre-pandemic period. However, during Omicron wave, RSV positivity was 7.1% (1/14) only seen in February 2022 (Figure 1A). During pre-pandemic period, hRV positivity rate was 12-51%. However, during Omicron wave, July 2022 its rate was detected as minimum (25%; 2/8) followed by May 2022 (81.8%; 9/11) (Figure 1B). Seasonal Inf A H3N2 was frequently detected during pre-pandemic period with positivity rate of 66.6% (2/3), 8.2% (5/61), 1.9% (1/53), 8% (2/25) and 8.3% (2/24) during October 2019, November 2019, December 2019, January 2020 and February 2020, respectively. However, during Omicron wave, the positivity of the same was recorded only in the month of March 2022 [30% (3/10)] (Figure 1C). HBoV was isolated only during the months of November 2019 (1.64%, 1/61) and December 2019 (1.89%, 1/53) during the pre-pandemic period though HBOV was consistently isolated during Omicron wave ranging from 4.76% to 20% (Figure 1I).

During the pre-pandemic period (n = 166), out of the seven states from where patients were admitted in the tertiary care hospital, Punjab showed the highest case burden with positivity/total samples screened i.e., (54/74) followed by Haryana (37/44) and Chandigarh (17/21) (Figure 2A). During the delta wave pandemic period and Omicron wave period, samples were received from 8 and 6 Indian states respectively (Figure 2B and 2C). Virus positivity/total samples screened from Punjab during delta wave pandemic period and Omicron wave period was (17/31) and (25/46) respectively.

## Discussion

It is postulated that if infants are exposed to infectious agents early in life, then there is a relatively lower risk of development of severe disease in later phase of life [20]. Even though non-pharmaceutical interventions (NPIs) used to curb COVID-19's spread may have halted the spread of influenza and other respiratory viruses, this may have put us in a precarious position for the future because the population that was less exposed to respiratory viruses is still susceptible to disease because it lacks adaptive immunity to

**Figure 2.** State wise distribution of respiratory samples and virus positivity.




Pre-pandemic period (October 2019 to February 2020) [A], Pandemic period Delta variant wave (July-December 2021) [B] and Omicron variant wave [C].

circulating viruses, which could increase the burden on our health care system [20]. Younger individuals in sub-Saharan African nations had comparatively low SARS-CoV2 morbidity rates, which was explained by their prior exposure to cross-reactive viruses, which enabled them to develop "trained immunity" to fight SARS-CoV2 [29]. During Inf A H1N1 pandemic, hRV infections might have delayed the spread of Inf A H1N1 infection in France and viral interference between respiratory viruses has attributed to the same, as they both share the same ecological niche [30], and that might help in explaining the reduced prevalence of other respiratory viruses during the current SARS-CoV2 pandemic [31]. In a prospective study conducted from 2013 to 2016 among patients with ALRTI, the infection rate of RSV was 40.68%, and a similar incidence, i.e., 51.2%, was recorded during the current study's pre-pandemic phase [32]. Studies have shown that NPIs such as lockdown and international travel restrictions to curb the trajectory of viral pandemics might have a profound effect on the prevalence of other viruses. NPI control measures during the 1918 influenza pandemic led to a 38% reduction in measles transmission [33] and public health measurements taken during the SARS-CoV epidemic in Hong Kong in 2003 resulted in reduction of influenza virus prevalence [34]. Interference between non-influenza respiratory viruses (NIRVs) and pdmH1N1 was observed during the first wave of pandemic influenza H1N1 2009, with NIRV infection rates higher in H1N1pdm negative patients than in H1N1pdm infected patients [35].

The patients in the current study came from a wide geographical area, covering seven states and a union territory that are commonly referred to this tertiary care hospital in north India. When compared to the pre-pandemic period (78.9%), there was a significant reduction in patients with any confirmed viral etiology of the studied viruses during the Delta wave (48.7%) and Omicron wave (55.8%) in the current study. While hRV was excluded from the current study's analysis, virus etiology positivity was 21.8% and 20.7% during the Delta and Omicron waves, respectively, compared to 59% in the pre-pandemic phase. The current study found a significant decrease in RSV infection rates, but no change in hRV and hBOV infectivity rates was found. The logical explanation for the observation is lockdowns and NPIs might not have impacted the circulation of non-enveloped viruses such as hRV and hBOV, which can be stable for a longer duration outside the host, and thus exhibited more forbearance than the enveloped ones such as influenza A and RSV [36]. Therefore, it is possible that non enveloped respiratory viruses escaped NPIs applied during the COVID pandemic in terms of hand washing/hand sanitization, surface decontamination, making them more prevalent in the SARS-CoV2 pandemic phase.

During the pandemic, infection prevention and control strategies for SARS-CoV2 such as mask use, enhanced hand hygiene, social distancing, restricted travel, closure of schools and day-care centres leading to limited exposure to other infants and children, and limited access to healthcare settings may have contributed to a decrease in the incidence, hospitalisation, and burden of respiratory infections. These pandemic containment strategies had ancillary effects, resulting in a decrease in the spread of other viruses with seasonal dynamics. When compared to previous years, Switzerland saw a significant reduction in almost all recorded infectious diseases during the pandemic year [37]. Other viral illnesses, such as measles, varicella, and rubella, had a lower burden in 2020. Although reduction in infectivity rate was primarily attributed to COVID control measures, under-reporting or misdiagnosis could also be inculpated as cause as the healthcare system was primarily focused on SARS-CoV2 management during the pandemic phase [38].

Since only hospitalized ALRTI patients were screened in the current study, this research is not based on surveillance, and as a result, it may not fully provide insight into the circulating respiratory viruses in the community. However, comparable findings indicating a decrease in the detection rate of respiratory viruses were found in other countries [39,40]. Since there was a significant decrease in admissions for AVB in our tertiary care hospital from March 2020 to July 2021 ($p = 0.001$) where no patients with AVB required PICU management or ventilator support, hence no laboratory investigations were requested for respiratory viral pathogens, except for SARS-CoV2, by clinicians [41]. As a result, the current study was unable to examine the burden of influenza virus and other NIRVs during that time period.

A limitation of the study is that the pre-pandemic trend of influenza and NIRVs was studied only for a period of 5 months before the inception of SARS-CoV2 pandemic in India. However, it is due to the fact that the pediatric hospital of the current study witnessed upsurge of ALRTI cases since October 2019 and samples were collected for the screening of Influenza and other NIRVs.

## Conclusions

The study highlights the alteration in circulating trend of respiratory viruses commonly associated with ALRTI in the pediatric population by comparing their incidence during pre-pandemic and pandemic years (post Delta wave upsurge and post Omicron wave upsurge) in a tertiary care hospital of north India catering to a large geographical region. This finding of the study possibly indicates that viral interference or introduction of NPIs may have played a role in the reduced prevalence of other respiratory viruses. The study focuses on the potential factors that led to containment of flu activity and other non-influenza respiratory viruses in this geographical area which in future could help in formulating prevention strategies.

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### Authors' contributions

MK: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted; SS: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted; SKA: analysis and interpretation of data, drafting the article, final approval of the version to be submitted; PS: acquisition of data, analysis and interpretation of data; MR: acquisition of data, analysis and interpretation of data; IB: conception and design of the study, drafting the article; RC: acquisition of data, analysis and interpretation of data; BS: acquisition of data, analysis and interpretation of data; JM: analysis and interpretation of data, final approval of the version to be submitted; RKR: conception and design of the study, analysis and interpretation of data, final approval of the version to be submitted.

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## Annex – Supplementary Items

**Supplementary Table 1.** Sequences of oligonucleotides used in the study for non-influenza respiratory virus screening.

| Name of oligonucleotide | Target gene | Sequence name (5'--3') | Expected amplified product size |
|---|---|---|---|
| RSV Fwd | RSV N gene | CTGTCATCCAGCAAATACAC | 683 bp |
| RSV Rev | | ACCATAGGCATTCATAAACAATC | |
| PIV-2 Fwd | PIV-2 N gene | GATGACACTCCAGTACCTCTTG | 197 bp |
| PIV-2 Rev | | GATTACTCATAGCTGCAGAAGG | |
| PIV-3 Fwd | PIV-3 N gene | GATCCACTGTGTCACCGCTCAATACC | 266 bp |
| PIV-3 Rev | | CTGAGTGGATATTTGGAAGTGACCTGG | |
| hMPV Fwd | hMPV N gene | GTGATGCACTCAAGAGATACCC | 199 bp |
| hMPV Rev | | CATTGTTTGACCGGCCCCATAA | |
| hBOV Fwd | hBOV N gene | GACCTCTGTAAGTACTATTAC | 354 bp |
| hBOV Rev | | CTCTGTGTTGACTGAATACAG | |
| hRV OS | hRV 5'UTR gene | HCAAGYACTTCTGTYWCCCCSG | 395 bp (External cycle) |
| hRV OAS | | GAAACACGGACACCCAAAGTAGT | |
| hRV AB IS | | CYAGCCTGCGTGGCKGCCWRC | 110 bp (Internal cycle) |
| hRV C IS | | GTAGCCYGCGTGGTGCCCWGC | |
| hRV IAS | | TTAGCCRCATTCAGGGGCCGG | |