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Original Article

Microbiology Section

Respiratory Syncytial Virus Infection in the Context of COVID-19: A Retrospective Study from Eastern India

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ABSTRACT

Introduction: Respiratory Syncytial Virus (RSV), a pathogenic virus, is one of the leading causes of acute respiratory tract infections, especially in infants, immunocompromised individuals and older adults. Prior to the Coronavirus Disease-2019 (COVID-19) pandemic, infections caused by this virus had a tendency to follow a predictable seasonal trend. The post-COVID picture of RSV has not been studied and there is a dearth of data, especially in the eastern part of the Indian subcontinent.

Aim: To describe the distribution of RSV in the eastern part of India in the context of COVID-19.

Materials and Methods: A retrospective study was carried out at a tertiary care hospital of West Bengal, namely Calcutta School of Tropical Medicine (Virology Unit) based on patient records of 954 Nasopharyngeal (NP) and Oropharyngeal (OP) swabs collected from indoor as well as outdoor patients between January 2021 and December 2024. The inclusion criterion was the presence of symptoms of lower respiratory tract infection, irrespective of age and gender. Following collection in appropriate Viral Transport Medium (VTM) containers and transport to the laboratory, maintaining cold chain, the samples were subjected to Ribonucleic Acid (RNA) extraction and real-time Reverse Transcriptase Polymerase Chain Reaction (rtRT-PCR).

Results: Out of 954 samples tested, 244 (25.6%) were found to be positive for RSV. In 2021, 50 out of 51 (98.04%) RSV positive samples showed presence of RSV A, while in 2022 and 2023, 155 out of 157 (98.7%) and 28 out of 32 (87.5%) RSV positive samples showed presence of RSV B, respectively.

Conclusion: The present study illustrates the behaviour of RSV in the context of the post-COVID scenario in eastern India, demonstrating the preponderance of RSV B since 2022 and showing its winter predominance in contrast to the summer predominance it displayed in the pre-COVID era.

Keywords: Molecular diagnostic techniques, Respiratory tract diseases, Reverse transcriptase polymerase chain reaction

INTRODUCTION

The RSV, a pathogenic virus, is one of the leading causes of acute respiratory tract infections. This Orthopneumovirus belongs to the *Pneumoviridae* family [1]. Initially named Chimpanzee Coryza Agent, the virus was later isolated from infants with lower respiratory illness and recognised as a human pathogen [2]. Its greatest impact is noted on infants, immunocompromised individuals and older adults [3]. It is the leading cause of infantile bronchiolitis [2]. It is a major cause of hospitalisations of asthmatic patients and an important cause of failure of lung and bone marrow transplants [4]. The virus occurs ubiquitously and almost every child is infected by the virus within two years of age [5]. It is one of the most widespread viral respiratory infections and its global prevalence continues to increase in children less than five-year-old [6]. Primary infection with the virus is usually associated with symptoms, but recent data points towards asymptomatic involvement as well [7]. The clinical manifestations may vary from severe, potentially lethal Lower Respiratory Tract Involvement (LRTI) to milder manifestations like mild Upper Respiratory Tract Illness (URTI) or otitis media [8]. Prior to the COVID-19 pandemic, infections caused by this virus had a tendency to follow a predictable seasonal trend. In temperate countries, these infections would have a preponderance for the winter months. In tropical countries, however, they occurred throughout the year, with outbreaks during the hot, humid summers and rainy months. In the pre-COVID era, pandemic RSV comprised the fourth cause of overall disability-adjusted life-years across all age groups [9].

Pre-existing studies tend to agree that infants under one year of age are not only the most commonly affected age group but also victims of the greatest burden of severe disease, needing hospitalisation, due to inadequate protection by maternal antibodies against RSV [5,10]. RSV has a single serotype and two antigenic subgroups A and B which may co-circulate, but usually there is the predominance of a single subtype [11,12]. The risk factors for RSV continue to be multidimensional and include young age, preterm birth, malnutrition, immunocompromised state, etc., [6]. The respiratory secretions of RSV-infected people can act as an important vehicle of viral transmission. The infection remains largely preventable by repeated handwashing and social distancing [13]. The seasonal pattern of RSV has been well-recorded in studies from the pre-COVID era, with a summer preponderance in temperate countries and a tendency of summer spikes in tropical countries [9,14,15]. However, there is insufficient data pertaining to the Indian scenario. Moreover, the post-COVID picture of RSV has not been studied exhaustively and there is a dearth of data, especially an under-representation of the eastern part of the Indian subcontinent.

The aim of the present study was to describe the distribution of RSV in the eastern part of India in the context of COVID-19. The primary objective of the study was to calculate the yearly estimate of RSV in a tertiary care hospital of West Bengal. The secondary objective was to identify seasonal patterns, if any, of infections with RSV in the context of COVID-19.

MATERIALS AND METHODS

A four-year long retrospective study was carried out at a tertiary care hospital of West Bengal, namely Calcutta School of Tropical Medicine (Virology Unit) between January 2021 and December 2024. The study was based on patient records of 954 NP and OP swabs collected from indoor as well as outdoor patients. The data was analysed in January 2025.

Inclusion criteria: Participants with of symptoms of lower respiratory tract infection irrespective of age and gender were included in the study.

Exclusion criteria: Participants with known history of tuberculosis were excluded from the study.

Sample size: In the absence of adequate data about prevalence of RSV, the prevalence was set at 50%. The acceptable error margin was set at 3%, and the confidence level was taken to be 95%. On calculation, sample size was found to be approximately 954.

Study Procedure

NP and OP swabs were collected in VTM from symptomatic individuals and transported to the Virology Unit of Calcutta School of Tropical Medicine (CSTM) maintaining proper cold chain. Once received, samples could be stored at 4°C for 48 hours and thereafter -80°C for longer preservation.

After thorough mixing of reagents (ensuring use of clean and dry nuclease-free tubes and tips), carrier RNA was reconstituted and a carrier RNA-lysis solution was prepared. The wash solution concentrate was diluted, carrier RNA-lysis solution was added to 140 µL of the cell-free sample and incubated at room temperature. Following centrifugation and binding, the lysate was loaded in the HiElute Miniprep Spin Column. After two rounds of washing (in the HiElute Miniprep Spin Column) and a final centrifugation (in a Micro Centrifugal Tube), pure RNA was obtained in the eluate in the Micro Centrifugal Tube.

Viasure RSV A and B Real-time kit PCR Detection [16], designed to identify and differentiate RSV A and B in respiratory samples from patients with symptoms of LRTI, was utilised to conduct the rtRT-PCR. The remaining RNA eluate was stored at -40°C. Each well in the kit contained all the necessary components for real time PCR assay (specific primer/probes, dNTPs, buffer, polymerase, reverse transcriptase) in a stabilised format, together with an Internal Control (IC) to monitor PCR inhibition. The detection procedure was done in a single step with reverse transcription and subsequent amplification of specific target sequence occur in the same reaction tube. The thermocycler was programmed as per the conditions listed below and the run was performed [Table/Fig-1].

Cycles	Steps	Time	Temperature
1	Reverse transcription	15 min	45°C
1	Initial denaturation	2 min	95°C
45	Denaturation	10 sec	95°C
	Annealing/extension	50 sec	60°C

[Table/Fig-1]: Steps for PCR.

RESULTS

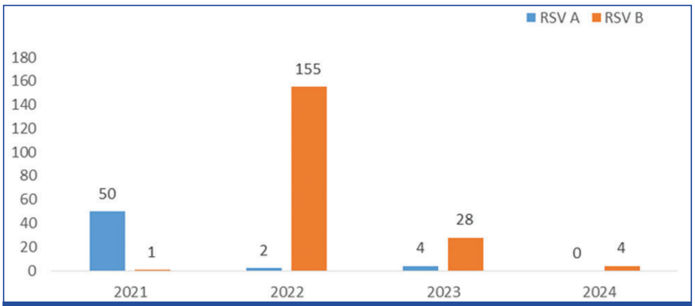
Out of 954 samples tested, 244 were found to be positive for RSV [Table/Fig-2].

Year	Number of samples tested	Number of samples found RSV-positive	Percentage of samples found RSV-positive
2021	62	51	82.26%
2022	499	157	31.46%
2023	266	32	12.03%
2024	127	4	3.15%

[Table/Fig-2]: Year-wise distribution of number of samples tested and the ones found RSV-positive.

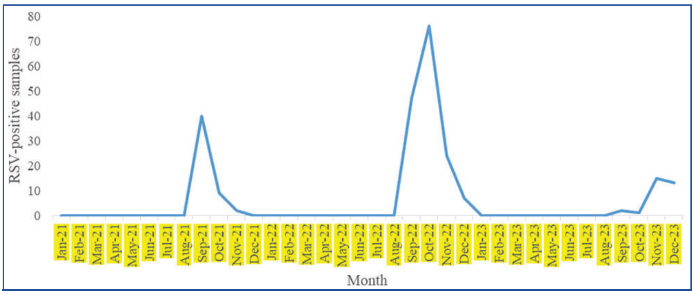
Out of the 244 samples found to be RSV-positive, 152 (62.3%) were from male patients and 92 (37.7%) from female patients. Most of the RSV patients (212 out of 244, i.e. 86.9%) were found to be in the 0-3 year age group, with 182 (74.6%) less than 1.5 years of age and 30 (12.3%) between 1.5 and 3 years of age. Interestingly, 154 infants were found to be RSV-positive, accounting for 63.11% of the positive samples.

In 2021, 50 out of 51 (98.04%) RSV positive samples showed presence of RSV A, while in 2022 and 2023, 155 out of 157 (98.7%) and 28 out of 32 (87.5%) RSV positive samples showed presence of RSV B, respectively [Table/Fig-3].



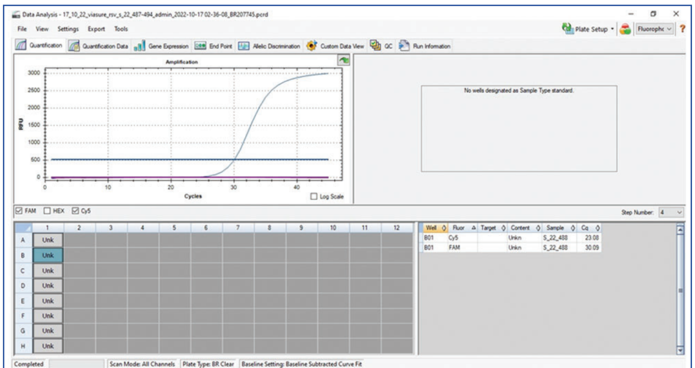
[Table/Fig-3]: Distribution of RSV A and RSV B among positive samples from 2021 to 2024.

The seasonal spikes as observed between 2021 and 2023 have been demonstrated in the line diagram. The months between September to December every year between 2021 and 2023 have demonstrated a distinct spike. The RSV-positive samples of 2024 have not been included in the graph due to paucity of RSV-positivity in the samples received in that year [Table/Fig-4].



[Table/Fig-4]: Line diagram showing distribution of RSV-positive samples for each month from January 2021 to December 2023.

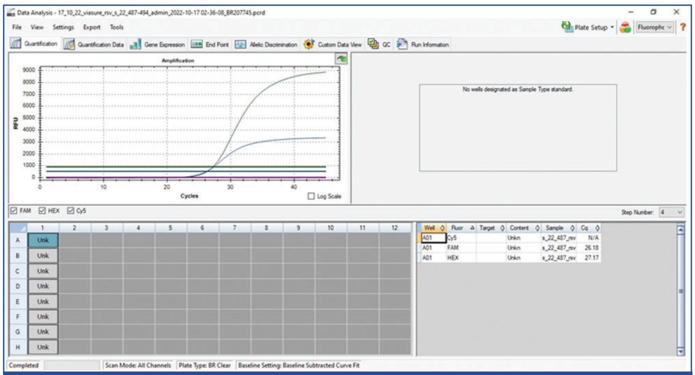
[Table/Fig-5] depicts real-time RT-PCR amplification curves for patient sample positive for RSV. This sigmoid-shaped curve corresponds to an individual clinical specimen, showing the expected exponential amplification phase followed by a plateau, consistent with successful target detection. The Ct value reflects initial viral load of the patient. This graph does not include an IC curve.



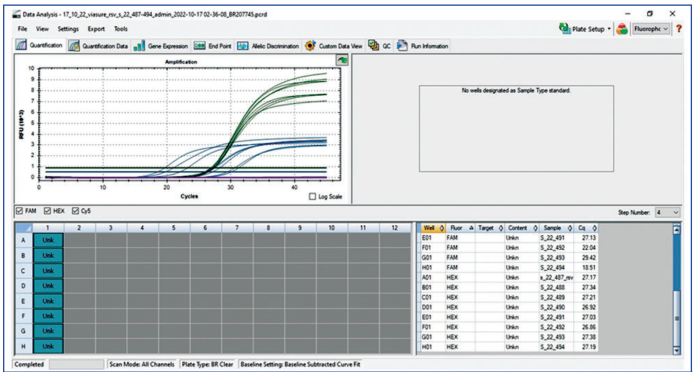
[Table/Fig-5]: Single positive graph without Internal Control (IC).

[Table/Fig-6] depicts real-time RT-PCR amplification curves for patient sample positive for RSV with an IC curve included to verify RNA extraction quality and rule out PCR inhibition in the reaction. Together, the patient, IC curves demonstrate the assay's accuracy, reliability and overall performance.

[Table/Fig-7] depicts real-time RT-PCR amplification curves for seven patient samples positive for RSV. Each sigmoid-shaped curve



[Table/Fig-6]: Single positive graph with Internal Control (IC).



[Table/Fig-7]: Sigmoid amplification curves obtained from real-time RT-PCR for seven RSV-positive patient samples.

corresponds to an individual clinical specimen, showing the expected exponential amplification phase followed by a plateau, consistent with successful target detection. Variation in Ct values reflects differences in initial viral load among patients. An IC curve is included to verify RNA extraction quality and rule out PCR inhibition in each reaction. The kit's positive control curve confirms reagent integrity and assay sensitivity. Together, the patient, IC and positive control curves demonstrate the assay's accuracy, reliability and overall performance.

DISCUSSION

The COVID-19 pandemic has caused changes in the typical epidemiology of RSV, which is still a major cause of LRTIs in children. The distribution, annual burden and shifting seasonal and subtype dynamics of RSV in the post-COVID context were all examined in this retrospective analysis, which was carried out in eastern India (West Bengal) between 2021 and 2024. The current cohort's RSV positivity rate peaked in 2021 (82.26%, 51/62) and then dropped to 31.46% in 2022, 12.03% in 2023 and 3.15% in 2024. The steep drop in 2024 points to either transient suppression or changed transmission dynamics most likely brought on by ongoing COVID-19 mitigation practices. This pattern is consistent with findings of Bhardwaj S et al., where prepandemic RSV positivity averaged about 7.2%, fell to 1.5% in 2020 and then increased to 32% in 2021 [17].

Children under one and a half-year-old (74.6%) accounted for a significant percentage of RSV-positive cases in this study, indicating that the youngest age groups are still the most vulnerable. Similarly, children aged 0-4-year-old had the highest RSV burden, with a slight male preponderance, according to Pune and rural West Bengal SARI surveillance [17]. These results underline the need for focused preventive interventions for this population and confirm that RSV still primarily affects young children despite pandemic-related disruptions in viral circulation. The present study had 154 (63.11%) positive samples coming from patients under one year of age, which was similar to the findings of pre-existing studies that infants are not only the most commonly affected age group but also victims of the greatest burden of severe disease needing hospitalisation. Thus these findings corroborate well with the observations of scholars like Bont L et al. and Piedimonte G, [5,10].

The current study, covering the East Indian scenario from the COVID-19 era to the immediate post-COVID period, shows a distinct spike in the months between September to December every year between 2021 and 2023, suggesting predominance in the relatively rainless winter months. This corresponds to outbreak data from southern India, where RSV cases clustered in September [18] and rural West Bengal Severe Acute Respiratory Infection (SARI) surveillance, which reported RSV peaking in autumn in 2022 [19]. The post-COVID-19 seasonal window in eastern India may have changed, possibly as a result of an interaction of changed social behaviours and climatic factors. Pre-COVID-19 studies in tropical regions frequently indicated summer or rainy season peaks [14,15].

Based on molecular analysis, RSV A predominance was 98.04% in 2021, followed by RSV B dominance in 2022 (98.7%) and 2023 (87.5%). In 2024, all positives were RSV B. There were no signs of RSV A and B co-infections. These results are consistent with previously reported single-serotype predominance patterns [11,12]. To correlate epidemiologic trends with disease severity, future directions should involve multicentric prospective surveillance throughout India and the integration of clinical outcome data, multiplex viral panels and genomic sequencing. In the changing post-pandemic environment, predictive modelling could help with proactive public health planning by enabling early warning systems, outbreak mitigation and knowledgeable paediatric respiratory care.

The practices of repeated handwashing and social distancing as preventive measures against COVID-19, in addition to the widespread use of N95 as well as surgical masks, are likely to have prevented larger-scale RSV outbreaks in the immediate post-COVID-19 era. The present study throws some light on the epidemiology of RSV as observed in the eastern part of the Indian subcontinent, which can help in preparedness for future infections, especially in infants.

Limitation(s)

The limitations of the present study include the limited sample size and the tertiary care hospital set-up of the study, which does not provide adequate information regarding the community scenario.

CONCLUSION(S)

The present study illustrated the behaviour of RSV in the context of the post-COVID scenario in eastern India, demonstrating the preponderance of RSV B since 2022 and showing its winter predominance in contrast to the summer predominance it displayed in the pre-COVID era.

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