

Original Research Article

MOLECULAR DETECTION AND SUBGROUP DISTRIBUTION OF RESPIRATORY SYNCYTIAL VIRUS AMONG CHILDREN UNDER FIVE YEARS WITH ACUTE LOWER RESPIRATORY TRACT INFECTION: A HOSPITAL-BASED PROSPECTIVE STUDY FROM EASTERN INDIA

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ABSTRACT

Background: Respiratory syncytial virus (RSV) is an important cause of acute lower respiratory tract infection (ALRTI) in young children, particularly infants. Molecular methods such as real-time reverse transcription polymerase chain reaction (RT-qPCR) provide sensitive detection and allow differentiation of RSV-A and RSV-B. However, region-specific data on RSV molecular detection and subgroup distribution among children with ALRTI remain limited. The objective is to detect Respiratory syncytial virus among children below five years of age with ALRTI using molecular methods and determine the distribution of RSV-A and RSV-B.

Materials and Methods: A hospital-based prospective observational study was conducted in a tertiary care hospital in eastern India from November 2023 to October 2025. A total of 189 children below five years of age with clinically diagnosed ALRTI were enrolled. Nasopharyngeal aspirates and/or nasopharyngeal swabs were collected and processed. Viral RNA was extracted and subjected to TaqMan real-time RT-PCR. The assay included separate targets for RSV-A and RSV-B, along with an endogenous RNase-P internal control. Demographic, seasonal, clinical and co-infection characteristics of RSV-positive children were analysed.

Results: Among the 189 children enrolled, RSV was detected in 36, yielding an overall molecular positivity rate of 19.0%. Of the 72 viral detections recorded in the study, 36 (50.0%) were RSV. RSV-A was detected in 26 (36.1%) viral detections, RSV-B in 7 (9.7%), and simultaneous RSV-A and RSV-B detection in 3 (4.1%). Among RSV-positive children, RSV-A alone accounted for 72.2%, RSV-B alone for 19.4%, and dual RSV-A/B detection for 8.3%. RSV was detected predominantly in children aged 0–12 months. Seasonally, RSV cases were most frequent during winter (41.7%), followed by pre-winter (25.0%). Prematurity was the most frequently recorded associated risk factor (30.0%). Co-detection with another pathogen occurred in 28 of 36 RSV-positive children (77.8%), with *Streptococcus pneumoniae* being the most frequently co-detected pathogen. Among RSV-positive children, 23 (63.9%) required oxygen therapy,

21 (58.3%) had prolonged hospital stays, 17 (47.2%) required ICU intervention, and 9 (25.0%) required mechanical ventilation.

Conclusion: Molecular testing showed that RSV accounted for a substantial proportion of viral ALRTI cases among children under five years of age in this tertiary-care population. RSV-A was the predominant molecular subgroup, and infections were concentrated among younger children, particularly infants, with a pronounced winter predominance. The high frequency of pathogen co-detection and the substantial need for respiratory and intensive care support underscore the clinical importance of RSV surveillance using molecular diagnostic methods.

Keywords: Respiratory syncytial virus; RSV-A; RSV-B; RT-qPCR; acute lower respiratory tract infection; children; molecular diagnosis; India.

INTRODUCTION

Acute lower respiratory tract infections (ALRTIs) remain a major cause of morbidity, hospitalization and mortality among children younger than five years, with a substantial burden in low- and middle-income countries. Viral pathogens account for an important proportion of childhood ALRTIs alongside bacterial agents, and the etiological spectrum varies with age, geography, season and host factors.^[1-3] The thesis similarly emphasizes the predominance of viral etiologies, particularly respiratory syncytial virus (RSV), in young children with ALRTI.

Respiratory syncytial virus (RSV) is among the most important viral causes of ALRTI in infancy and early childhood and is particularly associated with bronchiolitis and pneumonia.^[4,5] Global modelling estimated that, in 2015, RSV caused approximately 33.1 million episodes of ALRI. Among children younger than five years, there were 3.2 million hospital admissions and 59,600 in-hospital deaths.⁶ Updated estimates for 2019 further confirmed the substantial global burden of RSV-associated ALRI in this age group.⁷ The burden is especially important in resource-limited settings, where access to laboratory diagnosis and supportive respiratory care may be constrained.^[6,7]

In India, RSV epidemiology varies considerably by region and season. Hospital-based studies have reported differing RSV detection rates, reflecting differences in population, clinical definitions, season and diagnostic methods.⁸ A prospective study from southern India detected RSV in 24.5% of hospitalised children aged 1–59 months with ALRI, and more than 90% of RSV-positive children were younger than two years.⁹ Earlier community-based work from India also demonstrated RSV as the most frequently detected respiratory virus among ALRI episodes.¹⁰ Studies from other Indian regions, including northern India, have documented substantial RSV-associated severe respiratory illness and seasonal variation.^[11] Host and environmental factors influence susceptibility and severity of RSV-associated ALRTI. Prematurity, low birth weight, male sex, shorter breastfeeding, household crowding and tobacco-smoke exposure have been identified as important risk factors.¹² Severe RSV disease is also more likely in young infants and children with

underlying cardiopulmonary or other comorbid conditions.^[5,12] Repeated infection is common because immunity following RSV infection is incomplete, allowing reinfections throughout childhood.^[13]

RSV comprises two major antigenic subgroups, RSV-A and RSV-B, which may circulate simultaneously and vary temporally and geographically.^[8,14] Molecular assays, particularly real-time reverse-transcription polymerase chain reaction (RT-PCR), enable sensitive RSV detection and subgroup-specific identification.^[14,15] However, regional data on the distribution of RSV-A and RSV-B among children with ALRTI remain limited in eastern India. Therefore, this study aimed to characterize the respiratory etiological profile of children under five years of age with ALRTI, focusing particularly on the molecular detection and subgroup distribution of RSV-A and RSV-B at a tertiary-care hospital in eastern India.

MATERIALS AND METHODS

Study Design: This prospective observational study was conducted in a hospital setting to determine the etiological profile of acute lower respiratory tract infection (ALRTI) among children younger than five years, with particular emphasis on the molecular detection and subgroup distribution of respiratory syncytial virus (RSV).

Study Setting and Study Period: The study was conducted in the Department of Microbiology in collaboration with the Department of Paediatrics, M.K.C.G. Medical College and Hospital, Berhampur, Odisha. Children admitted to the paediatric wards and intensive care units with clinical features suggestive of ALRTI were evaluated for inclusion in the study. The study was carried out over a period of two years, from November 2023 to October 2025.

Study Population: The study population comprised children younger than five years who presented with clinical features suggestive of ALRTI and fulfilled the predefined eligibility criteria. Children were enrolled after written informed consent was obtained from their parents or legal guardians.

Sample Size: The required sample size was calculated using the standard formula for estimation of a proportion:

$$n = Z^2P(1-P)/d^2$$

where Z was taken as 1.96 for a 95% confidence level, P was the anticipated prevalence of ALRTI (40%)¹⁶, and d was the absolute precision (7%). Based on these assumptions, the calculated sample size was 189 children.

Inclusion Criteria: Children were eligible for inclusion if they were younger than five years and had a clinical diagnosis of ALRTI, manifested by one or more of the following: fever, cough, fast or difficult breathing, chest indrawing, wheezing or crepitations. Only children whose parents or guardians provided written informed consent were enrolled.

Exclusion Criteria: Children were excluded if their parents or guardians declined consent; if they were older than five years; if they had previously diagnosed bronchial asthma or other chronic pulmonary disorders; if they had chronic respiratory infections such as tuberculosis; or if they presented with an isolated acute upper respiratory tract infection without evidence of lower respiratory tract involvement. Children admitted for other illnesses who developed features of acute respiratory infection after 14 days of hospitalisation were also excluded.

Ethical Considerations: The study was conducted after approval from the Institutional Ethics Committee of M.K.C.G. Medical College and Hospital, Berhampur, vide reference no. EC/NEW/INST/2022/2934. The researchers secured written informed consent from the parents or guardians of every participating child. The study maintained patient confidentiality throughout and used the collected information exclusively for research purposes.

Data Collection: Demographic, clinical, radiological and laboratory information was collected using a pre-designed and pre-tested proforma. The demographic variables included age, sex and residence. Clinical history, presenting symptoms, physical examination findings, relevant radiological findings and laboratory investigation results were recorded for each participant.

Specimen Collection and Transport: Respiratory specimens, including sputum and/or nasopharyngeal specimens, were collected under strict aseptic precautions. In children requiring nasopharyngeal aspirate (NPA), a 5-French infant feeding tube cut to approximately 5 cm was used. Approximately 1.5 mL of sterile normal saline was instilled into each nostril and the aspirate was immediately collected using a sterile 2-mL disposable syringe. Nasopharyngeal swabs were collected using sterile flocked nylon swabs with a molded breakpoint. The swabs were placed in 3 mL of viral transport medium and transported immediately to the microbiology laboratory on ice packs, where they were stored at -70°C until processing. Sputum was collected from children who could expectorate.

Sample Processing: The Department of Microbiology, M.K.C.G. Medical College and Hospital, Berhampur, processed all respiratory specimens using standard biosafety and aseptic

precautions. Samples were received in sterile, leak-proof containers and labelled with the participant identification number, age, sex, date and time of collection. Specimens were processed as early as possible to minimize contamination, bacterial overgrowth and degradation of viral RNA.

Viral Specimen Processing: Nasopharyngeal aspirate or nasopharyngeal swab specimens were transferred aseptically into sterile tubes containing viral transport medium. The specimens were centrifuged at 3000 rpm for 10 minutes to concentrate viral particles before nucleic acid extraction and molecular testing.

RNA Extraction: Viral RNA was extracted from respiratory specimens using the TRIZIN-Xp RNA Isolation Kit. Approximately 200–400 μL of specimen was transferred into a 1.5-mL microcentrifuge tube, then 800 μL of TRIZIN reagent was added. After incubation and chloroform-mediated phase separation, the upper aqueous phase was collected, mixed with isopropanol and applied to an RNA-binding column. The column underwent sequential centrifugation and membrane-washing steps, and purified RNA was subsequently eluted with nuclease-free water. The extracted RNA was stored at -80°C until molecular analysis. DNase I treatment removed genomic DNA contamination.

Molecular Detection of Respiratory Viruses: Molecular detection of respiratory viruses was performed using TaqMan probe-based real-time reverse-transcription polymerase chain reaction (RT-qPCR) assays. The viral testing included RSV, influenza viruses, parainfluenza viruses and rhinovirus according to the respective assay protocols. The RSV assay specifically incorporated primer–probe targets for RSV-A and RSV-B, allowing subgroup-specific detection. An endogenous RNase-P internal control was included to assess specimen adequacy, extraction efficiency and the presence of PCR inhibitors.

RSV-A and RSV-B Detection by Real-Time RT-qPCR: RSV-A and RSV-B were detected using the DiAGSure RSV detection assay employing TaqMan real-time RT-PCR chemistry. The RSV primer–probe mix targeted RSV-A and RSV-B and included an internal control. The reaction mixture consisted of 13 μL of $2\times$ one-step master mix, 2 μL of primer–probe mix and 10 μL of extracted RNA or appropriate control, giving a final reaction volume of 25 μL .

The amplification protocol consisted of reverse transcription at 50°C for 10 minutes, initial denaturation at 95°C for 3 minutes, followed by 45 amplification cycles consisting of denaturation at 95°C for 10 seconds and annealing/extension at 58°C for 30 seconds. RSV-A and RSV-B were interpreted separately according to the fluorescence signals from their respective targets. A positive RSV-A signal with a valid internal control was interpreted as RSV-A infection, whereas a positive RSV-B signal with a valid internal control was interpreted as RSV-B infection. Simultaneous positivity for both targets was recorded as RSV-A and RSV-B co-detection.

Quality Control: Appropriate positive and negative controls were included during molecular testing. The endogenous RNase-P internal control was used to monitor specimen quality, RNA extraction efficiency and PCR inhibition. Samples that failed both the viral target and internal control were considered invalid and were retested according to the assay protocol.

RESULTS

Socio-demographic and baseline characteristics: The study included 189 children with ALRTI. As shown in [Table 1], of these, 81 (42.9%) were aged 0–2 years, while 108 (57.1%) were aged 3–5 years. Males predominated: 106 (56.1%) were male, and 83

(43.9%) were female. Most participants were from rural areas (113, 59.8%), compared with 76 (40.2%) from urban areas. Among the recorded risk factors, low socioeconomic status was most common (46, 24.3%), followed by malnutrition (37, 19.6%), incomplete vaccination (29, 15.3%), prematurity (23, 12.2%), overcrowding (21, 11.1%), contact history (19, 10.1%), and underlying/overlying disease (14, 7.4%). Regarding the level of care, 110 children (58.2%) were managed in the ward, whereas 79 (41.8%) required ICU care. An etiological pathogen was detected in 117 (61.9%) children, while none was identified in 72 (38.1%). Among pathogen-positive children, 83 (70.9%) had monomicrobial infection and 34 (29.1%) had polymicrobial infection.

Table 1: Socio-demographic variables of study participants

Variable	Category	n (%)
Age group	0–2 years	81 (42.9)
	3–5 years	108 (57.1)
Sex	Male	106 (56.1)
	Female	83 (43.9)
Residence	Rural	113 (59.8)
	Urban	76 (40.2)
Risk factors†	Low socioeconomic status	46 (24.3)
	Malnutrition	37 (19.6)
	Incomplete vaccination	29 (15.3)
	Prematurity	23 (12.2)
	Overcrowding	21 (11.1)
	Contact history	19 (10.1)
	Underlying/overlying disease	14 (7.4)
Level of care	Ward	110 (58.2)
	ICU	79 (41.8)
Pathogen detected	Yes	117 (61.9)
	No	72 (38.1)
Type of infection among pathogen-positive cases	Monomicrobial	83 (70.9)‡
	Polymicrobial	34 (29.1)‡

† Risk factors are not mutually exclusive, so percentages do not add up to 100%.

‡ Denominator = 117 pathogen-positive children.

Distribution of monomicrobial and polymicrobial etiological agents: As shown in [Table 2], among the 117 pathogen-positive children, 84 had monomicrobial infections, and 33 had polymicrobial infections. Among monomicrobial infections, the most frequently identified bacterial pathogen was *Klebsiella pneumoniae* (21 cases), followed by *Streptococcus pneumoniae* (17), *Staphylococcus aureus* (10), *Pseudomonas aeruginosa* (6), and

Acinetobacter baumannii (2). Among viral monomicrobial infections, influenza was detected in 12 cases, RSV in 8, rhinovirus in 4, and parainfluenza virus in 3. Polymicrobial infections included RSV with *S. pneumoniae* in 11 cases, RSV with rhinovirus in 7 cases, RSV with *S. aureus* in 5 cases, RSV with influenza virus in 4 cases, influenza virus with *P. aeruginosa* in 3 cases, influenza virus with *S. aureus* in 3 cases, and RSV with *Escherichia coli* in 1 case.

Table 2: Distribution of monomicrobial and polymicrobial etiological agents among pathogen-positive children (n=117)

Pathogen category	Etiological agents		Frequency (n)
Monomicrobial infection (n=84)	Bacterial isolates	<i>Streptococcus pneumoniae</i>	17
		<i>Staphylococcus aureus</i>	10
		<i>Klebsiella pneumoniae</i>	21
		<i>Pseudomonas aeruginosa</i>	6
		<i>Acinetobacter baumannii</i>	2
	Viral pathogens	RSV	8
		Influenza	12
		Parainfluenza virus	3
		Rhinovirus	4
Polymicrobial infection (n=33)	Mixed infection	RSV+ <i>Escherichia coli</i>	1
		RSV+ <i>S. pneumoniae</i>	11
		RSV+ <i>S. aureus</i>	5
		RSV+ Influenza virus	4
		RSV+ Rhinovirus	7
		Influenza virus + <i>Pseudomonas aeruginosa</i>	3
		Influenza virus + <i>S. aureus</i>	3

Distribution of respiratory viral pathogens: As shown in [Table 3], among the 72 children with a detected respiratory viral pathogen, RSV was the most common, identified in 36 cases (50.0%). RSV-A was detected in 26 (36.1%) children, RSV-B in 7 (9.7%), and both RSV-A and RSV-B simultaneously in 3 (4.1%). Influenza A was detected in 17 (23.6%)

cases and influenza B in 5 (6.9%), for 22 influenza-positive cases. Rhinovirus was detected in 11 (15.2%) children, and parainfluenza virus in 3 (4.2%). Overall, RSV represented the largest proportion of viral detections in the study population, with RSV-A being the predominant RSV subgroup.

Table 3: Prevalence of Viral etiological agents in study group (n = 72)

Viral Pathogen	Frequency (n)	Percentage (%)
RSV-A	26	36.1%
RSV-B	7	9.7%
RSV-A & RSV-B	3	4.1%
Influenza A	17	23.6%
Influenza B	5	6.9%
Parainfluenza virus	3	4.16%
Rhinovirus	11	15.2%

Distribution of RSV subgroups according to age:

As shown in Table 4, among the 36 RSV-positive children, RSV-A was detected in every age group, with the highest number in children aged 0–12 months (12 cases), followed by those aged 25–36 months (7 cases), 13–24 months (4 cases), 37–48 months (2 cases), and 49–60 months (1 case). RSV-B was detected mainly in younger children, including 6 cases in the 0–12-month group and 1 case in the 13–

24-month group; no RSV-B detections occurred among children aged 25–60 months. Dual RSV-A/B detection occurred only in the 13–24-month group, where 3 cases were identified. Overall, the 0–12-month group accounted for 18 of the 36 RSV-positive children, followed by 8 cases in the 13–24-month group, 7 in the 25–36-month group, 2 in the 37–48-month group, and 1 in the 49–60-month group.

Table 4: Distribution of RSV-A, RSV-B and dual RSV-A/B infection according to age group (n=36)

Age group	RSV-A (n)	RSV-B (n)	RSV-A+B (n)	Total
0–12 months	12	6	0	18
13–24 months	4	1	3	8
25–36 months	7	0	0	7
37–48 months	2	0	0	2
49–60 months	1	0	0	1

Distribution of RSV according to level of care and season: As shown in Table 5, among the 36 RSV-positive children, 17 (47.2%) were admitted to the ICU, while 19 (52.8%) were managed in the ward. RSV detection was concentrated in winter, with 15 (41.7%) cases, followed by the pre-winter period,

with 9 (25.0%) cases. Four (11.1%) cases occurred in autumn, 3 (8.3%) during the monsoon, 2 (5.6%) in spring, and 1 (2.8%) in summer. Overall, 24 of the 36 RSV cases (66.7%) were detected during winter and pre-winter.

Table 5: Distribution of RSV infection according to level of care and season(n=36)

Variable	Category	n	%
Level of care	ICU	17	47.2
	Ward	19	52.8
Season	Winter	15	41.7
	Pre-winter	9	25.0
	Autumn	4	11.1
	Monsoon	3	8.3
	Spring	2	5.6
	Summer	1	2.8

Risk factors, co-infections and clinical outcomes among RSV-positive children:

As shown in Table 6, among 36 RSV-positive children, prematurity was the most common risk factor (11, 30.6%), followed by incomplete vaccination (9, 25.0%), contact history (7, 19.4%), underlying/overlying disease (5, 13.9%), and overcrowding (4, 11.1%); factors were not mutually exclusive. As shown in Table 6 and fig 1, Co-infection occurred in 28 children (77.8%), most

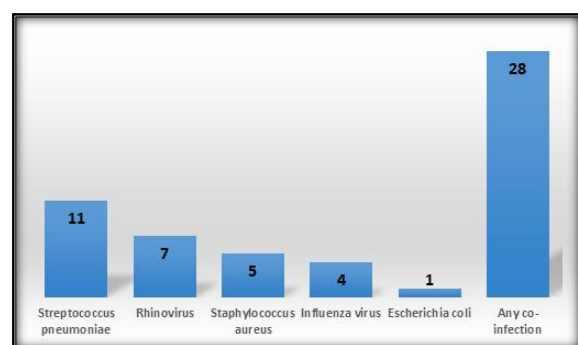
commonly with *S. pneumoniae* (11, 30.6%), rhinovirus (7, 19.4%), *S. aureus* (5, 13.9%), influenza virus (4, 11.1%), and *E. coli* (1, 2.8%); these were also not mutually exclusive. Outcomes included oxygen requirement (23, 63.9%), increased or prolonged hospitalization (21, 58.3%), ICU intervention (17, 47.2%), restrictive or obstructive complications (11, 30.6%), and mechanical ventilation (9, 25.0%).

Table 6: Risk factors, co-infections and clinical outcomes among RSV-positive children (n = 36)

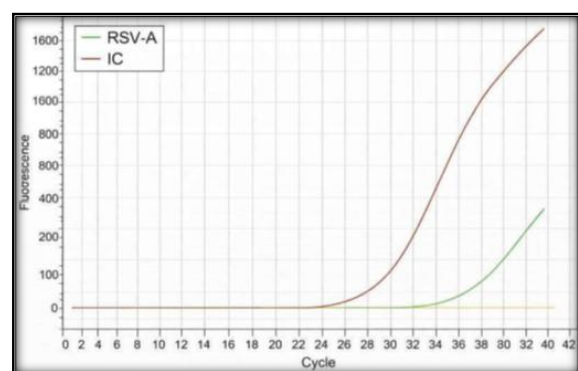
Domain	Variable	n	%
Associated risk factors†	Prematurity	11	30.6
	Incomplete vaccination	9	25.0
	Contact history	7	19.4
	Underlying/overlying disease	5	13.9
	Overcrowding	4	11.1
Co-infections‡	Streptococcus pneumoniae	11	30.6
	Rhinovirus	7	19.4
	Staphylococcus aureus	5	13.9
	Influenza virus	4	11.1
	Escherichia coli	1	2.8
	Any co-infection	28	77.8
Clinical outcomes	Oxygen requirement	23	63.9
	Increased/prolonged hospital stay	21	58.3
	ICU intervention	17	47.2
	Restrictive and obstructive complications	11	30.6
	Mechanical ventilation	9	25.0

† Risk factors were not mutually exclusive; therefore, percentages do not sum to 100%.

‡ Co-infections were not mutually exclusive. Overall, 28/36 (77.8%) RSV-positive children had at least one additional pathogen.

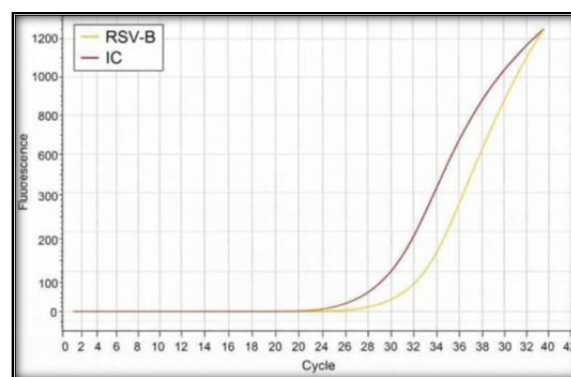
**Figure 1: Co-infections among RSV-positive children**

[Figure 2] shows the real-time RT-qPCR amplification profile for RSV-A, including a distinct amplification curve for the RSV-A target and amplification of the internal control (IC). The characteristic RSV-A amplification curve, together with valid IC amplification, supports the detection of RSV-A nucleic acid in the tested specimen and confirms the validity of the molecular reaction.

**Figure 2: Graph showing amplification curve of RSV-A virus with internal control**

[Figure 3] shows the real-time RT-qPCR amplification profile for RSV-B, featuring a distinct amplification curve for the RSV-B target along with amplification of the internal control (IC). The simultaneous amplification of both the target and

internal control confirms a valid RT-qPCR reaction and supports the molecular detection of RSV-B in the tested specimen.

**Figure 3: Graph showing amplification curve of RSV-B virus with internal control**

DISCUSSION

The present hospital-based prospective study demonstrates the substantial contribution of respiratory viruses, particularly respiratory syncytial virus (RSV), among children younger than five years presenting with acute lower respiratory tract infection (ALRTI). An etiological pathogen was identified in 61.9% of the 189 enrolled children, and a respiratory viral pathogen was detected in 72 children. Among the virus-positive children, RSV was the most frequently detected virus, accounting for 36 (50.0%) cases, followed by influenza viruses, rhinovirus, and parainfluenza virus. The predominance of RSV among hospitalized children with ALRTI is consistent with its established role as a major cause of lower respiratory infection in children worldwide. Large systematic reviews have identified RSV as one of the leading viral causes of ALRI in children under five years, with the greatest burden occurring in low- and middle-income countries.^[7,17] These findings therefore reinforce the importance of laboratory-based RSV surveillance among hospitalized children

with ALRTI, particularly in settings where distinguishing viral from bacterial respiratory infections clinically is challenging.

The detection of RSV in 36 of 189 children (19.0%) in the present study is comparable with the substantial RSV burden reported from other pediatric populations, although differences in study design, season, diagnostic method and case definition make direct comparison difficult. In a Southern Indian study by Kini et al., RSV was detected in 24.5% of 383 hospitalized children with ALRI, and more than 90% of RSV-positive children were younger than two years.⁹ In the present study, RSV accounted for 36 of 189 ALRTI cases, with half of RSV infections occurring in infants aged 0–12 months and 72.2% occurring in children younger than two years. The somewhat lower overall proportion compared with the Southern Indian study may reflect differences in geographic location, surveillance period, respiratory specimen type, diagnostic methodology and seasonal circulation. Nevertheless, the concentration of RSV infection in younger children in both studies supports the recognized vulnerability of infancy to RSV-associated lower respiratory disease.^[9]

The age distribution in the present study provides an important epidemiological observation. RSV-A was detected across all age groups, although most cases occurred among children aged 0–12 months. RSV-B was detected predominantly in infants, with six of seven RSV-B infections occurring during the first year of life. Notably, all three dual RSV-A/RSV-B detections occurred in the 13–24-month age group. The predominance of RSV infection during infancy is biologically and epidemiologically plausible, given the limited maturation of infant airway and immune responses and the increased susceptibility of young children to severe lower respiratory tract disease. In a prospective birth cohort from Kenya, Nokes et al. demonstrated substantial RSV-associated LRTI and severe LRTI during infancy, while also showing that clinically important RSV disease persisted into the second year of life.^[13] Similarly, the Southern Indian study by Kini et al. reported that more than 90% of RSV-positive children were younger than two years.^[9] Thus, the age pattern observed in the present study is broadly consistent with previous evidence identifying infancy and early childhood as key periods of RSV-associated morbidity.

A notable finding of the present study was the predominance of RSV-A, which constituted 26 of 36 RSV detections (72.2%), compared with seven RSV-B infections (19.4%) and three dual RSV-A/B detections (8.3%). Molecular and surveillance studies have also described the predominance of RSV-A, although the relative prevalence of RSV-A and RSV-B can vary geographically and temporally. Fodha et al. specifically evaluated RSV subgroup distribution in hospitalized infants using RT-PCR and found that disease severity was not significantly associated with RSV subgroup, emphasizing that subgroup distribution and clinical severity are not necessarily synonymous.^[14] Indian surveillance

literature has likewise demonstrated variation in RSV subgroup circulation across regions and seasons. The predominance of RSV-A in the present study therefore provides useful local molecular surveillance data from eastern India, while the detection of simultaneous RSV-A and RSV-B in three children highlights the possibility of concurrent circulation of both subgroups.

The seasonal distribution showed a clear concentration of RSV detections during winter and the pre-winter period, which together accounted for 24 of 36 cases (66.7%). Winter alone accounted for 41.7% of RSV cases, followed by pre-winter with 25.0%. This pattern is consistent with the recognized seasonality of RSV, although the timing of peak activity varies according to climate and geographic region. Broor et al. have emphasized considerable regional variation in RSV seasonality across India, with seasonal patterns influenced by climatic conditions.^[8] Similarly, surveillance from Kashmir reported predominant RSV detection during winter months, whereas studies from tropical regions have described RSV circulation during rainy or post-monsoon periods.^[18] The concentration of cases in winter and pre-winter in the present eastern Indian setting suggests that RSV activity may have a distinct seasonal pattern in this region. However, because the study was conducted at a single hospital, these findings should be interpreted as local surveillance observations rather than definitive estimates of community-wide seasonality.

The study also demonstrated a substantial burden of RSV-associated co-infection: 28 of 36 RSV-positive children (77.8%) had at least one additional detected pathogen. *Streptococcus pneumoniae* was the most frequently co-detected pathogen, followed by rhinovirus, *Staphylococcus aureus*, influenza virus, and *Escherichia coli*. This high frequency of co-detection is consistent with the increasingly recognized complexity of the microbiological landscape of childhood ALRTI. Studies using molecular diagnostic techniques have shown that respiratory infections in children may involve multiple pathogens rather than a single etiological agent.^[3,19] Similarly, the South African Drakenstein birth cohort identified multiple potential pathogens among children with pneumonia and highlighted the importance of considering polymicrobial etiologies in pediatric respiratory disease.^[20] The high co-detection rate in the present study may partly reflect the greater analytical sensitivity of molecular testing; importantly, detection of nucleic acid does not necessarily establish that every detected organism was causally responsible for the clinical illness.

The predominance of RSV-S. *pneumoniae* co-detection is clinically important. RSV-related disruption of respiratory epithelial integrity and host immunity may promote bacterial colonization or secondary infection; however, the study's cross-sectional design cannot establish temporal sequence or causality. Previous studies have linked RSV-bacterial co-infection with more severe pediatric

respiratory illness. These findings align with evidence that viral and bacterial pathogens may coexist in children with severe respiratory disease, but they indicate co-detection rather than confirmed sequential or synergistic infection.

Regarding associated risk factors, prematurity was the most frequently recorded characteristic among RSV-positive children (30.6%), followed by incomplete vaccination (25.0%), contact history (19.4%), underlying or coexisting disease (13.9%), and overcrowding (11.1%). These findings are broadly consistent with established evidence identifying prematurity and other host and environmental factors as determinants of RSV-associated ALRTI. A systematic review and meta-analysis by Shi et al. identified prematurity, young age, and several environmental and host factors as important risk factors for RSV-associated ALRI in children younger than five years.^[12] Fodha et al. likewise demonstrated an association between prematurity or low birth weight and disease severity among hospitalized infants with RSV infection.^[14] However, because the present study reports the frequency of these characteristics among RSV-positive children without a non-RSV control group, the findings should not be interpreted as measures of independent risk or causal associations.

The clinical outcomes demonstrate substantial morbidity associated with RSV infection in this hospitalized population. Oxygen supplementation was required in 63.9% of RSV-positive children, 58.3% experienced an increased or prolonged hospital stay, and 47.2% required ICU care. Respiratory complications occurred in 30.6%, while 25.0% required mechanical ventilation. These findings align with the established association between RSV and pediatric respiratory morbidity. Hall et al. reported that RSV contributes substantially to hospitalization and healthcare utilization among young children, while global estimates indicate that it causes millions of ALRI hospital admissions annually among children under five years.^{4,6} Importantly, this study describes outcomes only among RSV-positive children; it does not establish that RSV caused these outcomes or that they were worse than those in RSV-negative children.

The molecular findings shown in Figures 1 and 2 visually confirm the RT-qPCR detection of RSV-A and RSV-B, respectively, while amplification of the internal control supports the validity of each reaction. Molecular detection is particularly relevant because conventional clinical diagnosis cannot reliably distinguish RSV from other respiratory viral infections based on symptoms alone. Previous Indian and international studies have demonstrated the value of laboratory-based viral detection in defining the contribution of RSV to pediatric ALRTI.^[8,9] The present study builds on this approach by specifically identifying RSV-A and RSV-B, thereby providing subgroup-level surveillance data rather than treating RSV infection as a single undifferentiated entity.

CONCLUSION

Overall, RSV was the predominant respiratory virus detected among children with ALRTI in this eastern Indian hospital population. RSV-A predominated over RSV-B, infections were concentrated in infancy and early childhood, and cases clustered during winter and pre-winter months. Frequent pathogen co-detection and substantial oxygen and intensive-care requirements underscore the clinical complexity of RSV-associated ALRTI. These findings support routine molecular surveillance and RSV subgroup identification in pediatric hospitals. However, risk factors, co-infections, and outcomes remain descriptive because the analysis lacked a contemporaneous RSV-negative comparison group and cannot establish causality.

Limitations: The present study has certain limitations that should be considered while interpreting the findings. First, it was a single-centre hospital-based study, and therefore the observed frequency and seasonal distribution of RSV may not be directly generalizable to children with ALRTI in the community or to other geographic regions. Second, the sample size was relatively small (189 children), with only 36 RSV-positive cases, which limits the precision of estimates for RSV-A, RSV-B and dual RSV-A/B infections.

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