

Original Article

Risk factors and diagnostic accuracy in pediatric respiratory syncytial virus-associated acute respiratory illness: A cross-sectional study

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ABSTRACT

Objectives: Respiratory syncytial virus (RSV), an enveloped single-stranded ribonucleic acid virus with two subgroups (RSV A and B), is a major cause of acute lower respiratory tract infections (ALRTI) in young children. This study assessed RSV incidence, risk factors, and compared diagnostic methods: enzyme-linked immunosorbent assay (ELISA), rapid antigen test, conventional polymerase chain reaction (PCR), and real-time PCR.

Materials and Methods: A total of 104 nasopharyngeal and blood samples were collected from infants and young children with ALRTI. RSV antigen was detected using a rapid test. Real-time PCR and conventional PCR were performed on swab specimens, along with viral culture on Vero cell lines.

Statistical analysis: The Chi-square test was applied; $p < 0.05$ was considered significant.

Results: Of 104 samples, 65 (62.5%) were RSV positive, and 2 (1.92%) had influenza. The male: female was 1.8:1. The mean age was 10.4 ± 12.15 months; the median was 5 months. RSV peaked from October to January. Common symptoms included tachypnea (98%), fever (85%), and cough (83%). Risk factors significantly associated with RSV were overcrowding, lack of exclusive breastfeeding, and Neonatal Intensive Care Unit admission. Detection rates were Real-time PCR (61.5%), ELISA (56.9%), conventional PCR (46.1%), and rapid test (20%).

Conclusions: The variability in diagnostic performance underscores the need for sensitive and specific molecular assays for RSV detection.

Keywords: Enzyme-linked immunosorbent assay, Respiratory syncytial virus, Risk factors, Reverse transcription polymerase chain reaction

INTRODUCTION

Acute respiratory illness (ARI) are a major cause of child morbidity and mortality, with respiratory syncytial virus (RSV) being the most common pathogen in children <5 globally.^[1] In India, RSV prevalence in this age group is approximately 20-22%.^[2-4] RSV, an enveloped single-stranded ribonucleic acid (RNA) virus in the *Paramyxoviridae* family (genus *Pneumovirus*), can cause illnesses ranging from mild upper respiratory infections to severe bronchiolitis and

pneumonia.^[5,6] Outbreaks vary seasonally, peaking during the rainy season in tropical regions and in winter in temperate areas.^[7,8] RSV circulates as two antigenic subgroups, RSVA and RSVB, based on differences in glycoproteins.^[9]

Various diagnostic methods, such as enzyme-linked immunosorbent assay (ELISA), immunofluorescent assays, antigen detection tests, nucleic acid amplification tests, optical immunoassays, loop-mediated isothermal amplification, immunochromatographic tests, and viral culture, are currently available for the detection of RSV. Antigen-based assays often exhibit reduced sensitivity in populations with lower viral loads; however, real-time polymerase chain reaction provides high sensitivity, but its reliance on specialized equipment and trained personnel limits its availability in resource-constrained settings. Furthermore, advanced diagnostic methods can be expensive, posing challenges for widespread implementation, especially in low- and middle-income countries.

We aimed to determine the incidence of RSV in infants and young children presenting with acute lower respiratory tract infections in the Aligarh region of North India. Furthermore, the study aimed to identify the key risk factors associated with RSV infection in this population and finally to compare the diagnostic accuracy, specifically the sensitivity and specificity and practical utility in clinical settings, of ELISA, rapid antigen test, conventional reaction Reverse Transcription (RT-PCR), and real-time PCR in detecting the virus.

MATERIALS AND METHODS

Study population

The present study was performed on pediatric patients <5 years of age presenting with ARI/influenza-like illnesses (ILI) over 18 months from April 2023 to October 2024 in the pediatric outpatient department/inpatient department of J.N. Medical College Hospital, AMU, Aligarh. After taking informed consent from the patient's parents, they were enrolled in the study.

Definitions

ILI

An acute respiratory infection with a measured fever of $\geq 38^{\circ}\text{C}$ and cough, with onset within the past 10 days.^[10]

RSV clinical criteria

“Wheezing or apnea or cyanosis” (the classic manifestation of RSV); “severe chest indrawing with either cough or tachypnea” (severe acute lower respiratory infection); and “fever or any integrated management of childhood illness danger sign (lethargy or poor feeding confirmed by poor suck or seizure)” (severe pneumonia).^[11]

Clinical history and examination

A detailed clinical history was taken from the patient, which included fever, cough, coryza, nasal congestion, sore throat, headache, tachypnea, dyspnea, wheezing, chest retraction, bronchiolitis, ARI, and ILI. A total of 104 patients were included in the study.

Sample collection

Nasal/nasopharyngeal/throat swabs were collected from 104 patients in 3-4 mL viral transport media as per the World Health Organization protocol (WHO, 2011). The nasopharyngeal samples were divided into three aliquots: one for the rapid RSV antigen test, one for the conventional PCR, and one for the real-time PCR.^[12] Serum samples were taken to perform an immunoglobulin M (IgM) ELISA. The serum was separated by centrifugation, and then, it was stored at -20°C until tests were performed.

Rapid immunochromatographic test for RSV antigen

All 104 fresh nasopharyngeal swab samples were tested using the BinaxNOW RSV CARD (Abbott) rapid one-step card test. In this assay, 100 μL of the sample was applied to the nitrocellulose pad and incubated at room temperature for 15 min. A single pink control line indicated a negative result, whereas a positive result showed two pink lines (control and test regions) [Supplementary Figure 1].

Detection of anti-RSV IgM antibody through ELISA

All 104 samples were tested for anti-RSV IgM by ELISA Kit (DRG, Internationals). The procedure was done following the manufacturer's instructions. Each well's optical densities were measured at a wavelength of 450 nm and a reference filter of 620 nm. Interpretation of results: 10 DRG Unit (DU) is the cut-off value, for negative, it is <9 DU, and for positive, it is >11 DU.

RNA extraction and cDNA synthesis

A QIAmp viral RNA mini kit (QIAGEN kit) was used to extract viral RNA from nasopharyngeal samples from Acute Respiratory tract illness (ARTI) patients, as per the manufacturer's instructions. The extracted RNA was immediately used for cDNA synthesis, and the remaining RNA was stored at -20°C for real-time PCR.

cDNA synthesis

Synthesis of cDNA was done using[™] cDNA synthesis kit (Thermo Fisher scientific) in which each reaction comprised of 2 μL of $\times 10$ RT Buffer, 2 μL of $\times 10$ RT Random Primer, 0.8 μL of $\times 25$ dNTP Mix, 1 μL of MultiScribe Reverse

Transcriptase and Nuclease free water to make a master mix of 10 μ L, then 10 μ L of extracted RNA was added and a total volume of 20 μ L reaction was run on thermocycler at 25°C for 10 min followed by 37°C for 120 min and then 85°C for 5 min and final hold at 4°C, the cDNA synthesis was stored at -20°C until further use.

Conventional reverse transcriptase polymerase chain reaction

The PCR was performed using primers from Supplementary Table 1. The protocol included an initial denaturation at 96°C for 3 min, followed by 40 cycles of 96°C for 45 s, 58°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 5 min and a hold at 4°C.

Conventional PCR was standardized before sample testing. Optimization steps included gradient PCR to determine the ideal annealing temperature, adjustment of MgCl₂ concentration, primer concentration, and cycle number to enhance band clarity and minimize non-specific amplification. Amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide. A 268 bp band [Figure 1] indicated a positive result for RSV-RNA.

Amplification of subtypes detection by real-time PCR

Real-time PCR was performed using the RealStar[®] RSV RT-PCR Kit 3.0 (Altona Diagnostics) as instructed by the manufacturer. Results were interpreted using Cy[®] 5 and FAM[™] detection channels for RSV A and RSV B, respectively. According to the manufacturer, the kit demonstrates a sensitivity of 98.5% and specificity of 99.2% for RSV detection [Supplementary Figure 2].

Negative controls were included during all PCR and real-time PCR runs to monitor for contamination. A no-template control was used in each batch of amplification reactions. In addition, internal kit controls were employed according to manufacturers' protocols to check real-time PCR assay performance.

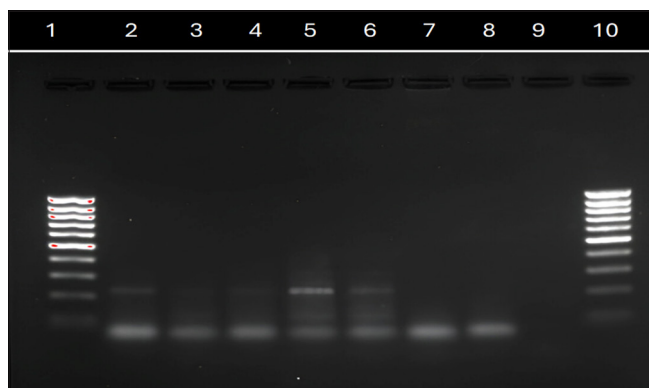


Figure 1: Gel Images showing respiratory syncytial virus-positive sample in lanes 2, 5, and 6 (282 bp) with ladder in lanes 1 and 10.

Viral culture

The viral culture was done on representative 16 samples. Viral culture was done on Vero cells with an overlay medium of Dulbecco's Modified Eagle's Medium with 10% fetal calf serum and 0.3% agarose. After 9-10 days, 4% paraformaldehyde and Crystal Violet dye were added, and the photographed image was captured using a gel documentation system [Figure 2].

Statistical analysis

The Chi-square test was employed to analyze the data comparison based on gender and clinical presentation. A 95% confidence interval (CI) was established as the significance level, rendering a $p < 0.05$ statistically significant. Continuous variables were analyzed using descriptive statistics. The diagnostic efficacy of different tests performed in the study was assessed by calculating the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and negative likelihood ratio using MedCalc statistical software.

RESULTS

Detection of RSV and RSV subtypes

During the study period of 18 months, 104 samples of nasopharyngeal swabs from patients aged <5 years who presented with signs and symptoms of ARI or bronchiolitis were collected, and among them, 65 (62.5%) samples were RSV positive and 2 (1.92%) were positive for influenza. RSV B was the main circulating subtype, present in all RSV real-time PCR-positive patients in this study. Among the RSV-positive samples, 42 (64.6%) were male, and 23 (35.3%) were female. The mean age of the patient positive for RSV was 10.4 ± 12.15 months and the median age was 5 (interquartile range: 0.46-54) months. The distribution of RSV-positive patients according to their age group is given in Supplementary Figure 3.

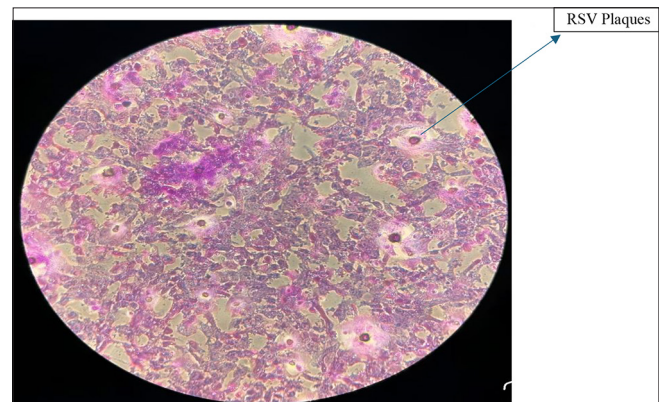


Figure 2: Respiratory syncytial virus (RSV) plaques on Vero cells from a RSV-positive sample Crystal Violet, (20x).

Clinical features and seasonality of RSV

During the study period, the seasonality of RSV showed a peak in circulation pattern from October to January, has been shown in Figure 3.

Tachypnea 64 (98.4%), followed by fever 55 (84.6%), cough 54 (83%), wheezing 53 (81%), and chest retraction 50 (76.9%), were the most common presenting complaints among RSV-positive patients in this study, as shown in Table 1. RSV was significantly associated with fever, cough, tachypnea, wheezing, chest retractions, bronchiolitis, bronchopneumonia, and anemia ($p < 0.05$). Furthermore, RSV-positive children were significantly associated with elevated levels of C-reactive protein (CRP), with a mean level of 87.6 mg/dL, while maximum and minimum values of CRP in these patients were 88 mg/dL and 0.37 mg/dL, respectively.

Risk factors and outcomes of RSV infection

In this study population, the risk factors associated with RSV infection were investigated. Overcrowding, absence of exclusive breastfeeding, and Neonatal Intensive Care Unit (NICU) admission were found to be statistically associated with RSV infection ($p < 0.05$) [Table 1]. Our analysis also revealed that RSV infection was associated with children who were not exclusively breastfed for 6 months. We also followed up on RSV-positive patients to assess outcomes. In our study, we found that 17 (26%) patients required ventilator support out of 65 RSV-positive patients, and among them, 11 (17%) died during the course of the disease and only 6 (9%) survived after a prolonged hospital stay.

Comparison between rapid antigen detection test, ELISA, viral culture, conventional RT-PCR, and real-time PCR

Among the 65 RSV-positive samples, 37 (56.9%) tested

positive by ELISA, 40 (61.5%) by real-time PCR, 13 (20%) using the rapid antigen test, and 30 (46.1%) by conventional RT-PCR. Viral culture was also performed on 16 representative samples, out of which 10 (62.5%) were positive.

Of the 104 total samples, 13 (27%) were RSV antigen positive by the rapid test [Table 2]. The rapid test demonstrated an overall sensitivity of 30.00% and a specificity of 98.44%. The PPV was 92.31% (95% CI: 61.86-98.89%), while the NPV was 69.27% (CI: 64.70-73.42%).

Conventional RT-PCR detected RSV-RNA in 30 out of 65 RSV-positive samples. In addition, 24 (60%) samples were positive for RSV-RNA by real-time PCR method but tested negative by conventional RT-PCR, categorizing them as false negatives. The overall sensitivity of conventional RT-PCR was 40%, with a specificity of 79.37%. The PPV was 55.17% (95% CI: 39.95-69.49%), while the NPV was 67.57% (CI: 61.10-73.43%).

Among the 65 RSV-positive cases, 37 (56.9%) tested positive by anti-RSV IgM Ab ELISA. ELISA test demonstrated an overall sensitivity of 30% and a specificity of 60.94%. The PPV was 32.43% (95% CI: 21.46-45.75%), while the NPV was 58.21% (95% CI: 51.23-64.88%), as shown in Table 2.

The diagnostic accuracy of real-time PCR was assessed using viral culture as the gold standard, as shown in Supplementary Table 2. Real-time PCR had a sensitivity and specificity of 28.57% and 100%, respectively.

On comparing different tests based on composite reference standard [CRS] [Table 3] (a sample was considered RSV positive if any of the two tests among four are positive), ELISA and Rapid tests showed moderate sensitivity (62.5%) and specificity (75%), with high PPV. Conventional PCR had perfect specificity and PPV, but lower sensitivity (50%), likely due to limited detection in low viral load samples.

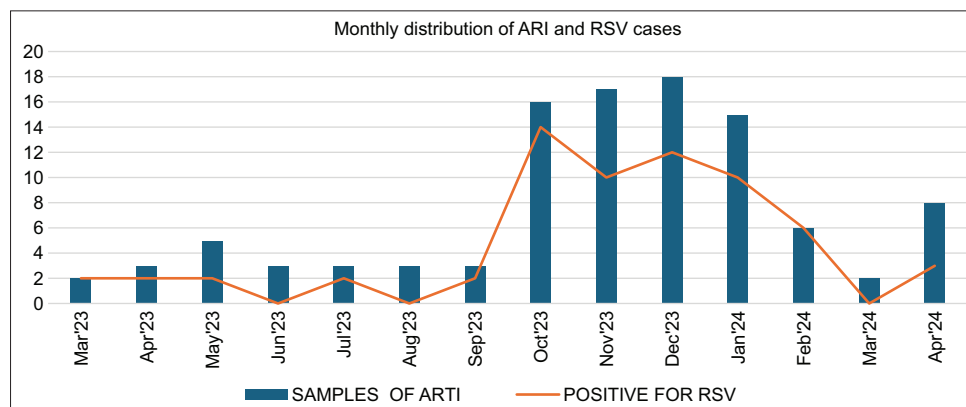


Figure 3: Seasonal distribution of respiratory syncytial virus in children (≤ 5 years) from March 2023 to April 2024. Number of cases are mentioned on Y axis and time period mentioned on X axis. ARI: Acute Respiratory Illness; RSV: Respiratory Syncytial Virus.

Table 1: Demographic and clinical presentation and risk factors of RSV-positive patients in the Aligarh region.

Characteristics	Total (n=104) (%)	RSV positive (n=65) (62.5%) (%)	RSV negative (n=39) (37.5%) (%)	p-value
Gender				
Male	63 (60.5)	42 (65)	21 (54)	0.277
Female	41 (39)	23 (35)	18 (46)	
Age				
<1 month	04 (4)	01 (1.5)	03 (8)	0.363
1-3 months	35 (34)	20 (31)	9 (23)	
4-6 months	22 (21)	13 (20)	15 (38.5)	
7 months-1 year	20 (19)	13 (20)	09 (23)	
2-3 years	19 (18)	15 (23)	07 (18)	
4-5 years	04 (4)	03 (5)	04 (10)	
			01 (3)	
Risk factors				
Gestational age at birth				
Preterm	75 (72)	48 (74)	27 (69)	0.805
Term	29 (28)	20 (30)	09 (23)	
LBW	23 (22)	14 (21.5)	09 (23)	0.297
NICU	24 (23)	17 (26)	07 (18)	0.041
Overcrowding	39 (37.5)	34 (52)	05 (13)	0.000
Not exclusively breastfed	87 (84)	53 (81.5)	34 (87)	0.042
Clinical presentation				
Fever	86 (83)	55 (85)	31 (79.5)	0.040
Cough	85 (82)	54 (83)	31 (79.5)	0.013
Rhinorrhoea	34 (33)	21 (32)	13 (33)	0.170
Tachypnea	100 (96)	64 (98)	36 (92)	0.007
Wheeze	82 (79)	53 (81)	29 (74)	0.008
Chest retraction	72 (69)	50 (77)	22 (56)	0.001
Malaise	17 (16)	09 (14)	08 (20.5)	0.808
Bronchopneumonia	75 (72)	49 (75)	26 (67)	0.008
Bronchiolitis	37 (35.5)	24 (37)	13 (33)	0.071
Anemia	69 (66)	48 (74)	21 (54)	0.001
Jaundice	19 (18)	10 (15)	09 (23)	0.819
CRP (<6 mg/dL)	53 (51)	26 (40)	27 (69)	0.890
CRP (>6 mg/dL)	51 (49)	45 (69)	06 (15)	0.0001

LBW: Low birth weight, NICU: Neonatal intensive care unit, CRP: C-reactive protein, RSV: Respiratory syncytial virus. They are bold because these p value are significant.

DISCUSSION

Different studies considered RSV as one of the most important viruses in infants and young children, causing

Table 2: Evaluation of ELISA, rapid test, and conventional RT-PCR test sensitivity and specificity considering real-time PCR as the gold standard.

Methods of diagnosis	Real-time PCR positive (%)	Real-time PCR negative (%)	Total (%)
ELISA test (n=104)			
Positive	12 (30)	25 (39.06)	37 (35.5)
Negative	28 (70)	39 (60.9)	67 (64.4)
Total	40 (100)	64 (100)	104 (100)
Statistics	Value		95% CI
Sensitivity	30.00		16.56-46.53
Specificity	60.94		47.93-72.90
Positive predictive value	32.43		21.46-45.75
Negative predictive value	58.21		64.88
Rapid test (n=104)			
Positive	12 (30)	1 (1.56)	13 (12.5)
Negative	28 (70)	63 (98.4)	91 (87.5)
Total	40 (100)	64 (100)	104 (100)
Statistics	Value		95% CI
Sensitivity	30.00		16.56-46.53
Specificity	98.44		91.60-99.96
Positive predictive value	92.31		61.86-98.89
Negative predictive value	69.27		64.70-73.42
PCR test (n=104)			
Positive	16 (40)	13 (20.6)	29 (28.1)
Negative	24 (60)	50 (79.3)	74 (71.8)
Total	40 (100)	63 (100)	103 (100)
Statistics	Value		95% CI
Sensitivity	40.00		24.86-56.67
Specificity	79.37		67.30-88.53
Positive predictive value	55.17		39.95-69.49
Negative predictive value	67.57		61.10-73.43

PCR: Polymerase chain reaction, ELISA: Enzyme-linked immunosorbent assay

respiratory infections worldwide, and reported its prevalence ranging from 2% to 42.3%^[13,14] aged <5 years. The prevalence of disease in India is 5-54%, as reported by different studies, and there are very few studies present regarding the prevalence of RSV in the northern region of India. In our study, the prevalence of RSV was relatively higher than in other studies, i.e., 62.5% was recorded in ages <5 who

Table 3: Diagnostic performance of ELISA, rapid test, and conventional PCR against composite reference standard.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ELISA	62.5	75.0	83.33	50.0
Rapid test	62.5	75.0	83.33	50.0
Conventional PCR	50.0	100.0	100.0	50.0

PCR: Polymerase chain reaction, ELISA: Enzyme-linked immunosorbent assay, PPV: Positive predictive value, NPV: Negative predictive value

presented with ARI and bronchiolitis.

RSV A and RSV B are two subtypes of RSV that are circulating worldwide,^[15] while in our study, only RSV B subtype was detected in all samples that were RSV positive by RT-PCR. This study is in concordance with a study from North India,^[16] which also reported RSV B to be the dominating subtype in this region, while RSV A was the most common subtype in South India in other reports.^[4]

Discussing the age group, we found that the mean age of RSV-positive patients was 10 ± 12.5 months which is in concordance with other studies^[17] showing infants were the most vulnerable group having the higher prevalence for RSV being the cause of hospitalization for infants, and as the age increases, their prevalence rate also declines (Centers for Disease Control and Prevention).^[18]

Comparing the male: female from our study to other studies, we found similar results showing male preponderance for RSV infections^[17,19] with a male: female of 1.8:1. Variation in the sex distribution is unclear, and the reason for this discrepancy may be due to reporting bias, as parents are more concerned for male children's health-related issues rather than female's.

In India, when the temperature falls, there is a rise in the number of RSV-positive cases during the month of winter, i.e., from October to January in our study which shows there is a correlation between temperature^[20,21] and transmission of the disease supporting our result as similar results have been reported by Hindupur *et al.*,^[4] Adivitiya *et al.*, and Choudhary *et al.*^[23]

Clinical manifestations shown by RSV infection are broader in range, such as cough, rhinorrhea, nasal congestion, and dyspnea, these are the most common presentations of RSV.^[24] In our study, we found tachypnea as the most common presentation among RSV-infected children, followed by fever, cough, wheezing, and chest retraction.

Preterm babies are more vulnerable to RSV infection, as reported by many researchers in their studies^[25,26] sustaining this fact, this study showed that 74% of the preterm babies presenting with ARI were positive for RSV infection.

Understanding these sequelae of prematurity and the reason for this may be inadequate defense mechanism against infection, an underdeveloped airway, and a low level of immunoglobulin G (IgG) isotype in preterm babies, in contrast to term babies. While in term babies, they have efficient IgG from the maternal placenta transported in the late gestational period.^[26]

Newborn children who are exclusively breastfed are always protected from infectious diseases, as proven by different studies.^[19,27] In 81.5% (53/65) of the children who had not exclusively breastfed were RSV positive in the study, confirming the fact that exclusive breastfeeding gives protection from infectious disease. 52% (34/65) of RSV-positive patients belong to overpopulated areas or live in large families. The above findings from our study corroborate results from different studies^[28,29] demonstrating that environmental factors such as living in overpopulated areas, low socioeconomic status, indoor air pollution, passive smoking, and living at high elevations, along with no exclusive breastfeeding, increase the susceptibility to RSV infection. Furthermore, the month of birth plays a role due to its link with RSV seasonality.

When comparing the RSV rapid antigen test to real-time PCR, it showed a 30% sensitivity and 98.4% specificity, while previous studies reported sensitivity from 5.7% to 72.3% and specificity from 90.5% to 99.9%.^[30] Low sensitivity may be a consequence of low antigen levels in samples or sample collection after the peak viral shedding, as they give rise to false-negative results.^[28] Hence, it was recommended that all negative rapid antigen tests should be confirmed by real-time PCR for accurate diagnosis.

More than half (56.9%) of the samples tested positive for anti-RSV IgM antibodies among all samples. When IgM ELISA was compared to real-time PCR, it gave 30% sensitivity and 60.9% specificity. the duration of illness ranges from 4 days to 15 days in RSV-positive patients and their sample collected during the hospital admission and thus the collection times give rise to this discrepancy: IgM antibodies against the virus appear after 1 week of infection and may persist for 20 days to 2-3 months,^[31] while viral shedding persists from day 1-7 in nasopharyngeal aspirates and up to 21 days in blood samples.^[24] Due to its low sensitivity and inability to pinpoint the stage of infection, serology alone is insufficient for diagnosing RSV; real-time PCR remains the preferred method for accurate detection.

When conventional PCR was compared to real-time PCR, it gave 40% sensitivity and 79.3% specificity, although 87% sensitivity and 100% specificity was reported in other studies.^[25] The viral culture was done for 16 representative samples, and then real-time PCR sensitivity and specificity were compared to viral culture, showing 28.8% sensitivity and 100% specificity, thus confirming the diagnostic accuracy.

Using a CRS defined as positivity by any 2 diagnostic tests, ELISA and rapid tests demonstrated moderate sensitivity and specificity, while conventional PCR showed perfect specificity but lower sensitivity. These findings highlight the variability in test performance and reinforce the need for combining methods to improve diagnostic accuracy in RSV detection.

CONCLUSIONS

This study highlights the significant burden of RSV among infants and young children in Aligarh, with peak circulation from October to January. RSV B was the predominant subtype, and common symptoms included tachypnea, fever, cough, wheezing, and chest retractions. Key risk factors were overcrowding, lack of exclusive breastfeeding, and prior NICU admission. Real-time PCR outperformed ELISA, conventional RT-PCR, and rapid tests, underscoring the need for sensitive molecular assays for accurate RSV detection and improved disease management.

Author's contributions: ZN: Formal analysis, Investigation, Methodology, Writing – original draft; HS: Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Validation; AR: Formal analysis, Investigation, Methodology, Supervision, Validation; PAK: Formal analysis, Investigation, Methodology, Writing – original draft; MA: Methodology; SGA: Methodology; KA: Supervision and validation; MAK: Statistical Analysis; YS: Methodology; MAA: Methodology and Supervision; Nazish Fatima: Supervision, Validation.

Ethical approval: The research/study was approved by the Institutional Review Board at the Institutional Ethics Committee, Faculty of Medicine, AMU, Aligarh, approval number IECJNMC/1077, dated 26th August 2023.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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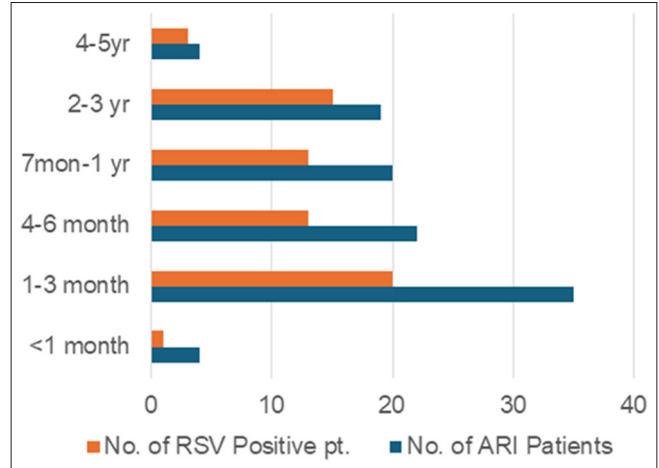
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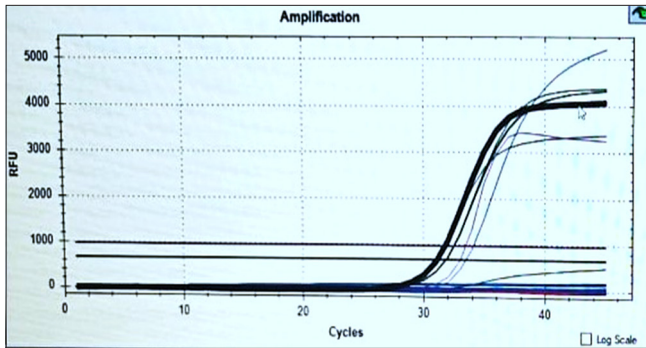
SUPPLEMENTARY TABLES AND FIGURES



Supplementary Figure 1: BinaxNOW Respiratory syncytial virus (RSV) Card showing positive result for RSV antigen from a patient sample.



Supplementary Figure 3: Age-wise distribution of respiratory syncytial virus-positive patients.



Supplementary Figure 2: Amplification curves obtained with real star respiratory syncytial virus (RSV) RT-PCR Kit 3.0, RSV A-specific ribonucleic acid (RNA) was detected at Cy[®]5channel; RSV B-specific RNA was detected at FAM[™] channel.

Supplementary Table 1: Selected primers for TaqMan amplification of viral RNA from RSV A and RSV B.

	The primer sequences
RSV A Forward	5'AGCCTACAGGAAAGCCAACC-3'
RSV A Reverse	5'GGCGATTGCAGATCCAACAC-3'
RSV B Forward	5' ACATTGGGGCAAATGCAACC-3'
RSV B Reverse	5' ATTTGATGTGGAGGGCTCGG-3'

RNA: Ribonucleic acid, RSV: Respiratory syncytial virus

Supplementary Table 2: Evaluation of real-time PCR test sensitivity and specificity to viral culture after considering culture as a gold standard.

Methods of diagnosis	Viral culture (n=16) positive	Viral culture negative	Total (%)
Real-time PCR (n=16)			
Positive	2	0	2
Negative	5	9	14
Total	7	9	16
Statistics	Value (%)	95% CI	
Sensitivity	28.57	3.6–70.96	
Specificity	100.00	66.37–100.00	
Positive predictive value	100.00	15.81–100.00	
Negative predictive value	64.29	52.98–74.20	

CI: Confidence interval, PCR: Polymerase chain reaction