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Detection and Epidemiological Characteristics of Respiratory Syncytial Virus, Influenza Virus, and SARS-CoV-2 Among Patients with Acute Respiratory Illness Using Multiplex RT-PCR: A Hospital-Based Cross-Sectional Study

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Abstract

Background: Acute respiratory infections remain a major cause of morbidity and hospitalization worldwide. Respiratory Syncytial Virus (RSV), Influenza viruses, and Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) are among the most important viral pathogens causing respiratory illness. Early and accurate identification of these viruses is essential for patient management, infection control, and surveillance. ***Methods:*** A hospital-based cross-sectional study was conducted on 110 respiratory specimens collected from patients with symptoms suggestive of respiratory viral infection. Multiplex RT-PCR was performed for simultaneous detection of RSV, Influenza A, Influenza B, Influenza A (H1N1), Influenza A (H3N2), and SARS-CoV-2. Demographic, clinical, seasonal, and hospitalization-related data were analyzed. ***Results:*** Among 110 respiratory samples tested, 12 (10.9%) were positive for at least one respiratory virus. RSV was the most frequently detected virus [7/110, 6.36%], followed by Influenza viruses [5/110, 4.55%]. No SARS-CoV-2-positive cases were identified. RSV infection predominantly affected children younger than one year (23.08%) and showed male predominance (71.4%). Influenza infections were confined to adults aged 19–60 years and were more common among females (60%). Cough was the most common symptom among RSV-positive (85.7%) and Influenza-positive patients (100%). Seasonal analysis demonstrated peak RSV and Influenza activity during the autumn season. Most positive cases were hospitalized. ***Conclusion:*** RSV emerged as the predominant respiratory virus among the studied population, particularly affecting infants, whereas Influenza infection was more common among adults. The absence of SARS-CoV-2 positivity suggests limited circulation during the study period. Multiplex RT-PCR remains a valuable diagnostic tool for respiratory virus surveillance and clinical management.

Keywords: Respiratory Syncytial Virus, Influenza Virus, Influenza A (H1N1) SARS-CoV-2, Multiplex RT-PCR, Respiratory Viral Infections, Hospital-Based Study, Respiratory Pathogens, Seasonal Distribution, Molecular Diagnosis, Acute Respiratory Illness (ARI)

INTRODUCTION

Acute respiratory tract infections (ARTIs) represent one of the leading causes of hospitalization and mortality globally, particularly among young children, older adults, and immunocompromised individuals. Respiratory viruses account for a substantial proportion of these infections and contribute significantly to the global healthcare burden. (1–4)

Respiratory Syncytial Virus (RSV) is recognized as the leading viral cause of lower respiratory tract infection among infants and young children. Globally, RSV is responsible for millions of hospitalizations annually and remains an important public health concern. (1–4,10–12,17) Influenza viruses continue to cause seasonal epidemics with substantial morbidity and mortality, especially among high-risk populations. (5–9,15) Since 2019, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has emerged as a major respiratory pathogen, highlighting the importance of continuous respiratory virus surveillance and strengthening respiratory disease monitoring systems worldwide. (9,14–16)

The clinical manifestations of RSV, Influenza, and SARS-CoV-2 frequently overlap, making laboratory confirmation essential for accurate diagnosis and appropriate patient management. (9,13,18,19) Multiplex real-time reverse transcription polymerase chain reaction

(RT-PCR) enables simultaneous detection of multiple respiratory pathogens with high sensitivity and specificity, thereby improving diagnostic efficiency, patient care, and epidemiological surveillance. (20–22)

The present study was undertaken to determine the prevalence and distribution of RSV, Influenza virus, and SARS-CoV-2 among patients presenting with respiratory illness and to assess demographic, clinical, and seasonal characteristics associated with these infections. Such information is important for understanding local epidemiological trends and guiding public health interventions aimed at reducing the burden of respiratory viral infections. (14–18)

MATERIALS AND METHODS

This hospital-based cross-sectional observational study was conducted over a period of September 2025 to March 2026 at the Virology Laboratory, Department of Microbiology, Pt. JNM Medical College, Raipur, Chhattisgarh, India. A total of 110 respiratory specimens were collected from patients presenting with symptoms suggestive of acute respiratory infection, including fever, cough, sore throat, breathlessness, nasal discharge, and other influenza-like illness manifestations. Respiratory samples were collected under aseptic conditions by trained healthcare personnel and transported to the virology laboratory following standard biosafety and specimen transport guidelines. (14–16) Viral nucleic acid extraction was performed according to the manufacturer's instructions, and all specimens were subsequently tested using multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) for the simultaneous detection of Respiratory Syncytial Virus (RSV), Influenza A virus, Influenza A (H3N2), Influenza B virus, and Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). (20–22) Demographic and clinical information, including age, gender, presenting symptoms, seasonal occurrence, and hospitalization status, were recorded and analyzed. The results were expressed as frequencies and percentages. Ethical approval for the study was obtained from the Institutional Ethics Committee prior to commencement of the study, and all patient information was kept confidential throughout the investigation.

Inclusion Criteria

Patients presenting with respiratory symptoms such as fever, cough, sore throat, breathlessness, nasal discharge, and other influenza-like illness manifestations.

Exclusion Criteria

Samples with inadequate clinical information or unsuitable specimen quality were excluded.

Statistical Analysis

Data were analyzed using descriptive statistics. Categorical variables were expressed as frequencies and percentages.

RESULTS

Distribution of RSV, Influenza and COVID-19

A total of 110 respiratory samples were analyzed using multiplex RT-PCR. Respiratory Syncytial Virus (RSV) demonstrated the highest positivity rate, with 7 positive cases (6.36%), followed by Influenza virus with 5 positive cases (4.55%). Among Influenza-positive samples, two cases were positive for Influenza B, two for Influenza A (H3N2), and one for Influenza A. No SARS-CoV-2-positive samples were detected. Overall, 12 of 110 samples (10.9%) were positive for at least one respiratory virus. RSV emerged as the predominant viral pathogen during the study period. Detailed distribution of viral positivity is presented in Table 1.

Table 1. Distribution of RSV, Influenza and COVID-19 among study participants (n = 110)

Virus	Total Tested	Positive n (%)	Negative n (%)
RSV	110	7 (6.36)	103 (93.64)
Influenza	110	5 (4.55)	105 (95.45)
COVID-19	110	0 (0.00)	110 (100.0)

Gender-wise Distribution of Positive Cases:

Among RSV-positive cases, males accounted for 71.4% (5/7), whereas females accounted for 28.6% (2/7). In contrast, Influenza infection was more common among females (60.0%) than males (40.0%). No COVID-19-positive cases were detected. Gender-wise distribution is shown in Table 2.

Table 2. Gender-wise distribution of positive cases

Virus	Male n (%)	Female n (%)	Total Positive
RSV	5 (71.4)	2 (28.6)	7
Influenza	2 (40.0)	3 (60.0)	5
COVID-19	0	0	0

Age-group-wise Distribution of Positive Cases

RSV infections were predominantly observed among infants aged less than one year, accounting for 6 cases (23.08%), while one case (8.33%) occurred in children aged 1–5 years. No RSV infections were observed among older age groups. All Influenza-positive cases occurred among adults aged 19–60 years (15.63%). No COVID-19-positive cases were identified in any age category (Table 3).

Table 3. Age-group-wise distribution of positive cases

Age Group	Total (n)	RSV Positive	Influenza Positive	COVID Positive
0-10 years	52	7 (13.47%)	0	0
11-20 years	11	0	0	0
21-30 years	6	0	1 (16.67%)	0
31-40 years	11	0	3 (27.27%)	0
41-50 years	8	0	1 (16.67%)	0
51-60 years	7	0	0	0
61-70 years	11	0	0	0
71-80 years	4	0	0	0

Symptom-wise Distribution of RSV-positive Cases

Among RSV-positive patients, cough was the most frequently reported symptom (85.7%), followed by breathlessness (57.1%) and sore throat (57.1%). Nasal discharge was observed in 28.6% of cases, whereas fever and sputum production were present in 14.3% of patients.

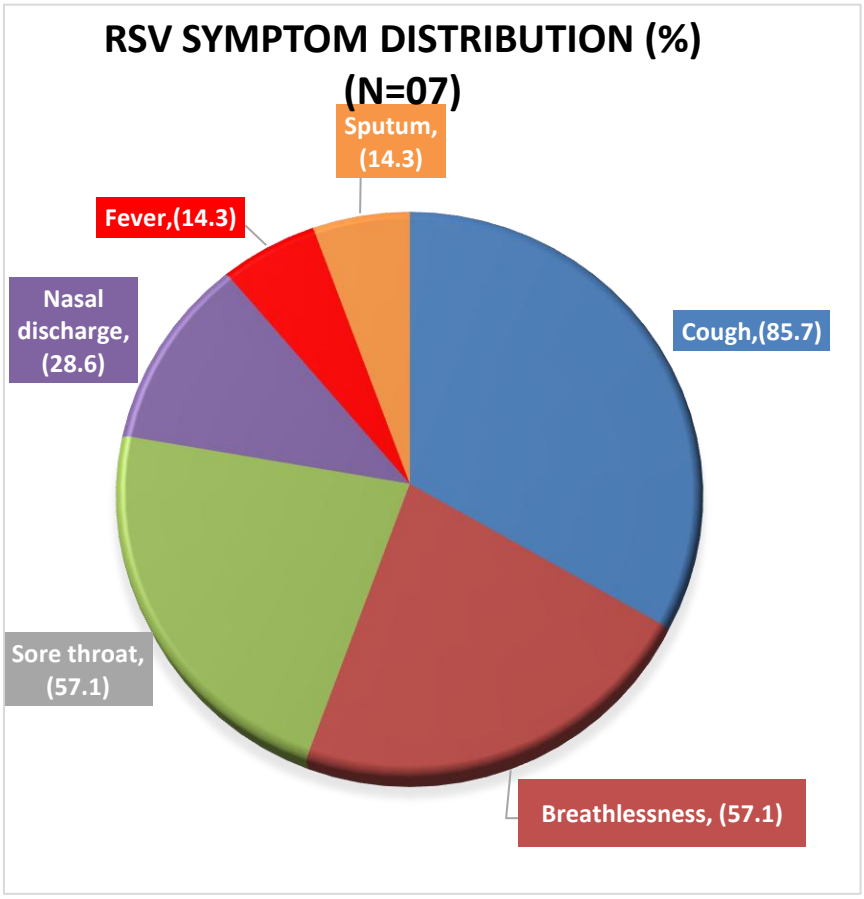

Figure 1. Symptom-wise distribution of Influenza-positive cases among study participants (n = 7).

Symptom-wise Distribution of Influenza-positive Cases

Clinical symptoms observed among the five patients who tested positive for Influenza virus infection, cough was the most common symptom, reported in all cases (100%), indicating that it was the predominant clinical manifestation of Influenza infection in the study population. Breathlessness and fever were the second most frequently observed symptoms, each occurring in 60% of patients. Sore throat and sputum production were present in 40% of cases each. Less frequently reported symptoms included nasal discharge, diarrhoea, vomiting, chest pain, and body ache, each observed in 20% of patients. The findings demonstrate that respiratory symptoms, particularly cough and breathlessness, were the dominant clinical features among Influenza-positive individuals, while gastrointestinal symptoms and generalized body aches were comparatively less common.

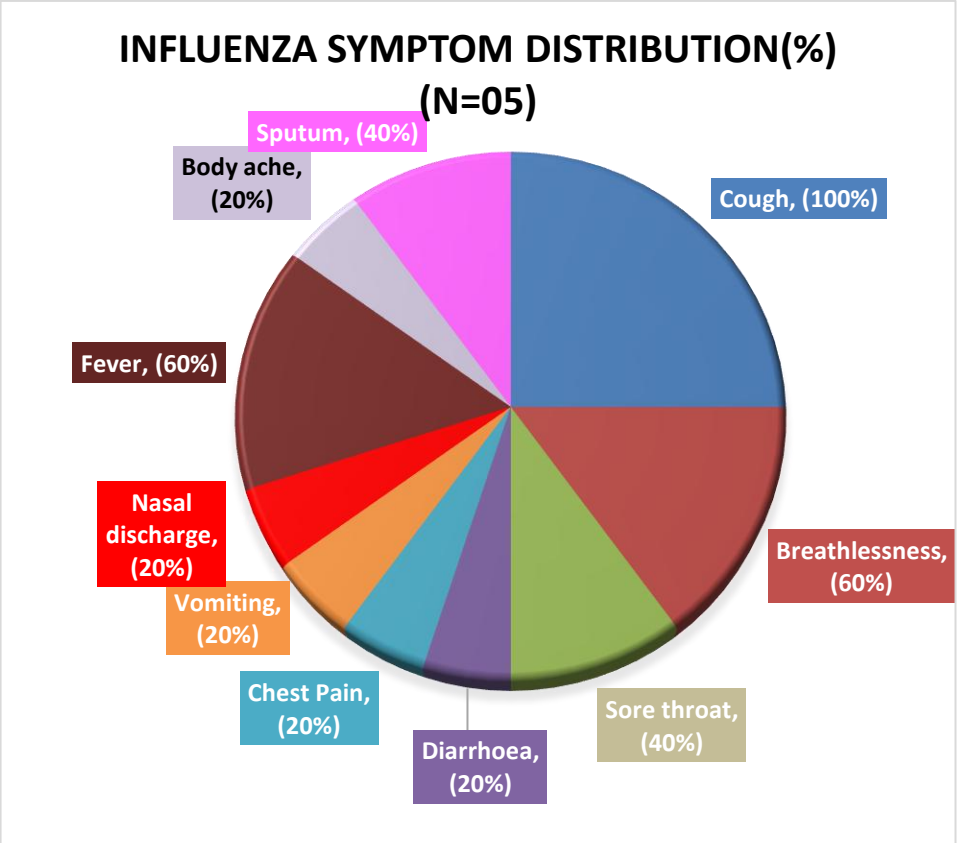

Figure 2. Symptom-wise distribution of Influenza-positive cases among study participants (n = 5).

Seasonal Distribution of RSV-positive Cases

RSV infections showed marked seasonality, with the majority of cases occurring during autumn (October–November), accounting for five cases, followed by two cases during the monsoon season.

Seasonal Distribution of Influenza-positive Cases

Influenza positivity was highest during autumn (60%), followed by winter and spring (20% each). No Influenza cases were detected during summer or monsoon seasons.

Hospitalization Status of Study Participants

Of the 110 study participants, 100 (90.9%) required hospitalization, while 10 (9.1%) were managed without admission. All RSV-positive patients and four of the five Influenza-positive patients were hospitalized.

Routine vaccination of the positive patients

All the 7 positive patients were children up to 2 years of age, youngest being 1 month old and oldest being 2 years old. All the children were immunized as per the routine vaccine schedule depending upon their age.

DISCUSSION

The present study identified RSV as the predominant respiratory virus among patients with acute respiratory illness. The observed RSV positivity rate aligns with previous studies from India and other countries reporting RSV as a major cause of respiratory infection among infants and young children. (1–4,10–12,17)

The exclusive detection of RSV among paediatric age groups highlights the susceptibility of infants to RSV-

associated lower respiratory tract disease. Similar age-specific trends have been reported globally, where RSV remains one of the leading causes of hospitalization among children younger than five years of age. (1–4,10–12)

Influenza infections were observed primarily among adults, consistent with reports demonstrating higher influenza activity among working-age populations. Detection of both Influenza A (H3N2) and Influenza B reflects the continued circulation of seasonal influenza strains reported through national and global surveillance programs. (5–9,15)

The predominance of respiratory symptoms such as cough, breathlessness, sore throat, and fever among RSV- and Influenza-positive patients observed in the present study is comparable to findings reported in previous clinical and epidemiological investigations of respiratory viral infections. (13,18,19)

The absence of SARS-CoV-2 positivity may indicate reduced community transmission during the study period, likely influenced by increasing population immunity, vaccination coverage, and evolving epidemiological patterns following the COVID-19 pandemic. Similar reductions in SARS-CoV-2 activity accompanied by the re-emergence of other respiratory viruses have been documented in surveillance studies worldwide. (9,14–16)

Seasonal peaks during autumn are consistent with previous observations regarding environmental and climatic factors influencing respiratory virus transmission. Seasonal variations in temperature, humidity, and population movement patterns have been shown to affect the circulation of RSV and Influenza viruses. (5,8,14–16)

The routine childhood vaccinations protect against critical viral diseases like Polio, Measles, Mumps, Rubella (MMR), Rotavirus, Varicella (Chickenpox), Hepatitis B, JE, fIPV etc has shown no correlation in protection from RSV vaccine as they target different pathogen which do not show any cross- protection.

The use of multiplex RT-PCR in the present study enabled rapid and simultaneous detection of multiple respiratory viruses with high sensitivity and specificity, supporting its utility in routine clinical diagnosis and respiratory virus surveillance. (20–22)

Continuous surveillance is therefore essential for preparedness, early outbreak detection, and implementation of timely public health interventions aimed at reducing the burden of respiratory viral infections. (14–16)

Strengths of the Study

- Use of multiplex RT-PCR for simultaneous viral detection.
- Inclusion of demographic, clinical, and seasonal analysis.
- Real-world hospital-based surveillance data.

Limitations

- Single-center study.
- Small sample size.
- Lack of viral load assessment.
- Absence of co-infection analysis.

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