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DETECTION OF MULTIPLE RESPIRATORY VIRUSES USING MULTIPLEX PCR PANELS

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Abstract

Background: Respiratory viral infections are a major cause of morbidity and hospital visits worldwide. Early and accurate identification of respiratory pathogens is essential for appropriate patient management, infection control, and reduction of unnecessary antimicrobial therapy. Multiplex polymerase chain reaction (PCR) assays have emerged as rapid and sensitive diagnostic tools capable of simultaneously detecting multiple respiratory viruses.

Aim: To detect respiratory viral pathogens using multiplex PCR panels among patients presenting with respiratory tract infections.

Materials and Methods: This prospective observational study was conducted in the Department of Microbiology and Molecular Biology at Leonard Hospital from October 2025 to December 2025. A total of 61 respiratory samples collected from patients with symptoms suggestive of respiratory viral infection were analyzed using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV multiplex PCR panel. The assay simultaneously detected SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) A+B. Data were analyzed using descriptive statistical methods.

Results: Out of 61 respiratory samples analyzed, viral pathogens were detected in 15 (24.6%) cases. Influenza A virus H3N2 was the predominant pathogen and was identified in 13 (21.3%) patients, while RSV A type was detected in 2 (3.3%) cases. No cases of Influenza B virus or SARS-CoV-2 infection were identified during the study period. Coinfections were not observed. The majority of samples, 46 (75.4%), were negative for all tested respiratory viruses.

Conclusion: Multiplex PCR using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel is a rapid, reliable, and sensitive method for detection of respiratory viral pathogens. The study demonstrated predominance of Influenza A virus H3N2 among respiratory infections during the study period. Multiplex molecular diagnostics can improve early diagnosis, optimize patient management, and strengthen infection control practices.

KEYWORDS: Multiplex PCR, Respiratory viruses, Influenza A H3N2, RSV, SARS-CoV-2, Molecular diagnosis

INTRODUCTION

Respiratory tract infections are among the most common infectious diseases worldwide and remain a major cause of morbidity and mortality across all age groups, particularly among children, elderly individuals, and immunocompromised patients [1]. Viral pathogens contribute significantly to both upper and lower respiratory tract infections and are responsible for substantial healthcare burden, hospital admissions, and economic loss globally [2]. Common respiratory viruses include Influenza viruses, Respiratory Syncytial Virus (RSV), Rhinovirus, Adenovirus, Human Metapneumovirus, Parainfluenza viruses, and Coronaviruses [3].

Early and accurate identification of respiratory viral infections is essential for appropriate clinical management, implementation of infection control measures, reduction of unnecessary antibiotic use, and optimization of antiviral therapy when indicated [4]. Conventional diagnostic methods such as viral culture, serological assays, and antigen detection techniques are often limited by lower sensitivity, prolonged turnaround time, and inability to detect multiple pathogens simultaneously [5]. These limitations may delay diagnosis and adversely affect patient care.

Molecular diagnostic techniques, particularly multiplex polymerase chain reaction (PCR), have revolutionized the diagnosis of respiratory viral infections in recent years [6]. Multiplex PCR assays allow simultaneous amplification and detection of multiple viral targets in a single reaction with high sensitivity and specificity [7]. Compared with traditional diagnostic approaches, multiplex PCR panels provide rapid results, improved diagnostic yield, and enhanced detection of coinfections [8].

The increasing availability of multiplex PCR panels has significantly improved laboratory diagnosis of respiratory pathogens and has become an important component of modern clinical microbiology practice [9]. Accurate viral identification using molecular methods also contributes to better antimicrobial stewardship by minimizing inappropriate antibiotic prescription and supporting targeted patient management [10].

The present study was conducted to evaluate the detection profile of respiratory viruses using multiplex PCR panels among patients presenting with respiratory symptoms at Leonard Hospital.

MATERIALS AND METHODS

Study Design
Prospective observational study.

Study Duration
October 2025 to December 2025.

Study Setting
The study was conducted in the Department of Microbiology and Molecular Biology at Leonard Hospital.

Study Population
Patients presenting with symptoms suggestive of acute respiratory tract infection were included in the study.

Sample Size
A total of 61 respiratory samples were analyzed during the study period.

Inclusion Criteria
1. Patients presenting with fever, cough, sore throat, breathlessness, nasal congestion, or other symptoms suggestive of respiratory viral infection.
2. Patients of all age groups attending outpatient or inpatient departments.
3. Patients providing informed consent for participation.

Exclusion Criteria
1. Inadequate or improperly collected respiratory specimens.
2. Samples with insufficient quantity for molecular testing.
3. Patients already receiving antiviral treatment before sample collection.

Sample Collection
Nasopharyngeal and/or throat swab specimens were collected under aseptic precautions using sterile flocked swabs. Samples were immediately transferred into viral transport media and transported to the molecular laboratory maintaining cold chain conditions.

Molecular Testing Procedure
Respiratory samples were processed using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel, a multiplex real-time PCR-based diagnostic assay designed for simultaneous qualitative detection of four major respiratory viral pathogens:
1. SARS-CoV-2
2. Influenza A virus
3. Influenza B virus
4. Respiratory Syncytial Virus (RSV) A+B

The assay was performed according to the manufacturer's instructions. The test cartridge integrates nucleic acid extraction, amplification, and detection within a closed automated system, minimizing contamination risk and reducing turnaround time.

After sample loading into the QIAstat-Dx analyzer, automated extraction and multiplex PCR amplification were carried out. Amplified targets were detected through fluorescence-based real-time monitoring, and results were interpreted using the software provided with the instrument.

Data Collection
Patient demographic details, clinical presentation, and laboratory findings were recorded in a structured data collection form. The results of multiplex PCR testing were documented and analyzed.

Statistical Analysis
Data were entered into Microsoft Excel and analyzed using descriptive statistical methods. Results were expressed as frequencies, percentages, tables, and charts wherever appropriate.

Ethical Consideration
Institutional ethical approval was obtained prior to commencement of the study from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), under reference number IEC-NI/24/FEB/91/22.

RESULTS
A total of 61 respiratory samples from patients presenting with symptoms suggestive of respiratory tract infection were analyzed using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel, which simultaneously detects four major respiratory viral pathogens including SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) A+B. Viral pathogens were detected in 15 (24.6%) cases, while 46 (75.4%) samples were negative for all tested respiratory viruses.

Table 1: Overall Viral Detection Status

Detection Status	Number of Cases	Percentage
Positive	15	24.6%
Negative	46	75.4%

Among the positive samples, Influenza A virus H3N2 was the predominant pathogen and was detected in 13 (21.3%) patients. RSV A type was detected in 2 (3.3%) cases. No cases of Influenza B virus or SARS-CoV-2 infection were identified during the study period.

Table 2: Distribution of Respiratory Viruses Detected

Viral Pathogen	Positive Cases	Percentage
Influenza A Virus H3N2	13	21.3%
RSV A Type	2	3.3%
Influenza B Virus	0	0%
SARS-CoV-2	0	0%
Negative for all pathogens	46	75.4%

Further analysis showed that all positive samples represented isolated single viral infections, and no mixed viral coinfections were detected in the study population.

Table 3: Viral Positivity Pattern

Viral Infection Pattern	Number of Cases
Influenza A H3N2 only	13
RSV A only	2
Influenza B only	0
SARS-CoV-2 only	0
Coinfection	0

The findings of the present study demonstrate that Influenza A virus H3N2 was the most frequently circulating respiratory viral pathogen during the study period, whereas RSV A positivity was comparatively lower. No Influenza B virus or SARS-CoV-2 infections were identified among the tested samples.

DISCUSSION

Respiratory viral infections continue to represent a major public health concern worldwide due to their high transmissibility, seasonal outbreaks, and associated morbidity. Rapid and accurate identification of respiratory pathogens is essential for timely clinical management, implementation of infection control measures, and reduction of unnecessary antimicrobial therapy. In the present study, multiplex PCR technology was utilized for simultaneous detection of major respiratory viral pathogens using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel.

Among the 61 respiratory samples analyzed, viral pathogens were detected in 24.6% of cases. Influenza A virus H3N2 was identified as the predominant pathogen, accounting for 21.3% of total cases, whereas RSV A type was detected in 3.3% of patients. No cases of Influenza B virus or SARS-CoV-2 infection were observed during the study period. These findings suggest active circulation of Influenza A H3N2 during the study duration and comparatively lower prevalence of other respiratory viral pathogens.

The predominance of Influenza A virus observed in the present study is consistent with several previous molecular epidemiological studies that reported Influenza A as a leading cause of seasonal respiratory viral infections [1,2]. Influenza A H3N2 strains are known to contribute significantly to seasonal influenza outbreaks and are frequently associated with increased hospitalization rates, especially among elderly individuals and patients with comorbidities [3].

RSV A positivity was observed in a smaller proportion of cases. RSV remains an important respiratory pathogen, particularly among pediatric and elderly populations, where it may lead to severe lower respiratory tract infections [4]. The lower RSV positivity in the present study may be related to seasonal variation and the limited study duration.

No SARS-CoV-2 positive cases were identified among the tested samples. This finding may reflect declining circulation of SARS-CoV-2 during the study period or effective public health control measures. Similarly, no Influenza B virus positivity was observed, suggesting lower community transmission of this subtype during the study duration.

Multiplex PCR assays offer several important advantages compared with conventional diagnostic methods such as viral culture and antigen detection tests. The QIAstat-Dx multiplex PCR panel enables simultaneous detection of multiple respiratory pathogens with high sensitivity, specificity, and shorter turnaround time [5]. The fully integrated cartridge-based system also minimizes manual handling and reduces the risk of laboratory contamination.

Another significant advantage of multiplex PCR is its ability to identify mixed viral infections. However, no coinfections were detected in the present study. This may be attributed to the relatively small sample size, limited circulation of multiple respiratory viruses, or seasonal epidemiological patterns during the study period.

A substantial proportion of samples (75.4%) tested negative for all targeted viral pathogens. This may indicate infections caused by bacterial pathogens or respiratory viruses not included in the testing panel. Additionally, timing of specimen collection and viral load may influence PCR positivity rates.

The findings of the present study highlight the clinical utility of multiplex molecular diagnostics in respiratory virus detection. Early and accurate viral identification can support appropriate patient isolation, reduce unnecessary antibiotic use, improve antimicrobial stewardship, and facilitate better infection control practices in healthcare settings.

Despite its advantages, multiplex PCR testing is associated with higher cost and requirement for specialized laboratory infrastructure, which may limit its widespread use in resource-limited settings. Nevertheless, its rapid turnaround time and high diagnostic accuracy make it an important diagnostic tool in modern clinical microbiology laboratories.

CONCLUSION

Multiplex PCR using the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel proved to be a rapid, sensitive, and reliable method for the detection of respiratory viral pathogens in patients with suspected respiratory tract infections. Influenza A virus H3N2 was identified as the predominant circulating virus during the study period, while RSV A positivity was comparatively lower. No cases of Influenza B virus or SARS-CoV-2 infection were detected.

The study highlights the importance of multiplex molecular diagnostics for early identification of respiratory viruses, enabling timely clinical decision-making, improved infection control practices, and reduction of unnecessary antimicrobial use. The ability to simultaneously detect multiple respiratory pathogens with a single assay makes multiplex PCR a valuable diagnostic tool in modern clinical microbiology laboratories.

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