

## Evaluation of viral respiratory pathogens detected in a tertiary university hospital between 2021 and 2025

Üçüncü basamak bir üniversite hastanesinde 2021-2025 tarihleri arasında saptanan viral solunum yolu etkenlerinin değerlendirilmesi

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### ABSTRACT

**Aim:** Infections caused by respiratory tract pathogens cause high morbidity and mortality and pose a significant threat to public health. In this study, we aimed to determine the prevalence and seasonal distribution of common respiratory viruses in patients admitted to our university hospital between 2021 and 2025.

**Material and Methods:** Between January 1, 2021 and January 1, 2025, 3131 nasal swab samples from patients with a preliminary diagnosis of acute respiratory tract infection were sent to the Molecular Laboratory of Ondokuz Mayıs University Faculty of Medicine Hospital. 24 different pathogens were detected by multiplex real time polymerase chain reaction method (Qiagen, Germany).

**Results:** Among the 3,131 samples analyzed, one or more viral agents were detected in 1,792 cases (57.23%). The most commonly identified viral pathogens were rhinovirus/enterovirus, respiratory syncytial virus A/B, SARS-CoV-2, and influenza A. The most frequently observed dual infection was influenza A combined with influenza A (subtype H1N1/2009), found in 22% of the co-infected cases. Seasonal distribution analysis showed that rhinovirus/enterovirus infections were more common in autumn, influenza A in winter, and respiratory syncytial virus A/B and SARS-CoV-2 in both autumn and winter months.

**Conclusion:** Determination of the viral pathogen by molecular methods in respiratory tract infections will be useful in preventing unnecessary use of antibiotics with correct treatment. It will also contribute to rapid and accurate management decisions with infection control measures and shorten hospitalization periods.

**Keywords:** Coinfection, multiplex PCR, respiratory viral panel

### ÖZ

**Amaç:** Solunum yolu patojenlerinin neden olduğu enfeksiyonlar, yüksek morbidite ve mortaliteye yol açmakta olup halk sağlığı açısından önemli bir tehdit oluşturmaktadır. Bu çalışmada, 2021-2025 yılları arasında üniversite hastanemize başvuran hastalarda yaygın solunum yolu virüslerinin prevalansının ve mevsimsel dağılımının belirlenmesi amaçlanmıştır.

**Gereç ve Yöntemler:** Ondokuz Mayıs Üniversitesi Tıp Fakültesi Hastanesi Moleküler Laboratuvarı'na 1 Ocak 2021-1 Ocak 2025 tarihleri arasında akut solunum yolu enfeksiyonu ön tanısı olan hastalardan alınan 3131 nazal sürüntü örneği gönderilmiştir. 24 farklı patojenin saptanabildiği multipleks real time polimeraz zincir reaksiyonu yöntemi (Qiagen, Almanya) ile viral etkenlerin varlığı araştırılmıştır.

**Bulgular:** Toplam 3131 örneğin 1792'sinde (%57,23) bir veya daha fazla etken tespit edilmiştir. Enfeksiyona neden olan en yaygın viral etkenler rinovirüs/enterovirüs, respiratuar sinsityal virüs A/B, SARS-CoV-2, influenza A'dır. En yaygın ikili etken ise %22 prevalans ile influenza A+influenza A (alt tip H1N1/2009) olmuştur. Mevsimsel olarak değerlendirildiğinde; sonbaharda rinovirüs/enterovirüs, kışın influenza A, sonbahar-kış aylarında ise respiratuar sinsityal virüs A/B ve SARS-CoV-2'nin daha sık enfeksiyona neden olduğu görülmüştür.

**Sonuç:** Solunum yolu enfeksiyonlarında moleküler yöntemlerle viral patojenin belirlenmesi, doğru tedavi ile antibiyotiklerin gereksiz kullanımını önlemede yararlı olacaktır. Aynı zamanda enfeksiyon kontrol önlemleriyle hızlı ve doğru yönetim kararlarının alınmasına ve hastanede yatış sürelerinin kısalmasına katkı sağlayacaktır.

**Anahtar Kelimeler:** Koenfeksiyon, multipleks PZR, solunum yolu viral panel

## Highlights

- The comprehensive prevalence of respiratory viruses in the region was determined with 3,131 samples during the 2021–2025 period.
- The viral positivity rate was 57.23%, with rhinovirus/enterovirus identified as the most dominant agent.
- High rates of co-infection were detected among influenza A subtypes, revealing simultaneous circulation dynamics.
- Distinct seasonal peaks were observed for rhinovirus/enterovirus in autumn and for influenza A in winter.
- Multiplex PCR was shown to provide rapid and accurate diagnosis, reducing unnecessary antibiotic use and improving clinical management.

## INTRODUCTION

Respiratory tract infections (RTIs) are more commonly observed in children but also cause morbidity and mortality in adults, representing a significant public health concern worldwide (1). While mortality due to respiratory viral infections is low among healthy individuals in developed countries, it is higher in less developed regions, particularly among children, with approximately 2 million children aged 0–4 years dying annually worldwide (2,3). RTIs can be caused by bacteria, viruses, fungi, and parasites; however, viruses are known to be the primary causative agents in most cases. The prevalence and seasonal distribution of these microorganisms can vary between countries and even between regions within the same country (4,5). The most commonly encountered viral pathogens include influenza viruses, rhinoviruses, respiratory syncytial virus (RSV), human coronaviruses, and parainfluenza viruses (6). Clinically and radiologically, RTIs often present with similar features, and the absence of pathogen-specific signs or symptoms makes it difficult to differentiate between causative agents. Definitive diagnosis largely relies on microbiological testing. High-sensitivity, high-performance tests capable of detecting multiple groups of microorganisms simultaneously and rapidly have become indispensable. Accurate and timely identification of RTI pathogens enables prompt initiation of antiviral therapy and prevents unnecessary antibiotic use, which is particularly valuable given the current challenges of bacterial resistance. Furthermore, it contributes to reducing hospital stay, the risk of nosocomial transmission, and treatment costs (7–10). The aim of this study was to investigate the prevalence and seasonal distribution of respiratory pathogens in patients presenting to Ondokuz Mayıs University Faculty of Medicine with a preliminary diagnosis of acute RTI between 2021 and 2025.

## MATERIAL and METHODS

Prior to the study, approval was obtained from the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine (Date: 2025, Decision No: 47). A to-

tal of 3,131 nasal swab samples collected between 2021 and 2025 at the Molecular Laboratory of the Department of Medical Microbiology, Ondokuz Mayıs University, were included in the study. For patients who provided multiple samples within a month, only the first sample was included. The samples, transported in viral transport medium (UTM, Copan Diagnostics, Italy) under cold chain conditions, were analyzed at the Medical Microbiology Laboratory using the respiratory syndromic panel kit (Qiagen, Germany) according to the manufacturer's instructions. The respiratory syndromic viral panel (Qiagen, Germany) was performed following the recommendations of the manufacturer. The panel was capable of detecting the following pathogens within approximately 70 minutes: Adenovirus, Bocavirus, *Bordetella pertussis*, *Chlamydomphila pneumoniae*, Coronavirus 229E, HKU1, NL63, OC43, Human metapneumovirus A/B, Influenza A, Influenza A (subtype H1), Influenza A (subtype H1N1/2009), Influenza A (subtype H3), Influenza B, *Mycoplasma pneumoniae*, Parainfluenza types 1–4, Rhinovirus/Enterovirus, RSV A/B, SARS-CoV-2, and *Legionella pneumophila*.

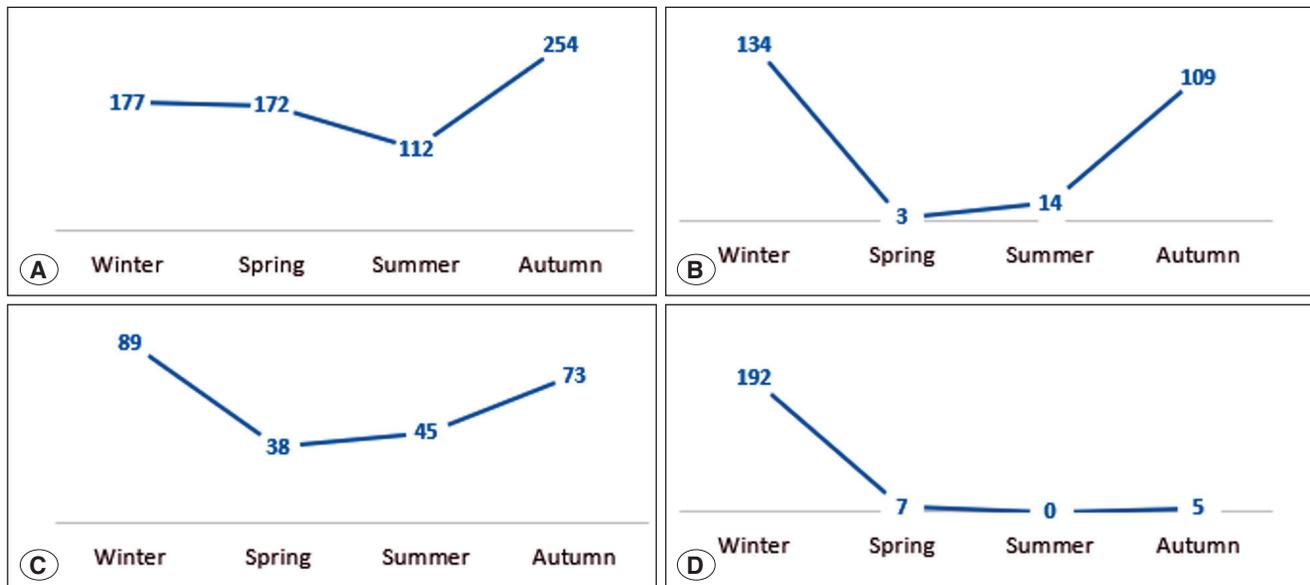
## RESULTS

Among the total 3,131 samples received in our laboratory, at least one viral pathogen was detected in 1,792 cases (57.23%). The detected pathogens were distributed as follows: Rhinovirus/Enterovirus in 39.8%, RSV in 14.5%, SARS-CoV-2 in 13.6%, and Influenza A in 11.3% (Table 1).

Dual infections were detected in 412 patients (23%), triple infections in 63 patients (3.5%), and quadruple infections in 10 patients (0.56%). Among dual infections, the most common combination was Influenza A + Influenza A (subtype H1N1/2009) at 22% (n=91), followed by Influenza A + Influenza A (subtype H3) at 16.5% (n=68), and Rhinovirus/Enterovirus + RSV A/B at 9.4% (n=39). Among triple infections, the most frequently observed combination was Influenza A + Influenza A (subtype H1N1/2009) + Rhinovirus/Enterovirus at 7.9% (n=5). The seasonal distribution of Rhinovirus/Enterovirus, SARS-CoV-2, RSV A/B, and influenza A infections is shown in Figure 1.

**Table 1:** Annual distribution of respiratory pathogens

Pathogen	2021		2022		2023		2024		Total	
	n	%	n	%	n	%	n	%	n	%
Rhinovirus/Enterovirus	117	40.3	176	37.2	213	43	209	39	715	39.8
RSV A/B	90	31	75	15.8	51	10.3	44	8.2	260	14.5
SARS-CoV-2	34	11.7	70	14.8	72	14.5	69	12.8	245	13.6
Influenza A	15	5.1	58	12.2	46	9.2	85	15.8	204	11.3
Adenovirus DNA	5	1.7	58	12.2	43	8.6	33	6.1	139	7.7
Parainfluenza 3	47	16.2	20	4.2	32	6.4	22	4.1	121	6.7
Influenza A (subtype H1N1/2009)	0	0	3	0.6	28	5.6	75	14	106	5.9
Human Metapneumovirus A/B	5	1.7	34	7.2	35	7	24	4.4	98	5.4
Influenza A (subtype H3)	15	5.1	54	11.4	16	3.2	4	0.7	89	4.9



**Figure 1:** Analysis of the seasonal variations of detected pathogens. **A)** Rhinovirus/Enterovirus, **B)** SARS-CoV-2, **C)** RSV A/B, **D)** Influenza A.

(A) Rhinovirus/Enterovirus cases remained high during winter and spring, showed a marked decrease in summer, and increased sharply again in autumn. (B) SARS-CoV-2 cases were elevated in winter, dropped sharply in spring, remained low during summer, and rose again in autumn. (C) RSV A/B infections were highest in winter, reached their lowest level in spring, showed a slight increase in summer, and remained at moderate levels in autumn. (D) Influenza A cases peaked in winter, declined substantially in spring and especially in summer, and showed a mild increase in autumn. Overall, the data demonstrate distinct seasonal patterns among respiratory pathogens.

**DISCUSSION**

Respiratory tract infections are among the most common infectious diseases worldwide, increasing healthcare vis-

its, hospitalizations, and work productivity losses, thereby imposing a significant economic burden (8). Acute RTIs account for a large proportion of all acute morbidities in developed countries. They are a leading cause of hospitalization among infants and young children in developed regions and a significant cause of mortality in developing countries (4). Studies using molecular diagnostic methods report detection rates of pathogens causing RTIs ranging from 30.9% to 96.1%, depending on the characteristics of the diagnostic kit, the demographic features of the patient population, and the timing of the study (11). While RTIs may be caused by bacteria, viruses, fungi, and parasites, viral agents are widely recognized as the predominant etiological factor (4). Accurate identification of viral pathogens cannot rely solely on clinical symptoms, as the manifestations are often non-specific and overlap among different viruses.

Therefore, laboratory-based tests are essential for etiological confirmation (7). The availability of specific antiviral treatments for influenza further emphasizes the importance of early and precise diagnosis. Recent advances in nucleic acid amplification techniques initially offered high sensitivity and rapidity, while modern multiplex systems now allow simultaneous detection of multiple pathogens, significantly improving diagnostic workflows (12, 13). Epidemiological data on respiratory pathogens show regional and seasonal variations. For example, RSV and influenza viruses are more common in winter, whereas rhinoviruses are prevalent from autumn through summer (5). Other studies have reported peaks in rhinovirus during October–November, and influenza virus, RSV, and human metapneumovirus during January–March (14). Influenza A is most frequent in winter, influenza B in spring, rhinovirus in autumn and spring, coronaviruses in summer, RSV in winter, and parainfluenza viruses in summer–autumn. Metapneumovirus and adenovirus infections also peak in winter (15, 16). In a study analyzing 5,102 clinical samples, approximately one-third of cases (33.4%) were positive for respiratory viruses, highlighting the dominant role of viral agents. Co-infections were observed in 18.6% of cases, with RSV + influenza A and RSV + parainfluenza being the most frequent combinations, indicating epidemiological co-circulation (5). In three-year data from Özdamar and Türkoğlu, at least one viral agent was detected in 83.5% of 283 samples, with the most common viruses being rhinovirus, adenovirus, influenza A/B, RSV A/B, human metapneumovirus A/B, and parainfluenza type 1 (17). Kuşkucu et al. analyzed 788 nasopharyngeal samples from patients diagnosed with acute RTIs between January 2010 and June 2018 using multiplex PCR. At least one viral agent was detected in 51.8% of samples, and multiple viruses in 7.2% of patients. The most frequently isolated agents were influenza A and B (11.4% and 5.5%), rhinovirus (14.9%), coronavirus (8.6%), RSV (7.1%), human metapneumovirus (4.1%), adenovirus (3.9%), and parainfluenza (3.6%) (15). Enterovirus and bocavirus were detected at rates below 1%. A single-center study at Süleyman Demirel University in 2019 examined 120 patients with suspected acute RTIs. At least one pathogen was detected in 59.2% (n=71) of cases, with RSV A/B and rhinovirus being the most frequent (16). In another study from March 2019 to December 2021, 8,825 respiratory samples were analyzed, and at least one viral agent was detected in 2,156 cases (24.4%). Single infections accounted for 85.5% of positive cases, predominantly rhinovirus, RSV A/B, human coronavirus, adenovirus, influenza virus, human metapneumovirus A/B, parainfluenza 1–4, influenza B, enterovirus, and bocavirus. Co-infections were observed in 14.5% of positive cases, with the most common combinations being adenovirus + rhinovirus, adenovirus + enterovirus, enterovirus + rhinovirus, rhinovirus + coronavirus, and RSV A/B + rhinovirus

(18). In our study, rhinovirus/enterovirus was detected in 39.8% of patients, with a higher prevalence in autumn and winter, consistent with the literature. Clinical manifestations are usually mild in healthy individuals but may be severe with a higher risk of complications in children under five, the elderly, and immunocompromised patients (6, 19). The use of respiratory viral panels provides valuable information to guide timely, targeted antimicrobial therapy, reduce unnecessary antibiotic use, and initiate antiviral treatment when appropriate (20). During pandemics, these panels can also serve as a clinical decision-support tool for predicting short-term outcomes and guiding patient management (21).

### Conclusion

Although our study is limited by its single-center design and the absence of comprehensive clinical correlation, continuous surveillance of circulating respiratory viruses remains critically important. Such monitoring not only contributes to the prevention of unnecessary or inappropriate antibiotic use, but also provides valuable guidance for clinicians in establishing timely and accurate treatment strategies, optimizing patient management, and supporting infection-control practices. Furthermore, population-level viral surveillance offers essential data for shaping public health policies, enabling early recognition of seasonal trends, outbreak preparedness, and resource allocation. In this context, multiplex real-time PCR assays for respiratory pathogens represent a highly effective diagnostic approach, as they allow for the rapid and sensitive identification of the etiologic agents of acute RTIs, thereby facilitating early initiation of targeted therapy, reducing hospital length of stay, and improving overall clinical outcomes.

### Author Contributions

Study conception and design: **Canberk Çınar, Sümeyye Özkaya, İlknur Bıyık**, data collection: **Yeliz Tanrıverdi Çaycı, Kemal Bilgin**, analysis and interpretation of results: **Demet Gür Vural, Asuman Birinci**, draft manuscript preparation: **Canberk Çınar, Kemal Bilgin, Asuman Birinci**. The author(s) reviewed the results and approved the final version of the article.

### Conflicts of Interest

The authors declare that there is no conflict of interest to disclose.

### Ethical Approval

Our study received ethical committee approval from the Ondokuz Mayıs University Faculty of Medicine Clinical Research Department, with decision number 2025/47.

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