ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Distribution of Respiratory Pathogens After the COVID-19 Pandemic: A Single-Center Pediatric Study From Türkiye

COVID-19 Pandemisinden Sonra Solunum Patojenlerinin Dağılımı: Türkiye'den Tek Merkezli Bir Pediatrik Çalışma

Elif Aydin[1], Guleser Guney Yilmaz[2], Neset Aydin[3], Yalcin Dicle[4], Duygu Percin Renders[5]

[1]Kütahya Health Sciences University Tavşanlı Health Services Vocational School, Department of Medical Services and Techniques, Kütahya, Türkiye; [2]Therapy and Rehabilitation Department, Kütahya Health Sciences University, Kütahya, Türkiye; [3]Kütahya Health Sciences University, Clinic of Pediatrics, Evliya Çelebi Training and Research Hospital, Kütahya, Türkiye; [4]Mardin Artuklu University, Faculty of Medicine, Department of Medical Microbiology, Mardin, Türkiye; [5]Kütahya Health Sciences University Faculty of Medicine, Department of Medical Microbiology, Kütahya, Türkiye

ABSTRACT

Introduction: *This study aimed to evaluate the frequency and typing of viral and bacterial pathogens in patients with a preliminary diagnosis of respiratory tract infection during the epidemic period in Türkiye.*

Material and Method: *The study included the respiratory pathogen analysis results of 215 patients aged 0–17 years admitted to the Pediatrics Department of Kütahya Health Sciences University Evliya Çelebi Training and Research Hospital between October 2022 and March 2023 with symptoms of respiratory tract infections. Twenty-three viruses and six bacteria in the respiratory panel were studied using multiplex real-time PCR. Samples were obtained from a nasopharyngeal swab in non-intubated patients and tracheal aspiration material in intubated patients.*

Results: *While 92.1% of the patients were positive for one or more agents, no agent was detected in 7.9%. Among the positive results, the rate of the single pathogen was 27.3% (n=54), while the rate of multiple pathogens was 72.7% (n=144). Respiratory syncytial virus was the most common viral agent with a rate of 23.3% and Adenovirus with a rate of 19.1%, while Haemophilus influenzae (48.8%) and Streptococcus pneumoniae (29.3%) were the most common bacterial agents.*

Conclusion: *In this study, viral and bacterial pathogens have been studied intensively in children. Simultaneous, rapid, and sensitive detection of these agents is essential in preventing unnecessary antibiotic use and infection control. This is also important in terms of reducing mortality and morbidity rates.*

Keywords: *COVID-19; pandemic; respiratory pathogens; respiratory syncytial viruses*

ÖZET

Giriş: *Bu çalışmanın amacı, Türkiye'de salgın döneminde solunum yolu enfeksiyonu ön tanısı alan hastalarda viral ve bakteriyel patojenlerin sıklığını ve tiplendirmesini değerlendirmektir.*

Materyal ve Metot: *Ekim 2022-Mart 2023 tarihleri arasında Kütahya Sağlık Bilimleri Üniversitesi Çelebıya Eğitim ve Araştırma Hastanesi Çocuk Sağlığı ve Hastalıkları Kliniğine solunum yolu enfeksiyonu semptomları ile başvuran 0–17 yaş arası 215 hastanın solunum yolu patojenleri analiz sonuçları çalışmaya dâhil edilmiştir. Solunum panelinde 23 virüs ve altı bakterinin varlığı multipleks gerçek zamanlı PCR kullanılarak çalışılmıştır. Örnekler entübe olmayan hastalarda nazofarengeal sürüntüden, entübe hastalarda ise trakeal aspirasyon materyalinden elde edilmiştir.*

Bulgular: *Hastaların %92,1'inde bir veya daha fazla etken pozitif bulunurken, %7,9'unda hiçbir etken saptanmamıştır. Pozitif sonuçlar arasında tek patojen oranı %27,3 (n=54) iken, çoklu patojen oranı %72,7 (n=144) idi. Respiratuvar sinsityal virüs %23,3 ve Adenovirüs %19,1 oranıyla en sık görülen viral etkenler olurken, Haemophilus influenzae (%48,8) ve Streptococcus pneumoniae (%29,3) en sık görülen bakteriyel etkenler olmuştur.*

Sonuç: *Bu çalışmada çocuklarda viral ve bakteriyel patojenler yoğun olarak saptanmıştır. Bu etkenlerin eş zamanlı, hızlı ve duyarlı bir şekilde saptanması gereksiz antibiyotik kullanımının önlenmesi ve enfeksiyon kontrolü açısından önemlidir. Bu da mortalite ve morbidite oranlarının azaltılması açısından önemlidir.*

Anahtar kelimeler: *COVID-19; pandemi; solunum patojenleri; respiratuvar sinsityal virüs*

İletişim/Contact: *Elif Aydın, Kütahya Health Sciences University Tavşanlı Health Services Vocational School, Department of Medical Services and Techniques, Kütahya, Türkiye* • ***Tel:*** *05053553846* • ***E-mail:*** *elif.aydin@ksbu.edu.tr* • ***Geliş/Received:*** *05.09.2023* • ***Kabul/Accepted:*** *09.11.2023*

ORCID: *Elif Aydın, 0000-0003-0877-453X • Güleser Güney Yılmaz, 0000-0003-1781-9381 • Neşet Aydın, 0000-0002-1437-835X • Yalçın Dicle, 0000-0002-7658-7763 • Duygu Perçin Renders, 0000-0002-4436-5226*

Introduction

Respiratory tract infections (RTIs) are the most common cause of both outpatient treatment and hospitalizations and antibiotic use in children. They are one of the leading causes of morbidity and mortality worldwide. The causative agents of these diseases may be of community or hospital origin[1]. The loss of labor force and treatment costs caused by RTIs show that they seriously burden national economies[2].

Although 60–80% of RTIs are reported as viral agents, the most common viruses are influenza virus, human rhinovirus (HRV), respiratory syncytial virus (RSV), human coronavirus (HCoV), and parainfluenza virus (PIV)[3]. The most common bacterial agents associated with RTI are *Streptococcus pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pyogenes*[1,4].

In RTIs, it is difficult to distinguish whether the pathogen is viral or bacterial without laboratory diagnosis. Since clinical symptoms that may be pathogen-specific are scarce in RTIs, the diagnosis is primarily based on laboratory investigation[5]. Tests that can rapidly identify various viruses simultaneously may help initiation of appropriate treatment. Multiplex real-time PCR (MRT-PCR) is an in vitro test that qualitatively detects the viruses and bacteria causing RTIs[6]. Rapid and accurate identification of these infectious agents is of great importance in terms of timely initiation of antiviral treatment and prevention of unnecessary antibiotic use, as well as reducing the duration of hospitalization, risk of nosocomial transmission, and treatment costs.

Our country faces a rapidly spreading epidemic that has seriously affected society in recent days. People have complaints such as fever, weakness, sore throat, and joint pain, which do not put them to bed but make it difficult to stand. For this reason, the number of hospitalizations with respiratory infections, which are more common in the 0–17 age group, has increased recently. In this study, we aimed to evaluate the frequency and typing of viral and bacterial pathogens in patients with a preliminary diagnosis of upper and lower respiratory tract infection in the pediatric population, which causes an increase in the number of hospital admissions in many regions of our country.

Material and Method

Study Population and Data Collection

This study included all children aged 0 to 17 years who underwent MRT-PCR of nasopharyngeal swabs for respiratory pathogens at a tertiary university hospital (Kütahya, Türkiye) between October 2022 and March 2023. The International Classification of Diseases Code was used to identify cases. Data was collected from computer databases and electronic medical records. Each patient's demographic information, symptoms, and laboratory results were documented. Respiratory tract samples sent from patients with a preliminary diagnosis of acute respiratory tract infection were analyzed for viral and bacterial agents by MRT-PCR. Patients were divided into age groups: 0–2, 3–5, 6–9, and 10–17 years. The age classification is based on the school system. Children under two years of age are kept separately at home or attend kindergarten; children from 3 to 5 years old attend kindergarten; children from 6 to 9 years old attend primary school. After age 10, children go to middle school and high school. The multiplex PCR was performed on nasopharyngeal swabs from patients presenting with symptoms attributable to respiratory tract infection (fever, myalgia, chills, weakness, sore throat, shortness of breath, cough, chest pain, headache, abdominal pain, nausea/vomiting, diarrhea, loss of taste, loss of smell, rash, and conjunctivitis). The hospital's pediatric emergency and inpatient services requested the test. This study protocol was approved by the local ethics committee (2023/01-39).

Nasopharyngeal Swab Collection and Genetic Material Extraction

Nasopharyngeal swabs were obtained from patients presenting to pediatric departments with RTI. For this purpose, rayon-tipped swabs with a bendable rod were used. The swab was inserted into the nasopharynx by inserting it through the nostril. The sample was obtained by gently holding it for 5 seconds, then gently rotating it, and finally transporting it to the microbiology laboratory in a Viral Nucleic Acid Buffer (vNAT) transport medium (Bioeksen, Türkiye), adhering to the cold chain regulations. Nasopharyngeal/oropharyngeal swab samples from patients in vNAT transport medium nucleic acid extracts were not pretreated. The study was performed on the CFX96 real-time PCR detection system (CFX96; Bio-Rad, USA).

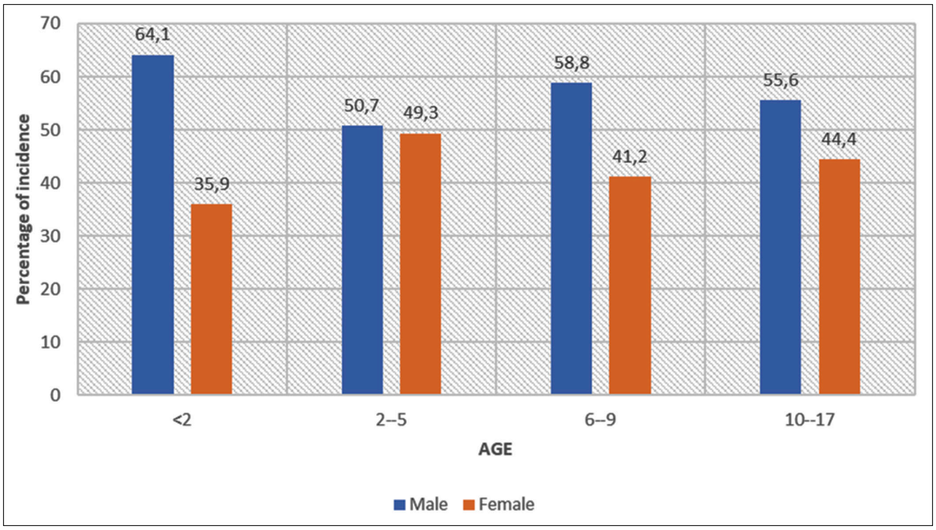

Figure 1. *Characteristics of children enrolled in the study.*

Multiplex PCR

Viral and bacterial nucleic acids (DNA or RNA) in respiratory tract samples were investigated with the Bio-Speedy® Respiratory Tract RT-qPCR MX-24S kit (Bioeksen, Turkiye). Based on the MRT-PCR technique, this system can simultaneously detect the presence of twenty-three viruses and six bacteria causative agents of respiratory tract infections. The agents that can be seen with the respiratory panel kit used are; Human bocavirus (HBOV), Adenovirus (AdV), Human coronavirus (HCOV-HKU1), Human coronavirus (HCOV-NL63), Human coronavirus (HCOV-OC43), Human coronavirus (HCOV-229E), Human enterovirus/Human rhino, Human metapneumovirus (HMPV A), Human parainfluenza virus 1–4, Human parechovirus (HPEV), Human rhinovirus (RV), Influenza A (FluA), Influenza A H1, Influenza A virus subtype (H1), Influenza A H3Nx, Influenza A H5Nx, Influenza A H37Nx, Influenza B (FluB), RSV, SARS-CoV-2, *Bordetella pneumoniae*, *Chlamydophila pneumoniae*, *H.influenzae, Legionella pneumophilia*, *Mycoplasma pneumoniae, S.pneumoniae*. *Streptococcus pyogenes*, the most common cause of RTI in children, was not evaluated as it was not included in the respiratory panel test list[1,4].

Data Analysis

Descriptive statistics, including mean and standard deviation, were provided for numerical variables exhibiting normal distributions. Percentage values and frequency tables were presented for specific variables. Statistical analyses were conducted using IBM Statistical Package for Social Sciences (SPSS) program version 23.0 (IBM Corp., Armonk, NY, USA). The study used the Phi correlation V test incorporating cross-table statistics to examine the relationship between positive and negative respiratory pathogens. The study defined the absolute value of the correlation coefficient as a "high correlation" between 0.7 and 1, a "moderate relationship" between 0.7 and 0.3, and a "low relationship" between 0.3 and 0. A significance level of $p<0.05$ was also considered statistically significant.

Results

Description of Cohort

From October 2022 to March 2023, 215 children, 125 (58.1%) boys and 90 (41.9%) girls, were tested for upper and lower respiratory tract pathogens. The demographic characteristics of the children included in the study are shown in Fig. 1.

The most common symptoms were fever (n=175, 81.4%), cough (n=168, 48.3%), weakness (n=131, 60.9%), and shortness of breath (n=121, 60.9%) (Table 1).

When respiratory pathogens were analyzed, more than one respiratory pathogen was found to be present at the same time. Among respiratory pathogens, it was determined that at least one pathogen was present in one child. In contrast, a maximum of 6 pathogens could be seen simultaneously. Among the positive results, the rate of the single pathogen was 27.3% (n=54), while the rate of multiple pathogens was 72.7% (n=144).

Table 1. Clinical findings of patients

	Age (years)				
	<2 n=92	2–5 n=71	6–9 n=34	10–17 n=18	Total Positive
Clinical Finding, N (%)					
Fever	81 (88)	58 (81.7)	27 (79.4)	9 (50)	175 (81.4)
Myalgia	0	13 (18.3)	5 (14.7)	5 (27.7)	23 (10.7)
Chills	0	27 (38)	13 (38.2)	5 (27.8)	45 (20.9)
Weakness	41 (44.6)	53 (74.6)	21 (61.8)	16 (88.9)	131 (60.9)
Sore throat	0	62 (87.3)	17 (50)	10 (55.6)	89 (41.4)
Shortness of breath	49 (53.3)	57 (80.3)	12 (35.3)	3 (16.7)	121 (56.3)
Cough	71 (77.2)	62 (87.3)	21 (61.8)	14 (77.8)	168 (78.1)
Chest pain	0	0	5 (14.7)	1 (5.6)	6 (2.8)
Runny nose	50 (54.3)	28 (39.4)	19 (55.9)	9 (50)	106 (49.3)
Headache	0	0	2 (5.9)	2 (11.1)	4 (1.9)
Abdominal pain	0	0	6 (17.6)	0	6 (2.8)
Nausea/vomiting	19 (20.7)	12 (16.9)	0	1 (5.6)	32 (14.9)
Diarrhea	7 (7.6)	16 (22.5)	0	0	23 (10.7)
Loss of taste	0	0	0	0	0
Loss of smell	0	0	0	0	0
Rash	9 (9.8)	3 (4.2)	0	0	11 (5.1)
Conjunctivitis	0	2 (2.8)	0	0	2 (0.9)

The most common concurrent pathogens were SARS-CoV-2 and *H.influenzae*, seen simultaneously in 14% (n=30) of children.

There was no significant correlation between the incidence of related respiratory pathogens according to gender (p>0.05). When the difference in the prevalence of pathogens between age groups was compared, a significant difference was found between AdV, *H.influenzae*, FluA, Human enterovirus/Human rhino, Influenza A virus Subtype H1, Influenza A H3Nx, and RSV according to age (p<0.05). No statistically significant difference was found between the frequency of other pathogens according to age groups (p>0.05).

The most common viruses were RSV (23.3%) and AdV (19.1%), while *H.influenzae* (48.8%) and *S.pneumoniae* (29.3%) were the most common bacteria. The pathogenicity of children divided according to age groups is given in Table 1, and the most frequent positivity was observed in the <2 years age group. This was followed by the 2–5, 6–9, and 10–17 age groups, respectively (Table 2).

Since the *H. influenza* and *S. pneumonia* agents we detected can normally colonize, it is thought that these bacteria may be the causative agents of the disease in 13 patients with only *H. influenza* positivity, six patients with only *S. pneumonia* positivity, and eight patients with both positive, in whom no other agents were detected in patients with clinical signs of respiratory tract infection.

Of the patients, 64 (29.8%) were hospitalized in the ward, and 11 (5.1%) were hospitalized in the pediatric intensive care unit (PICU). The mean duration of hospitalization in the ward was 2.3 days, and ten days in the PICU.

Finally, 51 (23.7%) patients received respiratory support. Of these, 43 (84.3%) received free-flow oxygen therapy, 5 (9.8%) high-flow nasal cannula (HFNC) oxygen therapy, 2 (3.9%) noninvasive ventilation therapy, and 1 (1.9%) endotracheal intubation.

Discussion

The COVID-19 pandemic has evolved rapidly and caused health problems worldwide. There was reportedly a decline in pediatric emergency department visits and visits due to RTIs during the pandemic period[7] and a significant decrease in the frequency of seasonal respiratory infections in the pediatric population during quarantine periods compared to previous years[8]. It has been observed that the number of applications to emergency and pediatric clinics with complaints such as fever, cough, sore throat, fatigue, joint pain, etc., in the pediatric population in our hospital between October 2022 and March 2023 increased excessively. We investigated the identification and incidence of

Table 2. *Distribution of respiratory pathogens by age groups*

	Age (years)					
	<2 n=92	2–5 n=71	6–9 n=34	10–17 n=18	Total Positive	p-value
Pathogens detected, N(%)						
AdV	7 (7.6)	19 (26.8)	13 (38.2)	2 (11.1)	41 (19.1)	**<0.001**
Bordetella pneumoniae	0	0	1 (2.9)	0	1 (0.5)	0.294
Haemophilus influenzae	37 (40.2)	45 (63.4)	18 (52.9)	5 (27.8)	105 (48.8)	**0.006**
HBOV	1 (1.1)	5 (7)	1 (2.9)	1 (5.6)	8 (3.7)	0.221
HCOV-NL63	2 (2.2)	2 (2.8)	0	0	4 (1.9)	0.511
HCOV-OC43	10 (10.9)	4 (5.6)	5 (14.7)	1 (5.6)	20 (9.3)	0.404
HCOV-229E	0	0	0	1 (5.6)	1 (0.5)	0.171
Human enterovirus/Human rhino	12 (13)	5 (7)	0	2 (11.1)	19 (8.8)	**0.037**
HMPV A	2 (2.2)	1 (1.4)	0	1 (5.6)	4 (1.9)	0.514
Human parainfluenza virus 3	15 (16.3)	5 (7)	1 (2.9)	1 (5.6)	2 (10.2)	0.065
Human parainfluenza virus 4	3 (3.3)	0	0	0	3 (1.4)	0.161
HPEV	1 (1.1)	0	0	0	1 (0.5)	0.636
FluA	5 (5.4)	19 (26.8)	8 (23.5)	2 (11.1)	34 (15.8)	**<0.001**
Influenza A H1	4 (4.3)	12 (16.9)	4 (11.8)	1 (5.6)	21 (9.8)	0.050
Influenza A virus subtype (H1)	5 (5.4)	13 (18.3)	4 (11.8)	0	22 (10.2)	**0.012**
Influenza A H3Nx	1 (1.1)	6 (8.5)	4 (11.8)	1 (5.6)	12 (5.6)	**0.045**
FluB	7 (7.6)	6 (8.5)	0	0	13 (6)	0.058
Streptococcus pneumoniae	29 (31.5)	21 (29.6)	7 (20.6)	6 (33.3)	63 (29.3)	0.639
RSV	38 (41.3)	10 (14.1)	1 (2.9)	1 (5.6)	50 (23.3)	**<0.001**
SARS-CoV-2	3 (3.3)	0	0	1 (5.6)	4 (1.9)	0.131
HCOV-HKU1	0	0	0	0	0	0
Chlamydophila pneumoniae	0	0	0	0	0	0
Human paraınfluenza virus **1**	0	0	0	0	0	0
Human paraınfluenza virus **2**	0	0	0	0	0	0
Influenza A H5Nx	0	0	0	0	0	0
Leginella pneumophilia	0	0	0	0	0	0
Mycoplasma pneumoniae	0	0	0	0	0	0

AdV: Adenovirus; HBOV: Human bocavirus; HCOV-HKU1: Human coronavirus-HKU1; HCOV-NL63: Human coronavirus-NL63; HCOV-OC43: Human coronavirus-OC43; HCOV-229E: Human coronavırus-229E; HMPV A: Human metapneumovirus; HPEV: Human parechovirus; FluA: Influenza A; FluB: Influenza B; RSV: Respiratory sinsityal virus.

respiratory tract pathogens to determine the causative agents of the disease and to organize the treatment for the causative agent during these dates when the number of applications intensified nationwide.

Timely detection of respiratory tract pathogens and initiation of appropriate antimicrobial treatment significantly reduces morbidity and mortality. MRT-PCR methods, which have recently been widely used in the detection of respiratory tract pathogens and used as a diagnostic tool that can detect many pathogens at the same time with high sensitivity, are essential in the application of correct and effective treatment by differentiating viral and bacterial infections and provide a better understanding of epidemiologic data on the distribution of these agents[9–10].

In this study, respiratory pathogen agent (s) were detected with a rate of 92.1% in samples obtained from patients admitted to pediatric departments within six months. Some studies reported positivity rates of 17.5%, 44%, 67.8%, and 73.5%. This study found a higher positivity rate of 92.1% than in the literature[11–14]. We think the positivity rate was high in this study because children stayed away from public areas such as schools, kindergartens, playgrounds, etc., during the pandemic. The spread of microbial agents in an epidemic manner was less with the use of masks, paying attention to distance and hygiene rules in this process, and these rules were relaxed after the pandemic. At the same time, it is thought that the high positivity rate may be due to the low body resistance of children who did not intensively encounter microorganisms during the pandemic. The distribution of respiratory tract pathogens by gender was compatible with the literature, and no significant correlation was found between boys and girls ($p>0.05$)[12,14,15].

This study's most common viral agents were RSV (23.3%) and AdV (19.1%). Our data are compatible with the data of studies conducted worldwide[13,16–19]. In contrast to similar studies, one study reported that HRV, and another said that HCoV was detected at the highest rate[20,21]. In a large-scale study, it was reported that 66.000–160.000 children under five years of age died from RSV infection-related complications[22]. Therefore, knowing the clinical effects and epidemiology of RSV, early detection, and giving the necessary importance to vaccination in the risky population are of great importance in preventing a severe RSV epidemic in the future. AdV is responsible for at least 5 to 10% of infections that frequently infect the lower or upper respiratory tract, conjunctiva, and gastrointestinal system and can remain in the bodies of infected children for months and require hospitalization[23]. In a study on gastroenteritis agents in the same city, AdV was the second most common agent[24]. To prevent exposure to AdV, which causes mortality and morbidity and poses a risk for childhood, it is essential to comply with hygiene rules in all living areas, especially in public places, and to provide education on these rules.

As a result of studies conducted in our country, the *S.pneumoniae* pathogen was reported as the most frequently detected bacterial agent from respiratory tract samples[14,25,26]. The most commonly isolated bacterial agents in these studies were reported to be *S.pneumoniae*, *S.aureus,* and *C.pneumoniae,* respectively[14]. In this study, *H.influenzae* (48.8%) was the most common bacterial agent, followed by *S.pneumoniae* (29.3%). Early detection and agent-directed treatment are essential to prevent antibiotic resistance against *S.pneumoniae* and *H.influenzae*, which are the agents of community-acquired bacterial pneumonia.

In studies on the simultaneous presence of more than one pathogen in the respiratory tract sample, RSV was reported to be the most common pathogen, and association with AdV, HBoV, and INF-A pathogens was reported[27]. Different studies said the most common pathogens accompanying HCoVs were HRV, RSV, HRV, HRV, and *S.pneumoniae*[28–30]. In this study, although the results were similar to the literature data, 27.3% of the analyzed samples were associated with a single agent and 72.7% were associated with 2–6 agents. *S.pneumoniae*, human influenza viruses, and *S.pneumoniae* and RSV synergies significantly exacerbate pneumonia's morbidity and mortality[31,32]. Large-scale studies are needed to investigate the mechanisms underlying these synergies and bacterial, viral, and host immune factors that increase susceptibility to infection.

Studies have shown that children <2 years of age appear more susceptible to infections, similar to our research[33,34]. In particular, rhinovirus, enterovirus, SARS-CoV-2, other coronaviruses, and Adenovirus were reported more frequently. Current assumptions relate to the lack of prior immunity[35] and the failure of children under 6 to use masks after schools reopen[36].

In conclusion, although our study was conducted briefly and in a single center during a nationwide epidemic, a high positivity rate was detected, and multiple agents were frequently found in the same patient. Respiratory tract infections are one of the most common reasons for using antimicrobials. Widespread and inappropriate use of antimicrobials is one of the reasons for the emergence of multidrug resistance, which continues to increase alarmingly[37]. The use of broad-spectrum antibiotics leads to more resistant strains. As resistance increases, there are delays in initiating appropriate and effective antibiotic treatment, and the frequency of sepsis and mortality rates increase. Detection of respiratory tract pathogens is also important to avoid delaying antibacterial treatment because the causative agents are viral during epidemic periods. For this reason, it is vital to prevent unnecessary and incorrect treatments with early diagnosis by using the MRT-PCR method (fast, sensitive, cost-effective, and capable of studying many agents) in detecting respiratory tract infections and pathogens.

This study had many limitations. It was a single-center study, and the most common pathogen, S. pyogenesis in RTI, was not included in the respiratory panel tests. Another limitation was the lack of data before and during the pandemic. We could not fully collect these data due to systemic change.

In conclusion, our data showed a change in the typical epidemiology of pediatric respiratory tract pathogens according to the time of the pandemic. Although our study was conducted in a single center shortly after the nationwide pandemic, RSV and/or influenza viruses, which decreased during the pandemic, were found after the pandemic, with a high rate of positivity and often more than one agent in the same patient. We think this is due to the transition to the normalization process and children's entry into the social environment.

Authors' Contributions

EA, YD ve NA performed experiments. DPR designed experiments supervised the team during the study, and prepared the manuscript. GGY analyzed statistics. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Data Availability

The datasets generated during and analyzed during the current study are not publicly available due to the potential compromise of individual privacy. Still, they are available from the corresponding author on reasonable request.

Code Availability

Not applicable to this submission.

Ethical Approval

Ethics committee approval for the study was obtained from the non-interventional ethics committee of Kütahya Health Sciences University with the letter dated 11.01.2023 and decision number 2023/01-39.

Competing İnterests

The authors declare no competing interests.

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