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Retrospective Evaluation of the Frequency of Respiratory Pathogens in Patients Admitted to Kafkas University Medical Faculty Hospital

Gulfem Nur Yildiz[1], Gizem Tukenmez[2], Ihsan Topaloglu[3], Ozgur Celebi[1], Murat Karamese[1]

1 Kafkas University, Faculty of Medicine, Department of Medical Microbiology, Kars, Turkey

2 Kafkas University, Faculty of Medicine, Department of Pediatrics, Kars, Turkey

3 Kafkas University, Faculty of Medicine, Department of Chest Diseases, Kars, Turkey

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Abstract

Objectives: This retrospective study was aimed to identify upper respiratory tract infection (URTI) pathogens in patients admitted to Kafkas University Medical Faculty Hospital between July 2023 and August 2024.

Methods: Nasopharyngeal swab samples from 1565 patients were analysed using the Multiplex Real-Time PCR (MRT-PCR) technique. Patient demographics, the month/season of hospital visits, and results of the respiratory agent tests were obtained from hospital archives and subjected to necessary statistical analyses. Chi-square and One-way ANOVA tests were used to analyse categorical and numerical data, respectively by SPSS v21.0.

Results: The data showed that 37.7% of the patients tested positive for at least one pathogen, while 62.3% were negative. The most frequently detected viral agents were Influenza B (34.3%), Influenza A (15.7%), and SARS-CoV-2 (14.4%). Streptococcus pyogenes was the most common bacterial pathogen (9.3%). Co-infection was observed in 9.14% of cases, with the most common combination being INF-B and INF-A. The seasonal distribution indicated that 40.3% of the positive cases occurred in the winter months (December 2023 - February 2024), and 39.1% in the spring (March - May 2024).

Conclusion: This retrospective study provides important epidemiological data on the identification and distribution of URTI pathogens in the region, contributing to the development of accurate approaches for diagnosis and treatment of infections.

Keywords: SARS-CoV-2, multiplex PCR, viral-bacterial infection, respiratory tract infection

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Correspondence / Yazışma Adresi: *Murat Karamese, Kafkas University, Faculty of Medicine Department of Medical Microbiology 36100, Kars, Turkey e-mail: murat_karamese@hotmail.com*

Kafkas Üniversitesi Tıp Fakültesi Hastanesine Başvuran Hastalarda Solunum Yolu Patojenlerinin Sıklığının Retrospektif Olarak Değerlendirilmesi

Öz

Amaç: Bu retrospektif çalışmada, Temmuz 2023-Ağustos 2024 tarihleri arasında Kafkas Üniversitesi Tıp Fakültesi Hastanesine başvuran hastalarda üst solunum yolu enfeksiyonu (ÜSYE) patojenlerinin belirlenmesi amaçlanmıştır.

Yöntemler: Multiplex Real-Time PCR (MRT-PCR) tekniği kullanılarak 1565 hastadan alınan nazofarengeal sürüntü örnekleri analiz edilmiştir. Hastaların demografik bilgileri, hastaneye başvurdukları ay/mevsim ve solunum ajanı testlerinin sonuçları hastane arşivlerinden elde edilmiş ve gerekli istatistiksel analizlere tabi tutulmuştur. Kategorik ve numerik verileri analiz etmek için SPSS v21.0 ile sırasıyla Ki-kare ve Tek yönlü ANOVA testleri kullanılmıştır.

Bulgular: Hastaların %37,7'sinde en az bir patojen saptanmış, %62,3'ünde negatif sonuç elde edilmiştir. En sık tespit edilen viral ajanlar İnfluenza B (%34,3), İnfluenza A (%15,7) ve SARS-CoV-2 (%14,4) olmuştur. Streptococcus pyogenes en yaygın bakteriyel patojen olarak bulunmuştur (%9,3). Ko-enfeksiyon oranı %9,14 olarak belirlenmiş ve en sık ko-enfeksiyon INF-B ve INF-A kombinasyonu olmuştur. Mevsimsel dağılıma göre, pozitif vakaların %40,3'ü kış aylarında (Aralık 2023-Şubat 2024) ve %39,1'i ilkbahar aylarında (Mart-Mayıs 2024) görülmüştür.

Sonuç: Bu retrospektif çalışma, bölgedeki ÜSYE patojenlerinin tanımlanması ve dağılımı hakkında önemli epidemiyolojik veriler sağlayarak enfeksiyonların tanı ve tedavisi için doğru yaklaşımların geliştirilmesine katkıda bulunmaktadır.

Anahtar kelimeler: SARS-CoV-2, multipleks PCR, viral-bakteriyel enfeksiyon, solunum yolu enfeksiyonu.

INTRODUCTION

Respiratory tract infections (RTIs) emerge in various forms each year, constituting a significant public health issue that affects millions of individuals globally. Whether acute or chronic, RTIs are highly prevalent among both adults and children[1]. Furthermore, RTIs are a major cause of morbidity and mortality worldwide, rendering them a global health concern[2]. In our country, the frequency and seasonal distribution of the pathogens responsible for these infections may vary between different geographic regions[3]. RTIs can be caused by bacteria, fungi, viruses, and parasites. Fungal infections are typically observed in immunocompromised patients who has primary immunodeficiency. Parasites rarely cause RTIs, whereas bacteria and viruses are more frequently implicated, even in healthy hosts[4]. URTIs refer to infections caused by bacterial and viral agents affecting areas such as the nasal mucosa, nasopharynx, oropharynx, auris media, sinuses, tonsils, and epiglottis[5].

URTIs are most commonly caused by viral agents, including Respiratory Syncytial Virus (RSV), Influenza Virus types A and B (INF-A, INF-B), Adenovirus (AdV), Parainfluenza Viruses (PIV types 1-4), Human Rhinovirus (HRV), Human Coronavirus (HCoV), Human Metapneumovirus (HMPV), and Human Bocavirus (HBoV)[6,7]. Fever, cough, sore throat, nasal congestion, and ear pain are the clinical symptoms of URTIs[8]. In the diagnosis of URTIs, molecular-based tests, including the polymerase chain reaction (PCR), are applied as well as conventional methods such as cell culture and direct fluorescent antibody testing[8-11].

The Multiplex-Real Time PCR (MRT-PCR) method is known for providing results in a shorter time frame, offering higher sensitivity and specificity, and allowing for the investigation of multiple parameters simultaneously. This technique enables the simultaneous detection of multiple respiratory pathogens in a single respiratory sample through a single reaction. Additionally, this method facilitates the identification of newer pathogens which are either difficult to culture

or cannot be cultured at all including HMPV, HBoV and some human coronaviruses (NL63 and HKU1)[11-13].

This study was aimed to retrospectively determine the respiratory pathogens using the MRT-PCR technique in patients who presented with upper URTI symptoms, including nasal discharge, nasal congestion, sore throat, cough, sneezing, low-grade fever, headache, and fatigue, at Kafkas University Faculty of Medicine Hospital, Kars, Turkey, between July 2023 and August 2024.

METHODS

This study was approved by the Ethics Committee for Non-Interventional Clinical Research at Kafkas University Medical Faculty, on [01.10.2024], with document number [80576354-050-99] and retrospectively conducted to determine the respiratory pathogens using the MRT-PCR technique in individuals who admitted to Kafkas University Medical Faculty Hospital, Kars, Turkey with at least one URTI symptoms, such as nasal discharge, nasal congestion, sore throat, cough, sneezing, mild fever, headache, or fatigue, between July 2023 and August 2024. Patient demographic data, the distribution of hospital visits by month/season and age, and the results of respiratory pathogen tests, were obtained from hospital archives and subjected to statistical analyses.

The total number of patients, the mean ages of them, the outpatient clinics and the season that the patients were admitted to hospital were recorded and the obtained data were used for statistical analysis. The pre-diagnosis and the symptoms were nearly same for all patients including URTI and sore throat/cough/sneezing/mild fever/headache, respectively.

Although this is a retrospective study, the following laboratory procedures had been applied for obtaining the patients' results. Respiratory samples (nasopharyngeal swabs or Broncho alveolar lavage specimens), collected from clinical and outpatient departments, were transported to the Medical Microbiology Laboratory in viral nucleic acid transport media (vNAT©, Bioeksen, Istanbul, Turkey) under cold chain conditions. Nucleic acid extraction was performed using the Bio-speedy Extraction Kit (Bioeksen, Istanbul, Turkey) on the EZ1 Zybio EXM3000 system, according to the manufacturer's instructions. The presence of viral and bacterial nucleic acids (DNA or RNA) in the respiratory samples was assessed using the Respiratory ID-1 Kit, a 7-pathogen multiplex PCR panel (Bioeksen, Istanbul, Turkey). The PCR process consisted of the following steps: (i) Reverse transcription at 52°C for 3 minutes; (ii) Hold at 95°C for 10 minutes; (iii) Touchdown cycling for 12 cycles, with denaturation at 95°C for 1 second, followed by annealing/extension at 67°C–56°C for 15 seconds; (iv) Final cycling for 30 cycles, with denaturation at 95°C for 1 second and annealing/extension at 95°C for 15 seconds. The amplification curves were obtained via four channels (FAM, HEX, ROX, and CY5) and evaluated using Sigmoida software (Sigmoida Analysis Software, Bioeksen, Istanbul, Turkey). Curves exceeding the threshold value were classified as "positive" while those without sigmoidal curves were labelled as "negative."

The multiplex PCR panel used in this study was capable of detecting viral agents such as INF-A, INF-B, RSVA/B, SARS-CoV-2, AdV, HRV, and the bacterial agent Streptococcus pyogenes (S. pyogenes).

Following data collection, the demographic and clinical characteristics of the patients, including the distribution of respiratory pathogens, were analysed using Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., NY, USA). Descriptive statistics such as percentages, means, and standard deviations (SD±) were calculated. Categorical data were analysed

using the Pearson Chi-square test, and one-way ANOVA was employed for the analysis of continuous variables. Statistical significance was set at $p<0.05$.

RESULTS

In this study, nasopharyngeal swab samples from 1565 patients (female=795 and male=770) who admitted to our hospital's clinics and outpatient departments with URTI complaints between July 2023 and August 2024 were retrospectively evaluated using the multiplex PCR method for the following pathogens; INF-A, INF-B, RSV-A/B, SARS-CoV-2, AdV, HRV, and S. pyogenes. Of 1565 samples, 591 (37.7%) were positive for either a single pathogen (n=537), two pathogens (n=52), or three pathogens (n=2), while 974 (62.3%) were negative. Among the positive cases, 287 (48.9%) were female, and 304 (51.1%) were male. Statistical analyses revealed no significant difference between gender and positivity rates (p=0.092).

A total of 1565 patients aged between 0 and 95 years were evaluated; 736 (47.03%) were children (under 18 years), and 829 (52.97%) were adults (over 18 years). The overall mean age was 26.98±15.07. Positivity was detected in 305 (41.46%) of the pediatric patients and 286 (34.51%) of the adult patients (Table 1). No statistically significant difference was found between the groups in terms of pathogen positivity (p=0.14).

Table I: The positivity and age distribution of patients

	Child (0-18 age)	Adult (18 and over)	Total
Positive cases	n = 305 (41,46%)	n = 286 (34,51%)	n = 591 (37,76%)
Negative cases	n = 431 (58,74%)	n = 543 (65,49%)	n = 974 (62,24%)
Total	736	829	1565

The distribution of the identified pathogens among positive samples was as follows; INF-A: n=93 (15.7%), INF-B: n=203 (34.3%), RSV-A/B: n=35 (5.9%), SARS-CoV-2: n=85 (14.4%), AdV: n=47 (8%), S. pyogenes: n=55 (9.3%), and HRV: n=73 (12.4%). 36.1% (287/795) and 39.5% (304/770) were positive female and male rates, respectively.

The distribution of patients according to hospital departments, as well as positivity rates for each department, is presented in Table 2. The majority of patients were from Department of Pediatrics (48%) and Department of Chest Diseases (22.8%).

Table II: Distribution of patients according to the units they were admitted to the hospital

Department	Positive Samples (%)	Positivity Rate (%)
Paediatrics	48% (752/1565)	42.15% (317/752)
Pulmonary Medicine	22.81% (357/1565)	32.77% (117/357)
ENT Clinic	6.19% (97/1565)	42.26% (41/97)
Emergency Department	2.93% (46/1565)	32.60% (14/46)
Coronary Intensive Care Unit	0.95% (15/1565)	46.66% (7/15)
Palliative Care Unit	0.57% (9/1565)	22.22% (2/9)
Cardiology Clinics/Service	0.51% (8/1565)	37.5% (3/8)
General Surgery Department	0.44% (7/1565)	28.57% (2/7)
Anaesthesia and Reanimation	0.12% (2/1565)	50% (1/2)
Orthopaedics Department	0.12% (2/1565)	50% (1/2)
Urology Department	0.12% (2/1565)	50% (1/2)
Ophthalmology Department	0.06% (1/1565)	0% (0/1)
Neonatal Intensive Care Unit	0.06% (1/1565)	0% (0/1)

The seasonal distribution of 591 positive cases was as follows; 238 (40.3%) was during winter (December, January, February), 231 (39.1%) was in spring (March, April, May), 108 (18.3%) was in summer (June, July, August), and 14 (2.4%) was in autumn (September, October, November) (Figure 1).

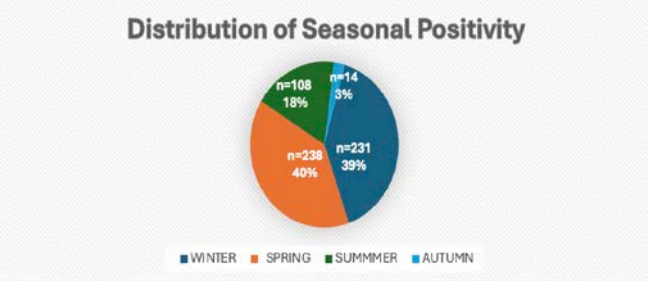

Figure 1: Distribution of seasonal positivity

No statistically significant difference was detected between positivity rates and seasons (p=0.852) or months (p=0.901). The detailed distribution of positivity and negativity rates by season is shown in Table 3.

Table III: Positive and negative rates of patients according to seasons

Parameters	Seasons				
	Winter	Spring	Summer	Autumn	Total
Inf-A	36	37	21	1	95
Inf-B	68	91	41	3	203
RSV-A/B	16	14	5	1	36
SARS-CoV-2	39	32	22	2	95
AdV	23	18	4	2	47
S. pyogenes	30	21	4	2	57
HRV	30	24	17	3	74
Negative	379	391	162	26	958
Total	**621**	**628**	**276**	**40**	**1565**

Co-infection was detected in 54 (9.14%) patients. Of those, 52 samples had two pathogens, and 2 samples had three pathogens. The most frequent detected co-infection agents were INF-B+INF-A (n=12), SARS-CoV-2+S. pyogenes (n=7, 11.8%) and INF-B+RSV-A/B (n=6, 10.1%). The detailed information about co-infections are presented in Table 4.

Table IV: Co-infection agents detected in positive cases.

Co-infections	Amount (n/%)
INF-B+ INF- A	12 (2.03)
SARS-COV-2+ *S. pyogenes*	7 (1.18)
INF-B+ RSV-A/B	6 (1.01)
INF-B + HRV	5 (0.84)
INF-B + SARS-COV-2	5 (0.84)
INF-B+ *S.pyogenes*	4 (0.67)
S.pyogenes+HRV	4 (0.67)
INF-B +AdV	2 (0.33)
HRV+AdV	2 (0.33)
INF-B+SARS-COV-2+*S. pyogenes*	1 (0.16)
S.pyogenes +AdV	1 (0.16)
INF- A+ HRV	1 (0.16)
RSV- A/B+SARS-COV-2	1 (0.16)
RSV+AdV	1 (0.16)
S.pyogenes+SARS-COV-2+RSV-A/B	1 (0.16)
TOTAL	54 (100)

DISCUSSION

In respiratory tract infections (RTIs), timely detection of viral and bacterial agents and initiation of appropriate treatment significantly reduces morbidity and mortality[3]. According to UNICEF's 2018 report, approximately 800,000 children worldwide die from pneumonia annually, which corresponds to an average of 2,200 children per day (Pneumonia in Children Statistics - UNICEF DATA access date: 2022-04 21[14]. Therefore, rapid and accurate detection of bacterial and viral agents causing upper respiratory tract infections is of great importance[15]. MRT-PCR method plays an important role in both directing the correct treatment and obtaining epidemiological data by detecting multiple respiratory viruses and bacterial agents with high sensitivity[3].

In this study, respiratory tract samples obtained from 1,565 patients admitted to Kafkas University Hospital with URTI symptoms between July 2023 and August 2024 were analysed. In our study, nearly half of the positive cases (305/591 – 41.46%) were the patients aged between 0 and 18, while the others (286/591 – 34.51%) were aged between 19 and 95. The positivity was nearly same both in children and adults. When current literature was checked, it was seen that the rate of positivity according to the age was parallel with our study. Cicek et al. performed. a study between 2002 and 2014 and reported the positivity of URTIs agent as 35.4% in children (under age 18) and 27.3% in adults (above age 18)[8]. Another study was also reported similar data that the positivity rate was detected as 45% and 62% in pediatric and adult patients, respectively[3].

On the other hand, at least one or more viral or bacterial agents were detected in 37.7% (n=590) of these samples, while no agent was detected in 62.3% (n=975). The most frequently detected viral agents were INF-B (n=203), INF-A (n=93), SARS-CoV-2 (n=85), HRV (n=73) and AdV (n=47), while the only bacterial agent (S.pyogenes) positivity was 9.3% (n=55). In a study conducted by Kuşkucu et al. (16) on adult patients in Istanbul, at least one viral or bacterial agent was found in 408 of 788 samples (51.78%). In that study, the most frequently

detected agents were IFN-A/B (n=133) and RSV (n=117), respectively. Özdamar et al[17]. conducted a study in Kocaeli and Istanbul, where one or more agents were isolated in 236 of 283 samples (83.45%), with HRV, AdV, and INF-A/B being the most common agents. Similarly, Biçer et al.[18] found RSV and AdV as the two most frequent agents in their study, while Akçalı et al.([19] identified RSV (61%) and HRV (36%) as the most common agents. In a study conducted by Dingmei Zhang et al.[20] a total of 14,237 nasopharyngeal swab samples were collected, and 5,582 (39.24%) of these tested positive. Among the most frequently detected pathogens, RSV was identified in 1,120 samples (7.86%), PIV in 494 samples (3.47%), and AdV in 493 samples (3.47%). Additionally, in a study performed by Kanberoğlu et al.[21] in İzmir, the most commonly identified bacterial agents were reported as S. pneumoniae (n=23), S. aureus (n=12), and M. pneumoniae (n=5). According to the national data most frequently detected viral agent was generally Inf-A/B and bacterial agent was S. pneumoniae. On the other hand, international studies also presented similar data that most frequently detected viral agent was generally Inf-A/B and bacterial agent was S. pneumoniae[22-24].

Co-infections were also examined in this study. Among 591 positive samples, 9.1% (n=54) had co-infections, with double (n=52) or triple agents (n=2). The most common co-infection combination was INF-B and INF-A (n=12). Our co-infection rate was consistent with the rate of current literature which was between 1-25%. For example, multiple infections were detected in 18.6% (n=318) of 1,705 patients in a study performed by Cicek et al.[8] The most common co-infections were RSV+INF-A (12.6%, n=40), and RSV+PIV (10.4%, n=33). Another multiple infection was detected in 114 of 788 patients (14.46%) in a study performed Özkarataş et al. The most common co-infection was HCoV+RSVA/B, observed in 7.23% (n=57) of the cases[16]. Similar results can be seen in current literature that shows the co-infection rate in URTIs screening tests up to approximately 25% and the most common agents were generally different combinations of viral agents[25].

When analysing seasonal distribution, respiratory viruses were generally more dominant during the winter and spring months. In our study, INF-B and SARS-CoV-2 were the most frequently detected agents during these seasons. INF-A, HRV, and S. pyogenes also caused more frequent infections in the winter. However, no statistically significant seasonal variation was found (p=0.852 for INF-A, p=0.901 for HRV) between the positivity and the season. A seasonal evaluation revealed by Cicek et al.[8] reported that the INF-A virus was more frequently detected during the winter months, while INF-B was detected in the spring. HRV was commonly identified in the spring and autumn, HCoV in summer, and RSV A/B in winter. PIV was more frequently observed in the late summer and autumn, while HMPV and ADV were predominantly detected in winter. In a study performed by Özkarataş et al.[16] pointed out that the influenza A virus was most commonly detected during the winter months, while influenza B was prevalent in the spring. Data from other studies also indicate that the seasonal distribution of respiratory pathogens in Turkey shows similarities across different geographic regions.

In conclusion, this study retrospectively evaluates the detection of pathogens, regional prevalence, and seasonal distribution using MRT-PCR in patients presenting with URTI symptoms at Kafkas University Hospital's clinics and outpatient services over the span of one year. While the single-center nature of the study, the relatively limited sample size, the absence of clinical evaluations, and the lack of age-based group divisions (children, adults, elderly) present certain limitations, we believe

this research contributes valuable epidemiological data to the literature.

Ethics Committee Approval: This study was approved by the Ethics Committee for Non-Interventional Clinical Research at Kafkas University Medical Faculty, on [01.10.2024], with document number [80576354-050-99].

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