ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Investigation of Viral and Bacterial Agents in Samples Taken from Patients with Suspected Upper Respiratory Tract Infection

Üst Solunum Yolu Enfeksiyonu Şüphesi Olan Hastalardan Alınan Örneklerde Viral ve Bakteriyel Etkenlerin Araştırılması

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ABSTRACT

Aim: *Upper respiratory tract infection (URTI) complaints are the most common causes of admission to outpatient clinics and rapid detection by Multiplex-PCR method contributes to the monitoring and control of infection by easily evaluating the etiology of viral and bacterial agents regionally. This study aimed to evaluate the etiology of pathogens with a 24-X viral and bacterial respiratory Multiplex-PCR panel in patients admitted to Kafkas University Health Research and Application Hospital with URTI symptoms between November 2023 and February 2024.*

Materials and Methods: *The presence of viral and bacterial pathogens was analyzed by Multiplex-PCR method in nasopharyngeal swab samples obtained from 100 patients diagnosed with URTI by physical examination such as allergic rhinitis and acute bronchitis. The respiratory panel contained 24 different microorganisms such as SARS CoV-2, Influenza-A/B, Human Rhinovirus/Enterovirus, Human Metapneumovirus, Respiratory Syncytial Virus A/B, Human Parainfluenza Virus-1/2/3/4, Human Coronavirus 229E/OC43/NL63/HKU1, Human Parechovirus, Adenovirus, Human Bocavirus, Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila and Bordotella pertussis.*

Results: *According to the findings, the highest number of applications was in January. Out of 100 patients, 73% (n=73) were positive and 27% (n=27) were negative, the most common viral agent was INF A (n=33, 26.9%) while the most common bacterial agent was S.pneumoniae (n=19, 15.4%). Of 73 positive patients, 38.3% (n=28) had co-infections, 11% (n=8) had only bacterial agents, and 50.7% (n=37) had viral agents.*

Conclusions: *In conclusion, URTI agents were rapidly detected by Multiplex-PCR and their incidence was investigated in this study. Finally, it aims to prevent possible epidemics, unnecessary antibiotic use, mortality and morbidity and to contribute to other academic studies with rapid diagnosis and treatment of URTI infections.*

Key words: *URTI; SARS-CoV-2; influenza; multiplex PCR*

ÖZET

Amaç: *Üst solunum yolu enfeksiyonu (ÜSYE) şikâyetleri polikliniklere en sık başvuru nedenidir. ve Multiplex-PCR yöntemi ile hızlı tespit, viral ve bakteriyel etkenlerin etiyolojisini bölgesel olarak kolayca değerlendirerek enfeksiyonun izlenmesine ve kontrolüne katkı sağlamak oldukça önem arz etmektedir. Bu çalışmanın amacı, Kasım 2023-Şubat 2024 tarihleri arasında Kafkas Üniversitesi Sağlık Araştırma ve Uygulama Hastanesine ÜSYE semptomları ile başvuran hastalarda 24-X viral ve bakteriyel Solunum Yolu Multiplex-PCR paneli ile patojenlerin etiyolojisini değerlendirmektir.*

Gereç ve Yöntem: *Alerjik rinit ve akut bronşit gibi ÜSYE ön tanısı konulan 100 hastadan alınan nazofarengeal sürüntü örneklerinde viral ve bakteriyel patojenlerin varlığı Multiplex-PCR yöntemi ile analiz edilmiştir. Solunum paneli SARS CoV-2, Influenza-A/B, Human Rhinovirus/Enterovirus, Human Metapneumovirus, Respiratory Syncytial Virus A/B, Human Parainfluenza Virus-1/2/3/4, İnsan Coronavirus 229E/OC43/NL63/HKU1, İnsan Parechovirus, Adenovirus, İnsan Bocavirus, Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila ve Bordotella pertussis gibi 24 farklı mikroorganizma içermekteydi.*

Bulgular: *En yüksek başvuru sayısı ocak ayında gerçekleşmiştir. Yüz hastanın %73'ü (n=73) pozitif, %27'si (n=27) negatif olup, en sık görülen viral etken INF A (n=33, %26,9), en sık görülen bakteriyel etken ise S. pneumoniae (n=19, %15,4) olarak raporlanmıştır. Pozitif 73 hastanın %38,3'ünde (n=28) en az iki ajanın etken olduğu ko-enfeksiyon mevcuttu.*

Sonuç: *Bu çalışmada ÜSYE etkenleri Multiplex-PCR ile hızlı bir şekilde tespit edilmiş ve görülme sıklıkları araştırılmıştır. Sonuç olarak, ÜSYE enfeksiyonlarının hızlı tanı ve tedavisi ile olası salgınların, gereksiz antibiyotik kullanımının, mortalite ve morbiditenin önlenmesi ve diğer akademik çalışmalara katkı sağlanması amaçlanmıştır.*

Anahtar kelimeler: *ÜSYE; SARS-CoV-2; influenza; multiplex PCR*

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Introduction

The human respiratory system consists of the upper respiratory tract (URT) and lower respiratory tract, harboring a diverse microbial community. The URT includes the nose, mouth, sinuses, pharynx and larynx[1]. Upper respiratory tract infection (URT) ranks first among diseases that affect the economy, resulting in a loss of workforce and treatment costs. If not treated appropriately, it can cause serious complications and sequelae[2]. Viral and bacterial agents generally cause respiratory tract infections (RTIs), and both have similar symptoms, including fever, dry cough and sore throat[3]. These infections can manifest as otitis media, pharyngitis, laryngitis, rhinitis, and nasopharyngitis. Additionally, URTs can commonly be detected during the winter season[4], pediatric patients constitute 44% of the total number of patients admitted to hospitals with URTI, and 21% were prescribed antibiotics[1].

It is known that avian and swine-origin Influenza A viruses, animal reservoir-independent Influenza B viruses, Respiratory Syncytial Virus (RSV), Human Parainfluenza viruses, Human Metapneumovirus, Human Parechovirus, Human Rhinovirus, Human Bocavirus, Adenoviruses, Human Coronaviruses, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Chlamydophila pneumoniae* are circulating as bacterial and viral agents for URTIs in our country. These pathogens are primarily detected between October and April and can also be circulating from spring through winter[5–8]. The disease occurs nearly 2–4 and 6–10 times in a season in adults and children, respectively. The transmission typically occurs through three primary routes: direct and indirect contact, aerosol transmission, and droplet transmission[6,9,10]. For any infectious disease, including RTIs, the most suitable diagnostic methods are those based on rapid and accurate nucleic acid molecular diagnostic techniques such as Polymerase Chain Reaction (PCR), which rely on fully automated and integrated molecular diagnostic systems that are cost– and time-efficient, while also ensuring sensitivity and accuracy[3]. The Multiplex PCR technique should be used to determine multiple agents, especially in RTIs.

This study aimed to evaluate the etiology of pathogens with a 24-X viral and bacterial respiratory Multiplex-PCR panel in patients admitted to Kafkas University Health Research and Application Hospital with URTI symptoms between November 2023 and February 2024.

Material and Methods

A total of 100 patients aged 18–65 years who admitted to the outpatient clinics and emergency departments of Kafkas University Health Research and Application Hospital between November 2023 and February 2024 and who had at least one of the clinical symptoms of acute bronchitis, pneumonia, pain, cough, or acute upper respiratory tract infections, were included in the study. Nasopharyngeal swab samples were previously collected for routine analysis in the Medical Microbiology Laboratory. The samples were stored at -80°C until the experimental day, and then they were used for multiplex PCR analysis targeting 24 viral and bacterial respiratory pathogens. Clinical data, including the admission department, gender, age, and medical history of the patients, were obtained from the hospital archive and recorded. After a laboratory technician collected the nasopharyngeal swab samples, they were immediately placed in vNAT® transfer tubes, then numbered and sent to the laboratory on the same day. Ethical approval for the study was obtained from the Clinical Research Ethics Committee of the Faculty of Medicine, Kafkas University (Date: 26.04.2023, Decision No: 212).

The Bio-speedy Extraction kit (Bioeksen, Türkiye), based on the magnetic bead method, was used according to the manufacturer's instructions for nucleic acid extraction from the nasopharyngeal swab samples. The extraction was performed using the Zybio EXM 3000 device (Bioeksen, Türkiye). The respiratory tract RT-qPCR MX-24L panel (Bioeksen, Türkiye) included 18 viral and 6 bacterial pathogens: Sars-CoV-2, EV/HRV, HPeV, HPiV (1/2/3/4), AdV, HBoV, HMPV, INF A/B, HCoV (OC43, HKU1, 229E, NL63), RSV A/B, S. pneumoniae, *Haemophilus influenzae*, *Bordetella pertussis*, *M.pneumoniae*, *C.pneumoniae*, and *Legionella pneumophila*. The "SY-1 Rxn and SY-2 Rxn" strips were placed on a cooling block at -22°C, and 10 µl of "template nucleic acid" patient samples were pipetted into each. The strips were carefully sealed and placed in the Micro-PCR (BMS Mic qPCR cycler, Bioeksen, Türkiye). The amplification curves were examined for each reaction well to determine the Cq values. Sigmoidal curves above the threshold value were interpreted as "positive," while non-sigmoidal curves were considered"negative."

Statistical analysis of the data was performed using IBM Statistical Package for Social Sciences (SPSS) program software version 22. Pearson's Chi-square test

was used to compare categorical data between groups. One-way ANOVA-Duncan's test should be used if the data showed normal distribution; so, the Mann-Whitney U test was applied for continuous data that did not show a normal distribution. Data were considered significant at $p<0.05$.

Results

Among the 100 patients studied, 47% were male, and 53% were female, with a mean age of 47.74±18.89 years. No statistically significant differences were detected between groups in terms of age and gender (Chi-square test, p=0.823). Of the patients, 73 (73%) were positive for one or more viral and bacterial pathogens, while 27 (27%) were negative. Co-infections were observed in 28 (38.3%) of 100 patients. The most frequently detected co-infections were INF A/B – *S.pneumoniae* (n=5, 18.6%) and SARS-CoV-2/ *S.pneumoniae* (n=3, 11.1%). The pathogens involved in co-infections and their numerical distribution are shown in Table 1.

A total of 73 patients were diagnosed as positive, 37 (50.7%) were caused solely by viral agents, while 8 (10.9%) were caused by bacterial agents. SARS-CoV-2 (n=14, 11.4%) and INF A/B (n=33, 26.8%) were the most frequently detected viral agents. On the other hand, *S.pneumoniae* (n=19, 15.5%) was the most commonly identified bacterial pathogen. The numerical distribution of the other detected pathogens by month is shown in Table 2.

Discussion

The respiratory system is a complex structure divided into the upper and lower respiratory tracts. If the microbiome of the URT, which has a high bacterial load, becomes imbalanced, the invasion of opportunistic pathogens can lead to serious infections[1,11]. The innate immune system is the first defense against these invading pathogens[12]. Viral upper respiratory infections include viral pharyngitis, sinusitis, otitis media, and viral rhinitis[13].

Respiratory infections affect vulnerable populations, such as pregnant women, infants, and the elderly,

Table 1. *Pathogens involved in co-infections and their distribution*

Co-Infection Pathogens		N	%
INF A/B	S.pneumoniae	6	21.4 %
SARS-CoV-2	S.pneumoniae	3	10.7 %
HRV / EV	H.influenzae	2	7.2 %
SARS-CoV-2	AdV	1	3.5 %
SARS-CoV-2	HPiV 3	1	3.5 %
INF A/B	RSV A/B, L.pneumophila, HPiV 2, H.influenzae, HBoV, HRV/EV	1	3.5 %
INF A/B	S.pneumoniae HPiV4	1	3.5 %
INF A/B	H.influenzae	1	3.5 %
INF A/B	B.pertussis	1	3.5 %
HRV / EV	C.pneumoniae, L.pneumophila, HPeV	1	3.5 %
HRV / EV	HBoV, RSV A/B, S.pneumoniae, C.pneumoniae	1	3.5 %
HRV / EV	S.pneumoniae, RSV A/B	1	3.5 %
AdV	H.influenzae	1	3.5 %
RSV A/B	HCoV-OC43	1	3.5 %
RSV A/B	S.pneumoniae, HPiV 4	1	3.5 %
HCoV-HKU1	H.influenzae, S.pneumoniae	1	3.5 %
HCoV-HKU1	HCoV-NL63, M.pneumoniae HCoV-OC43 RSV A/B	1	3.5 %
HCoV-HKU1	HPiV2, HCoV-229E, HCoV-OC43, HRV/EV	1	3.5 %
HCoV-229E	HPiV4	1	3.5 %
HCoV-HKU1	HCoV-OC43, HCoV-NL63, HPiV3, INF A, AdV	1	3.5 %
Total Number of Patients with Co-infection		28	100 %

Table 2. *Distribution of detected pathogens by month*

Monthly Distribution of Detected Pathogens																	
Year	2023								2024								
Months	November				December				January				February				
Weeks	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	TOTAL
SARS-CoV-2		1				3		1	1	2	1	2		1		2	14
INF A/B								1	5	7	6	11	1		1	1	33
HRV/EV									1		2	1	1	1	2		8
HMPV																	-
RSV A/B										3		3		1	2		9
HPIV-1																	-
HPIV-2													1				1
HPIV-3						1						1			1		3
HPIV-4										2						1	3
HCoV-229E													1			1	2
HCoV-OC43												1	1				2
HCoV-NL63												2					2
HCoV-HKU1											1	3	1				5
HPeV											1						1
AdV								1	1		1	1					4
HBoV														1	1		2
S. pneumoniae		1						2	1	4	4	3		2	1	1	19
H. influenzae									1	1	3	2			1		8
C. pneumoniae											1			1			2
B. pertussis									1			1					2
M. pneumoniae												1					1
L. pneumophila											1				1		2
Total	-	2	-	-	-	4	-	5	11	19	21	32	6	7	10	6	123

and are the most common seasonal infectious diseases worldwide, often leading to epidemics and pandemics[14]. Common respiratory viruses, such as HRVs, can cause milder infections, while SARS-CoV-2 and seasonal Influenza A/B viruses can lead to more serious or even fatal illnesses in at-risk populations[15–17]. Because the specific clinical symptoms of URIs are often minimal, it is difficult to diagnose viral and bacterial infections based on clinical signs and radiological findings[18]. Approximately 60–80% of URIs are viral, with the most frequently detected viruses being INF A/B, HRVs, RSV, HCoVs, and HPiVs. Similar reports can be seen in current studies conducted in our country and worldwide. Talay et al. reported that 82 (95.3%) and 4 (4.6%) of all patients were viral and bacterial, respectively, while Aydin et al. reported these rates as 60.4% and 39.4% for viral and bacterial agents, respectively[19,20]. A study conducted between 2009 and 2019 in China showed that viral positivity was detected as 46.9% and bacterial was 30.9% in children under 5-years. Additionally, Kwiyolecha et al. reported that 46.9% and 40.4% of all participants were positive regarding viral and bacterial pathogens, respectively. Like others, a Shenzhen Children's Hospital study found that 49.1% had either a single bacterial or viral pathogen among 273 positive cases[21–23]. Our study observed that 73.9% of patients had only viral infections, while 26.1% had only bacterial infections. Based on our findings and the current literature, it is seen that viral pathogens are the major cause of URIs when compared to bacterial pathogens and have maintained their activity over time.

In studies from Türkiye, the most commonly detected viral and bacterial pathogens were respectively HRV (23.3%), *S.pneumoniae* (18.6%), and HCoVs

(17.4%). A study reported the most commonly seen agents in URTIs as follows; *H.influenzae* (48.8%), *S.pneumoniae* (29.3%), RSV (23.3%), and AdV (19.1%), while another study was reported as follows; RSV (40.7%), and AdV (23.26%)[18,19,24]. Similar studies are available in current literature that show the most commonly detected viral and bacterial agents. The detected agents in our country were generally INF A, SARS-CoV-2, HCoV, HRV/EV, RSV A/B, *S.pneumoniae*, and *H.influenza* [25,26]. The viral and bacterial agents were nearly same (INF A/B, RSV, HCoV, HRV, and *S.pneumoniae*) in other countries such as China, Mexico, Tanzania[21,22,27,28].

In our study, we detected INF A (26.2%), SARS-CoV-2 (11.1%), RSV (7.14%), *H. influenzae* (7.14%), and *S.pneumoniae* (15.9%) as the most common pathogens. Our findings were consistent with other studies both globally and in Türkiye. When comparing our data with the literature, we can conclude that the circulation and prevalence of respiratory pathogens fluctuate over time.

Co-infection of respiratory viruses and bacteria contributes to disease severity[29]. INF A/B infections are generally associated with severe immunopathology in immunocompromised individuals, infants, and elderly patients, leading to secondary viral or bacterial co-infections and lower respiratory tract infections[16]. SARS-CoV-2 can also cause co-infections with bacteria or viruses by damaging respiratory epithelial surfaces, leading to inflammatory and immune dysregulation. A meta-analysis emphasized the role of co-infections and superinfections in SARS-CoV-2 patients. Thus, viral and bacterial co-infections are thought to worsen the clinical presentation of COVID-19, including in children[17,30]. Girgin's study found viral co-infections in 23.5% of 413 patients and triple viral co-infections in 11 cases, with co-infection rates for RSV/HRV, HRV/HPiV, HRV/EV, and HRV/HBoV being 21%, 10.5%, 11.6%, and 12.8%, respectively[2]. Kuşkucu et al. identified multiple pathogens in 408 samples, with the following agents; HCoV/RSV, HMPV/AdV, HPiV/EV, and HBoV (7.23%, 6.47%, 0.63%, and 0.13%, respectively)[14]. Şirin et al. found co-infection rates of 10.8% (n=13) in 120 patients, with the highest co-infection rate for HRV and *S.pneumoniae* (23.1%), followed by HRV and HCoV-229E (15.4%)[18]. Additionally, Aydin et al. found that 72.7% (n=144) of children had multiple pathogens combined with SARS-CoV-2 and *H.influenzae* (14%)[20]. These studies indicate that co-infection rates should not be overlooked. In our study, we found that 37% (n=27) of patients had co-infections, with the most common combinations as INF A – *S.pneumoniae* (18.5%), SARS-CoV-2 – *S.pneumoniae* (11.1%), and HRV/EV – H. influenzae (7.4%).

In conclusion, the prevalence of respiratory pathogens observed in our study is consistent with findings from both national and international researches. Our goal in identifying the viral and bacterial agents responsible for URIs was to provide etiological data and contribute to understanding which pathogens are circulating and causing infections. High-sensitivity and specificity tests like Multiplex-PCR are crucial for providing quick diagnoses, which can help in the rapid administration of antiviral treatments and the prevention of unnecessary antibiotic use, thus reducing the development of antibiotic resistance. These methods are essential for preventing pandemics and epidemics and reducing the economic and social burden of these infections. Therefore, we recommend that PCR-based diagnostic methods, such as Multiplex-PCR, be used effectively and widely in hospitals to improve health system efficiency, reduce healthcare costs, and facilitate the management of respiratory infections.

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