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Original Article / Özgün Araştırma

The Comparison of Two Different Multiplex Respiratory PCR Panels and The Evaluation of the Viral and Bacterial Agents

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Abstract

Objective: Respiratory tract infections (RTIs) are among the leading causes of morbidity and mortality in children worldwide. This study aimed to compare the diagnostic performance of two multiplex PCR (MT-PCR) panels, 7-pathogen and 24- pathogen, for detecting viral and bacterial pathogens in pediatric patients having upper respiratory tract infection (URTI) symptoms.

Methods: The study was conducted between January and July 2024. A total of 61 pediatric patients aged 0-16 years and admitted to Kafkas University Health Research and Application Hospital were enrolled. Nasopharyngeal swab samples were collected and analyzed using MT-PCR panels. The 7- pathogen panel detected ADV, INF A/B, HRV, RSV A/B, SARS-CoV-2, and S. pyogenes, while the 24- pathogen panel included an extended range of pathogens, such as HCoV, HPIV, HBoV, S. pneumoniae, and H. influenzae. Comparative analyses focused on positivity rates, co-infections, and pathogen distribution.

Results: Pathogens were detected in 96.7% of the samples using the 24-pathogen panel compared to 83.6% with the 7-pathogen panel. Viral pathogens dominated the infections, with RSV A/B (10.3%) being the most frequently detected pathogen in 24- pathogen panel and INF-B (27.1%) in 7-pathogen panel. The 24-pathogen panel identified more bacterial pathogens, notably S. pneumoniae (22.4%). Co-infections were significantly higher with the 24-pathogen panel (62.3%) compared to the 7-pathogen panel (18.3%).

Conclusion: The 24-pathogen MT-PCR panel demonstrated superior diagnostic capabilities, highlighting the importance of comprehensive pathogen detection for accurate diagnosis and effective treatment of URTIs. This study underlines the necessity of using advanced molecular diagnostic tools to improve clinical outcomes and support public health strategies in managing respiratory tract infections among pediatric populations.

Keywords: Respiratory Tract Infections, Multiplex PCR, Viral Pathogens, SARS-CoV-2, Bacterial Co-infections, Pediatrics, URTI

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İki Farklı Multiplex Solunum PCR Panelinin Karşılaştırılarak Viral ve Bakteriyel Etkenlerin Değerlendirilmesi

Öz

Amaç: Solunum yolu enfeksiyonları (SYE) dünya çapında çocuklarda morbidite ve mortalitenin önde gelen nedenleri arasındadır. Bu çalışmanın amacı, üst solunum yolu enfeksiyonu (ÜSYE) semptomları olan çocuk hastalarda viral ve bakteriyel patojenleri tespit etmek için 7 patojen ve 24 patojen olmak üzere iki farklı multipleks PCR (MT-PCR) solunum panelinin tanısal performansını karşılaştırmaktır.

Yöntemler: Çalışmaya, Ocak-Temmuz 2024 tarihleri arasında Kafkas Üniversitesi Sağlık Araştırma ve Uygulama Hastanesi'ne başvuran 0-16 yaş arası toplam 61 çocuk hasta dahil edildi. Nazofarengeal sürüntü örnekleri toplanmış ve hem 7-patojen hem de 24-patojenli MT-PCR solunum yolu panelleri kullanılarak analiz edilmiştir. Panellerde başta ADV, INF A/B, HRV, RSV A/B, SARS-CoV-2 ve *S. pyogenes*'i olmak üzere çok sayıda patojen mevcuttur. Karşılaştırmalı analizler pozitiflik oranlarına, ko-enfeksiyonlara ve patojen dağılımına göre gerçekleştirilmiştir.

Sonuçlar: 24-patojen PCR paneli kullanılarak örneklerin %96,7'sinde pozitiflik tespit edilirken, bu oran 7-patojen PCR panelinde %83,6'dır. Viral patojenler enfeksiyonların büyük kısmını oluştururken, 24 patojenli panelde en sık tespit edilen ajan RSV A/B (%10,3) ve 7 patojenli panelde en sık tespit edilen patojen INF-B (%27,1) olmuştur. 24 patojenli panelde başta S. pneumoniae (%22,4) olmak üzere fazla sayıda bakteriyel patojen tespit edilmiştir. Ko-enfeksiyonlar 7-patojenli panele (%18,3) kıyasla 24-patojenli panelde (%62,3) önemli ölçüde daha yüksek olarak tespit edilmiştir.

Tartışma: 24 patojenli MT-PCR paneli, ÜSYE'lerin doğru teşhisi ve etkili tedavisi amacıyla kapsamlı patojen tespitinin önemini vurgulayan bir paneldir. Bu çalışma, klinik sonuçların değerlendirilmesini iyileştirmek ve pediatrik popülasyonlar arasında solunum yolu enfeksiyonlarının yönetiminde halk sağlığı stratejilerini desteklemek için gelişmiş moleküler tanı araçlarının kullanılması gerekliliğinin altını çizmektedir.

Anahtar kelimeler: Solunum Yolu Enfeksiyonları, Multipleks PCR, Viral Patojenler, SARS-CoV-2, Bakteriyel ko-enfesiyonlar, ÜSYE.

INTRODUCTION

Respiratory tract infections (RTIs) encompass a group of diseases affecting the upper and lower respiratory tracts, characterized by high prevalence and significant public health burden due to a wide variety of pathogens[1]. RTIs are among the leading causes of morbidity and mortality globally, ranking fourth in terms of global mortality based on recent WHO data[2,3]. The etiological agents of RTIs include a wide range of pathogens such as viruses, bacteria, and fungi[1]. The respiratory pathogens responsible for infections vary regionally due to climatic, cultural, and geographic differences[4].

In pediatric patients, etiological agents of respiratory infections are generally Adenovirus (AdV), Human Bocavirus (HBoV), Human Enterovirus (HeV), Human Rhinovirus (HRV), Respiratory Syncytial Virus (RSV A and B), Human Metapneumovirus (HMPV), Human Parainfluenza Virus (HPiV 1/2/3/4), Influenza viruses (IFN A/B), and Human Coronavirus (HCoV) alongside bacterial pathogens such as *Streptococcus pyogenes* (*S.* pyogenes), *Streptococcus pneumoniae* (*S. pneumoniae*), *Mycoplasma pneumoniae* (*M. pneumoniae*), and *Haemophilus influenzae* (*H. influenzae*). Some viruses, like RSV and IFN A/B, exhibit significant seasonal variations, while others persist throughout the year[5]. RTI symptoms range widely, from simple colds to severe conditions like pharyngotonsillitis, sinusitis, and laryngotracheitis[6].

The upper respiratory tract (URT) is frequently exposed to external microorganisms, and hosts a resident microbiota. The nasal and oral cavities are good entry points for pathogens into the lower respiratory and gastrointestinal tracts. Disruptions in microbial balance, increased antimicrobial exposure, and loss of beneficial microorganisms can predispose the URT to pathogen colonization, increasing susceptibility to Upper Respiratory Tract Infections (URTIs)[7]. Symptoms of URTIs, including sore throat, fever,

nasal discharge, and cough, affect many individuals, leading to increased healthcare utilization and significant medical costs[8]. Risk factors for URTIs include early exposure to infectious agents, immunosuppression, indoor and outdoor pollution, secondhand smoke exposure, atopic reactions, allergies, and low socio-economic status. These infections can recur and sometimes require hospitalization[9]. WHO estimated approximately 5 million deaths in children under five years due to RTIs in 2022, emphasizing the preventable nature of these deaths through timely treatment[10].

Diagnostic methods for these infections are generally culture (for especially bacterial agents), immunochromatographic tests, cell culture (for especially viral agents), direct fluorescent antibody tests, ELISA, and molecular techniques such as Polymerase Chain Reaction (PCR)[11,12]. Multiplex PCR (MT-PCR) panel tests, offering high sensitivity and rapid turnaround times, are increasingly used for the simultaneous detection of various pathogens in a single sample. Viral and bacterial co-infections in pediatric patients are frequently identified using MT-PCR[13].

This study was aimed to determine the positivity rates of AdV, INF (A/B), HRV, RSV (A/B), SARS-CoV-2, and S. pneumoniae using a 7-pathogen MT-PCR panel and AdV, HCoV (NL63, OC43, HKU1, 229E), INF (A/B/A H1N1 2009/A H3), HBoV, HMPV, HeV, HRV, RSV (A/B), SARS-CoV-2, HPiV 1/2/3/4, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, *B. pertussis*, *M. pneumoniae*, and *L. pneumophila* using a 24-pathogen MT-PCR panel among pediatric patients presenting with URTI symptoms between January and July 2024. Additionally, it was also aimed to compare the findings of these two molecular panels.

METHODS

Between January and July 2024, nasopharyngeal swab samples were collected from 61 pediatric patients presenting with URTI symptoms at Kafkas University Health Research and Application Hospital, Pediatric outpatient clinic. Before initiating the study, clinical data, including clinic, gender, and age were recorded. Ethical approval was obtained from the Clinical Research Ethics Committee of Kafkas University Faculty of Medicine (Decision No: 2024/368).

Nasopharyngeal swab samples were collected by the clinicians from the nasopharyngeal region under hygienic conditions and transported to the laboratory on the same day in vNAT® transfer tubes. Nucleic acid extraction was performed using the Bio-speedy Extraction kit (Bioeksen, Istanbul, Turkey) based on a magnetic bead method, following the manufacturer's instructions, on the Zybio EXM 3000 device (Zybio, Bioeksen, Istanbul, Turkey). Samples were processed in sterile conditions for MT-PCR analysis after obtaining high-purity nucleic acid (RNA-DNA) from the lysate, nucleic acid adsorption, magnetic bead transfer, washing, and elution steps. All patients' samples were evaluated first 7- pathogen PCR panel and then 24-pathogen PCR panels. All results were recorded and compared with eachothers.

The Respiratory RT-qPCR MX-24L panel detected 18 viral and 6 bacterial agents, including SARS-CoV2, EV, RHV, HPIV (1/2/3/4), AdV, HBoV, HMPV, INF A/B, INF A H1N1 2009, INF A-H3, INF A-H1, HCoV-OC43, HCoV-HKU1, HCoV-229E, HCoV-NL63, RSV A/B, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, *B. pertussis*, *M. pneumoniae*, and *L. pneumophila*.

Simultaneously, the 7-pathogen Respiratory Panel used the same method to detect INF A/B, SARS-CoV2, RHV, RSV A/B, AdV, and *S. pyogenes*. "SY-1 Rxn and SY-2 Rxn" strips were placed on a cooling block at -22°C. To each strip, 10 µL of "Template Nucleic Acid" was added from patient samples using precise pipetting techniques. The strips were sealed and placed into the Micro-PCR (BMS mic qPCR cycler, Bioeksen, Istanbul, Turkey) device for analysis. Table 1 outlines the amplification steps of the 7- and 24-pathogen Respiratory Panels using Multiplex-PCR.

Table I: Amplification steps for 7- and 24-Pathogen Respiratory Panels

Steps	Cycle Count	Temperature	Duration
Reverse Transcriptase	1	52°C	3 min.
Holding	1	95°C	10 min
Denaturation	(12) Touchdown Cycle	95°C	1 sec.
Touchdown Cycle		67°C-56°C	15 sec.
Denaturation	30	95°C	1 sec.
Attachment/extension		95°C	15 sec.
Detection		(FAM-Green) (HEX-Yellow) (ROX-Orange) (CY5-Red)	

The obtained amplification curves were analyzed using the Sigmoida software, based on the Cq values. For each reaction well, the FAM, HEX, ROX, and CY5 channels were examined, and curves above the threshold value were considered "positive" while non-sigmoidal curves were evaluated as "negative."

RESULTS

Nasopharyngeal swab samples from 61 pediatric patients who presented URTI symptoms and admitted to Kafkas University Health Research and Application Hospital Pediatric outpatient clinic were collected. These samples had previously been tested using a 7-pathogen respiratory panel in routine Microbiology laboratory and we retested the same samples by using a 24-pathogen panel. The data were compared with the 7-pathogen panel results.

A total of 61 patients were enrolled in this study. Of those, 31 (50.82%) were female, and 30 (49.18%) were male, with a mean age of 4.41±0.50 years. As seen in Table 2, the most seen detected microorganism was *S. pneumoniae* by 24-pathogen panel, while it was INF-A by 7-pathogen panel (Table 3). According to the PCR results, 13 of the 61 samples showed only viral infections, 8 showed only bacterial infections, and 38 had co-infections, while no pathogens were detected in 2 sample. By the way, 44 of the 61 samples showed only viral infections, 5 showed only bacterial infections, and 11 had co-infections, while no pathogens were detected in 10 sample in 7-pathogen panel. All number and percentage information about microorganism can be seen both in Table 2 and Table 3.

One or more additional microorganism was detected in 32 patients by 24-pathogen PCR panel when compared to the results of 7-pathogen PCR panels. The result was negative in two patients by 24-pathogen PCR panel, while a microorganism was detected by 7- pathogen PCR panel in the same patients. While the cause of this situation is unknown; it is estimated that it may be due to a mistake in the steps of isolation and/or manipulation. On the other hand, the result was negative in 10 patients by 7-pathogen PCR panel, while at least one microorganism was detected by 24-pathogen PCR panel in the same patients.

Table II: Pathogen distribution of the 24-pathogen Respiratory Panel

Pathogens	n	%	Pathogens	n	%	Pathogens	n	%
S. pneumoniae	26	22,4	SARS-CoV-2	4	3.4	*L. pneumoniae*	-	-
RSV-A/B	12	10,3	INF-A H3	4	3.4	HPiV-4	-	-
H. influenza	11	9,5	INF-B	4	3.4	HBoV	-	-
INF-A	10	8,6	HCoV-229E	2	1.7	HCoV-NL63	-	-
S. pyogenes	10	8,6	HCoV-HKU1	2	1.7	EV	-	-
RHV	9	7,8	HMPV	1	0.9	HPiV-1	-	-
INF-A H1N1 2009	6	5,2	HCoV-OC43	1	0.9	HPiV-3	-	-
AdV	6	5,2	HPiV-2	1	0.9			
M. pneumoniae	6	5,2	*B. pertussis*	1	0.9			

According to the Chi-square test results, no significant differences were found between the 7-pathogen PCR results and gender (p=0.578), or between the 24-pathogen PCR results and gender (p=0.362). Similarly, no significant differences were found between PCR results and age (p=0.783).

Table III: Pathogens and Co-infection distribution of the 7-pathogen Respiratory Panel

Pathogens	n	%	Co-Infection	n	%
INF-A	10	14.3	RSV/INF-B	4	36.4
INF-B	19	27.1	*S. pyogenes*/SARS-CoV-2/RSV	1	9.1
SARS-CoV-2	3	4.3	AdV/*S. pyogenes*	2	18.2
S. pyogenes	8	11.4	INF-A/INF-B	1	9.1
RHV	8	11.4	RHV/INF-B	3	27.3
AdV	10	14.3			
RSV	12	17.1			
Total	70		**Total**	11	18,3

Among the 61 samples included in the study, a pathogen was detected in 59 (96.7%), while no pathogen was observed in 2 (3.8%). In 13 samples (22.03%), only viral pathogens were detected, and in 8 samples (13.56%), only bacterial pathogens were identified. Co-infections were detected in 38 samples (64.41%), with 22 samples (57.9%) having two pathogens, and 16 samples (42.1%) having three or more pathogens (Table 4). The most commonly detected co-infection was RSV A/B and *S. pneumoniae* (n=5, 13.2%). The left side of Table 4 is presenting the distribution of three or more co-infection agents, while the right side of it is presenting the distribution of two co-infection agents.

Table IV: Co-infection distribution of the 24-pathogen Respiratory Panel

Pathogens	n	%	Pathogens	n	%
SARS-CoV2 /RSV/INF-B	1	2.6	RSV/*S. pneumoniae*	5	13.2
INF-A/INF-A H3/*S. pneumoniae*/AdV	1	2.6	RHV/*S. pneumoniae*	2	5.3
INF-B /*S. pneumoniae/H. influenzae*	1	2.6	SARS-CoV2/*S. pyogenes*	2	5.3
RHV/*H. influenzae*/HPiV-2	1	2.6	AdV/HCoV-HKU1	1	2.6
AdV/*S. pneumoniae/H. influenzae*	1	2.6	INF-A/INF-A H3	1	2.6
HMPV/*H. influenzae/S. pneumoniae*	1	2.6	INF A/*H. influenzae*	1	2.6
HCoV-OC43/*S. penumoniae/S. pyogenes*	1	2.6	RHV/*M. pneumoniae*	1	2.6
RSV/*M. pneumoniae/S. pneumoniae*	1	2.6	RSV/*H. influenzae*	1	2.6
INF-A/INF-B/INF-A H1N1 2009/*S. pneumoniae*	1	2.6	*M. pneumoniae/S. pneumoniae*	1	2.6
INF-A/INF-A H3/*M. pneumoniae/H. influenzae*	1	2.6	INF-B /*S. pneumoniae*	1	2.6
INF-A H1N1 2009/*S.pneumoniae*	1	2.6	*S. pyogenes/S. pneumoniae*	1	2.6
INF-A/INF-A H1N1 2009/*S. pneumoniae*/HCoV-229E	1	2.6	*H. influenza/S. pyogenes*	1	2.6
RHV/*H. influenzae/S. pneumoniae*	1	2.6	RSV/*M. pneumoniae*	1	2.6
INFA/ INFA H1N1 2009/*S.pneumoniae/B.pertussis/H.influenzae*	1	2.6	INF-A/INF-A H1N1 2009	1	2.6
RSV/*H.influenzae/S.pneumoniae*	1	2.6	AdV/*S.pyogenes*	1	2.6
INF-A/INF-A H1N1 2009/*S.pneumoniae*	1	2.6	HCoV-HKU1/RHV	1	2.6
Total	16	41.6	**Total**	22	62.3

DISCUSSION

RTIs continue to pose a significant burden on global healthcare systems with the emergence of SARS-CoV-2[12]. URTI is characterized by one or more symptoms such as fever, cough, nasal discharge, nasal congestion, and respiratory distress, and can lead to respiratory system diseases such as laryngotracheitis, bronchiolitis, the common cold, otitis, tonsillitis, sinusitis, acute bronchitis, and laryngitis[14]. These infections increase hospital visits and create a significant economic burden on healthcare systems by causing workforce loss due to treatment processes and hospitalizations[15,16]. The simultaneous presence of multiple viral and bacterial pathogens can complicate the diagnostic process due to the similar symptoms caused by these pathogens. At this point, molecular methods such as RT-PCR tests emerge as an effective tool for diagnosis due to their high sensitivity[17]. RT-PCR, the ability to detect both viral and bacterial pathogens simultaneously, not only allows for accurate and effective treatment but also contributes to obtaining epidemiological and prevalence data to understand the distribution of pathogens. Various multiplex PCR test panels are used today for the detection of respiratory viruses[17-19]. Moreover, it contributes to accurate treatment processes by increasing the diagnosis rate up to 90%[15].

When the positivity rates of viral and bacterial agents causing URTI in the literature were checked, it was seen that most of studies had parallel findings. Şen et al. examined 21 pathogens using RT-PCR in 120 patients, and detected one or more agents in 71 (59.2%) patients, while no pathogens were detected in 49 (40.8%). Of those agents, 69.8% were viral and 30.2% were bacterial[18]. Another study performed by Aydin et al. also reported similar data that 60.4% of detected agents were viral and 39.4% were bacterial[20]. Karabulut et al. performed a study in 9354 patients and detected 23 different respiratory pathogens by using multiplex respiratory panel kit. They detected viral pathogens in 3,779 (48.41%) patients and bacterial pathogens in 1254 (16.06%) patients[2]. Duclos et al. detected viral pathogens in 558 patients (99.1%) and bacterial pathogens in 5 patients (0.9%) out of 1334 patients[21]. In our study, the viral pathogen rate with 7-pathogen panel was detected as 88.6%, while the bacterial pathogen rate was 11.4%. In 24-pathogen panel, these rates were determined as 53.4% and 46.6%, respectively. The results of our study are consistent with other studies in the literature that viral pathogens are more dominant than bacterial pathogens among circulating URTIs agents. This may due to the fact that URTI is generally of viral origin, and molecular methods such as RT-PCR allow for extensive pathogen detection.

The use of a multiplex pathogen approach in the detection of agents causing URTI will accelerate the identification of co-infections, which are common in children under the age of 5, and help understand their prevalence. Since co-infections leads an increase in severity and mortality rates, rapid diagnosis is advantageous as it saves time, costs, and provides faster and more specific treatments[13]. Aydoğan et al. detected 2,156 (24.4%) pathogens in 8,825 samples. The most frequent pathogens in these positive samples were HRV (n=586, 31.8%) and RSV (n=302, 16.4%), respectively[22]. In another study, the frequencies of pathogens were as follows: HRV (n=20, 23.3%), *S. pneumoniae* (n=16, 18.6%), HCoVs (n=15, 17.4%), INFs (n=9, 10.4%), and *S. aureus* (n=9, 10.4%)[17]. Zhao et al. detected the following agents in their study; *S. pneumoniae* (9.66%), *M. catarrhalis* (5.80%), PiV (4.83%, including types[1-4], and HMPV (3.38%)[23]. Similarly, another study reported that the most frequently detected pathogens were HRV/EV (25.13%) and RSV A/B (24.42%)[24]. In our study, the most

frequently detected pathogens for the 24-pathogen panel were *S. pneumoniae* (n=26, 22.4%) and RSV A/B (n=12, 10.3%), while they were INF-B (n=19, 27.1%) and *S. pyogenes* (n=8, 11.4%) for 7-pathogen panel. According to the current literature, it is understood that the positivity rates of viruses such as INF, HCoV, RSV A/B, HRV/EV, HPMV, and bacteria including *S. pneumoniae*, *H. influenzae*, and *S. pyogenes* in URTI can vary over time and in different regions. The understanding of this variability and the reliability of the prevalence data obtained significantly depend on the contribution of molecular methods, especially techniques like RT-PCR.

The co-occurrence of agents causing URTI (co-infection) increases the severity of the disease and exacerbates the clinical process. The impact of viral and bacterial co-infections has become more pronounced, particularly during the COVID-19 pandemic[25,26]. Kuşkucu et al. detected multiple pathogens in 57 samples (7.23%) and reported co-infection rates for HCoV/RSV, HMPV/AdV, HPiV/EV, and HBoV as 7.23%, 6.47%, 0.63%, and 0.13%, respectively[15]. Türe et al. detected co-infection in 37 of 119 patients (31.1%). They most frequently observed the combination of HRV and INF-A (33.3%) and HRV and RSV-A/B (27.3%)[27]. Aydoğan and colleagues detected multiple pathogens in 313 of 2156 patient samples (14.5%). The most frequently detected combinations were AdV/RV (10.9%) and EV/HRV (9.6%)[22]. A study performed in our country reported the co-infection rate detected by 7-pathogen Multiplex PCR panel for URTI as 9.14%[28]. In our study, the co-infection distributions in patients infected with multiple agents were RSV/S. pneumoniae for the 24-pathogen panel (n=38, 62.3%) and RSV/INF-B for the 7-pathogen panel (n=11, 18.3%).

MT-PCR enables to detect the co-infection in diseases and to test the greater number of pathogens. In this context, it is possible to say that pathogens such as *H. Influenzae*, *S. pneumoniae*, HRV, EV, RSV, INF, and AdV often form co-infections with other respiratory agents both n our country and globally. Depending on the type of pathogen, co-infection case/mortality rates, bacterial and viral infection rates in developing countries were range from 16-18%, 10-14%, and 1-7.3%, respectively[29].

Co-infections have the potential to pose a global public health threat. In this context, the MT-PCR method enables the rapid and accurate identification of pathogens, facilitating timely and effective treatment. At the same time, it contributes to reducing unnecessary antibiotic use and preventing the development of multidrug resistance. This reduces hospital admissions and mortality risk, thereby alleviating the burden on healthcare systems[30]. Unnecessary antibiotic prescriptions in viral infections are a critical factor that leads to the development of resistance. Therefore, molecular tests like MT-PCR are of great importance in determining the etiology of the pathogen and implementing the correct treatment strategies. Additionally, MT-PCR enables timely diagnosis, allowing for the rapid implementation of necessary measures to control the spread of the infection[31].

In conclusion, respiratory pathogens which exhibit seasonal variations and are more active during the winter and spring months in our country infect pediatric patients, and no similar study has been conducted in the Kars region before, as seen in the literature. It is believed that sharing the data obtained from this study will contribute scientifically to the literature. In this study, we compared the rates of viral and bacterial infections detected by two different PCR panels (7-pathogen and 24-pathogen) in children aged 0-16 years with URTI. The 24-pathogen MT-PCR panel allowed us to detect the presence of pathogens not found in the 7-pathogen panel. The results suggest that

working with more comprehensive multi-plex panels rather than narrow ones, which are a rapid and effective method for identifying URTI pathogens, is crucial for the accurate determination of pathogen etiology and prevalence. Moreover, a comparison in terms of cost, performance, and technical aspects guides the decision-making process in choosing which analysis method to use.

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