

# Does the Use of Multiplex PCR Contribute to the Management of Paediatric Emergency Physicians in <2-Year-old Children with Acute Respiratory Infections?

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## Abstract

Multiplex polymerase chain reaction (PCR) is used to detect respiratory viruses in pediatric emergency departments, but its indications and interpretation of results must still be clear. In the present study, we examined the effect of detecting a viral agent with multiplex PCR on patient management. Infants and toddlers, aged between 1-24 months, who presented to the pediatric emergency department with respiratory tract infection complaints and underwent multiplex-PCR between 1 January 2014 and 28 February 2020 were included in the study. Patients with at least one agent detected were considered as the study group, and patients without detection were considered as the control group. The same design was implemented only for patients with chronic diseases. A total of 1106 patients were recruited [median age: 6.7 months (range: 2.9-13.0 months)]. Seven hundred and eighty-nine in the study group and 317 in the control group. There were no significant differences between the groups in hospital admissions (study group: 271 admissions; control group: 89 admissions;  $p=0.055$ ), length of hospital stay duration [mean  $\pm$  standard deviation:  $3.09\pm 7.87$  days (study group) and  $2.6\pm 7.79$  days (control group);  $p=0.045$ ], or antibiotic use [234 patients (study group) and 77 patients (control group);  $p=0.078$ ]. When these variables were examined only for those with chronic diseases, there was no difference again. Although multiplex PCR is an ideal method with high sensitivity, specificity, and cost-effectiveness, the limits of its clinical application need to be clarified. We did not observe significant differences in the treatment of patients with detected viral agents.

**Keywords:** Pediatric acute respiratory infection, pediatric emergency, polymerase chain reaction



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## Introduction

Acute respiratory infections (ARI) in children are one of the most common causes of emergency department visits and paediatric hospitalisations. Almost 40-60% of hospital visits and 30-40% of hospitalisations among children seem to be due to ARI.<sup>1,2</sup>

Also, these infections are one of the most important causes of death in children under the age of 5.<sup>3</sup> Although most of the causes of ARI are viral, unnecessary antibiotic prescriptions continue.<sup>4,5</sup> This increases antibiotic resistance and is a concern for the future.

The viral causes of ARI can be detected by viral culture, direct immunofluorescence assays (DFA), rapid antigen tests, and nucleic acid tests.<sup>6</sup> Apart from nucleic acid tests, other tests are no longer preferred due to their important limitations. Viral culture is a time-consuming method and requires expertise rather than advanced technology. Direct immunofluorescence tests and rapid antigen assays can give results in relatively short time compared to viral cultures. However, their sensitivity and specificity are lower, and they can only be used for specific viruses.<sup>7</sup>

Monoplex polymerase chain reaction (PCR) test is one of the nucleic acid-based methods. It can detect single viral pathogens such as RSV and influenza viruses. Because it can detect one pathogen at a time, it is a time-consuming method for ARI. Another nucleic acid-based method is the multiplex PCR test. With multiplex PCR tests, it is possible to detect a wide range of respiratory viruses.<sup>8</sup> Its specificity and sensitivity are higher than other methods. In addition, it requires a shorter time and is cost-effective.

Based on clinical features, it is not always possible to distinguish between viral and bacterial infections in patients with ARI. It is aimed to make this distinction with rapid viral diagnostic tests. Studies on diagnostic methods other than PCR tests have shown that these tests provide a reduction in antibiotic use, hospitalisation, and length of stay.<sup>9,10</sup> Similar results are expected with PCR tests, but studies show that multiplex PCR tests do not lead to decreases in hospital admissions, shorter hospital stays, or less antibiotic use for children with ARI.<sup>11,12</sup>

In appropriate indications, we take multiplex PCR tests from some patients with ARI symptoms, like many hospitals. The aim of this study is to determine whether the multiplex PCR results of patients with respiratory symptoms affect patient management in the paediatric emergency department.

## Materials and Methods

In a controlled clinical trial, nasal wash specimens (NWS) were obtained to evaluate multiplex PCR diagnostic

method for ARIs in pediatric patients. From 1 January 2014 to 28 February 2020, NWS was obtained from children with ARI symptoms such as fever, rhinorrhea, nasal congestion, cough, or respiratory distress. Infants and toddlers were included in the study, so patients younger than two years and older than one month were included.<sup>13</sup> Since the latest guideline published by the American Academy of Pediatrics does not recommend using NWS in newborns, patients younger than one month were excluded from the study.<sup>14</sup> The study was conducted in Hacettepe University Hospital Pediatric Emergency Department where approximately 70000 patients presented annually. The study was approved by the Institutional Review Board of the Hacettepe University (date: 15.03.2022, decision no: 2022/05-02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Multiplex PCR (Bosphore Respiratory Pathogens Panel Kit v4, Anatolia Gene Works, Turkey) was used to detect viral agents in NWS following the

manufacturer's instructions. Multiplex PCR assays were performed for 17 pathogens (RSV A, RSV B, influenza viruses A and B, adenovirus, parainfluenza viruses 1, 2, 3, and 4, human bocavirus, coronaviruses 229E, HKU1, OC43, and NL63, human metapneumovirus, rhinovirus, enterovirus). Patients with at least one viral agent as a result of PCR were considered the study group, and patients without any agent were considered the control group.

Demographic characteristics, presenting symptoms at admission, vital findings, laboratory and imaging tests, treatment methods, type of respiratory support (if done), hospital admission, admission to the intensive care unit, length of hospital stay, and clinical outcomes of patients were retrospectively investigated from medical records. Additionally, results of multiplex PCR for respiratory tract pathogens in the NWS were recorded.

ARI was defined as a new episode of respiratory symptoms of the upper and/or lower airways. Upper respiratory tract infection was defined as respiratory symptoms without abnormalities in lung auscultation (e.g., rhinorrhea, nasal congestion, sore throat, erythematous pharynx, earache or erythematous eardrum). Lower respiratory tract infection (LRTI) was defined as respiratory symptoms with abnormalities in lung auscultation (e.g., rales, crackles, crepitations, wheezing, or prolonged expiration).

The hospital admissions of the patients with the same complaints related to respiratory tract infection within 7 days after the first hospital admission were accepted as revisit. It was not accepted as a revision due to other symptoms and diagnoses.

### Highlights

- Multiplex-polymerase chain reaction (PCR) is a method with high sensitivity and specificity in rapid viral diagnosis and allows more than one factor to be studied simultaneously.
- Few studies on its effect on patient management have presented different results.
- Clinicians did not make any changes in patient management on antibiotic use and hospital admission with the results of multiplex PCR.
- In patients with chronic disease, antibiotic use, hospitalisation, and length of hospital stay were higher.

## Statistical Analysis

Statistical Package for Social Sciences (SPSS) for Windows 22.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Variables were investigated using visual (histogram, probability plots) and analytical methods (Kolmogorov-Smirnov) to determine whether they were normally distributed. Numerical measurements were presented with mean and standard deviation or medians with interquartile range based on distribution; qualitative data with numbers and percentages. According to the distribution of numerical variables, a paired sample t-test or Mann-Whitney U was performed to investigate the differences between the groups. For categorical variables, a chi-square test or Fisher exact test was performed. The possible factors determined by the univariate analysis were then analysed with a multiple logistic regression model. The p-value <0.05 was considered statistically significant.

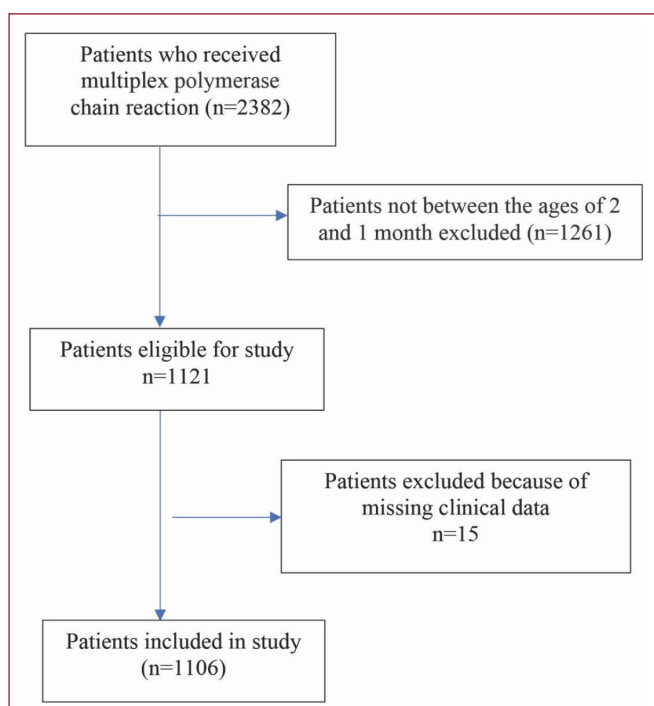
## Results

### Patient Enrollment

The medical records of patients administered to the paediatric emergency department between 2014 and 2020 were retrospectively reviewed using the hospital database. Patients who obtained NWS due to suspected respiratory tract infection were screened. During the 6-year period, NWSs were performed on 2382 patients. According to the inclusion criteria of our study, the number of patients aged 1-24 months who obtained NWS were 1121. Fifteen of them were not included in the study because of missing clinical data. A total of 1106 patients were recruited in this study. **Figure 1** shows the flowchart of patient enrollment.

### Main Characteristics of Patients

Demographic and clinical characteristics are presented in **Table 1**. At least one viral agent was detected in



**Figure 1.** Flowchart of patient enrollment

789 (71.3%) patients. Seven hundred and eighty-nine patients were included in the study group and 317 (28.6%) patients were in the control group. The median age, sex, chronic disease, and revisit rates of both groups were found to be close to each other. Cough, rhinorrhea and wheezing were statistically more found in the study group ( $p<0.001$ ). The rash was greater in the control group ( $p<0.001$ ). When laboratory test results were compared, the neutrophil-lymphocyte ratio was higher in the study group ( $p=0.016$ ), while C-reactive protein was higher in the control group ( $p=0.024$ ).

### Outcomes

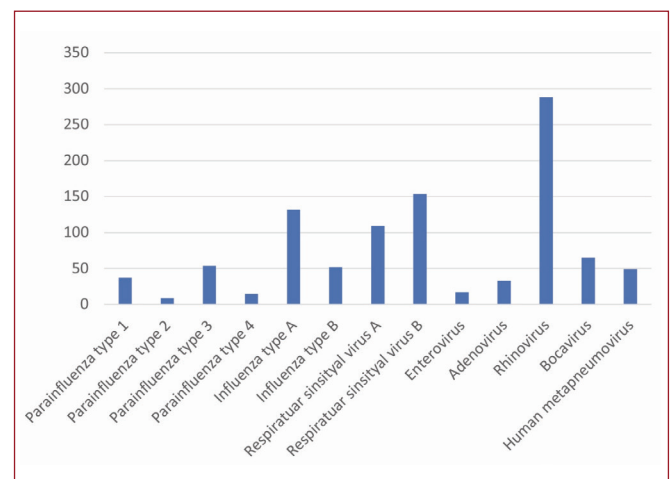
Regarding the main objectives of this study, chest X-ray, inhaler salbutamol, oseltamivir, and oxygen therapy with mask were higher in the study group ( $p=0.001$ ,  $p<0.001$ ,  $p<0.001$ ,  $p=0.026$ , respectively). In addition, the duration of hospitalisation was longer and statistically significant ( $p=0.045$ ). Although antibiotic treatment and hospitalisation were higher in the study group, no statistically significant differences were found (**Table 1**).

Among viral agents, rhinovirus was detected most frequently in 288 (26.0%) patients, while RSV A/B was detected in 263 (23.7%) patients, and influenza A/B was detected in 184 (16.6%) patients (**Table 2**). The coinfection rate was 13.8%. Coinfection was detected with two agents in 121 (10.9%) of the patients and three agents in 14 (1.2%) (**Table 3, Figure 2**).

Two hundred and seventy-six patients in the study had at least one chronic disease. The distribution of these chronic diseases is as follows: cardiovascular disease 84, pulmonary disease 45, neuromuscular disease 44, renal disease 24, gastrointestinal disease 29, hematological disease 43, metabolic disease 14, malignancy 13 patients. Humanrhinoviruses are the most common viruses detected in patients with chronic diseases. Antibiotic use, hospitalization, and the length of hospital stay were higher in the study group, but no statistically significant difference was found.

## Discussion

This study is one of the rare studies examining the utilization of multiplex PCR in the pediatric emergency department. Multiplex PCR testing has replaced the old methods, and its routine use in practice has become



**Figure 2.** Distribution of viral agents

widespread in the last decade.<sup>15</sup> Studies on the subject mostly show the distribution of detected viruses according to seasons and years.<sup>6,16-18</sup> In addition, there are studies investigating other rapid viral diagnosis methods, such as DFA and viral culture, and showing that hospitalization rates and hospital stays of patients are shortened.<sup>9,10</sup> However, at the time of these studies, multiplex PCR was not used. Furthermore, DFA and viral

culture were compared in these studies, and the DFA result time may be shorter than the viral culture. Studies on the clinical effect of the multiplex PCR assay are limited. The main goals are to determine the indications of virus PCR and to improve our ability to interpret the results from a clinical point of view.

The present study observed that the detection of a viral agent as a result of multiplex PCR did not affect the

**Table 1.**  
*Demographic and clinical characteristics of the patients*

	Viral agent (+) (n=789)	Viral agent (-) (n=317)	p-value
<b>Age, month (median, IQR)</b>	6.5 (2.8-13.5)	7.1 (3.6-13.2)	0.737
<b>Sex</b>			
Male	443 (56.1%)	175 (55.2%)	0.775
Female	346 (43.9%)	142 (44.8%)	
<b>Chronic disease</b>	195 (24.7%)	81 (25.5%)	0.685
<b>Revisit</b>	45 (5.7%)	15 (4.7%)	0.540
<b>Symptoms</b>			
Fever	482 (61.1%)	204 (64.4%)	0.266
Highest measured fever, °C, (median, IQR)	38.5 (38.0-39.0)	38.6 (38.0-39.3)	0.325
Cough	588 (74.5%)	160 (50.5%)	<0.001
Rhinorrhea	388 (49.2%)	112 (35.3%)	<0.001
Wheezing	198 (25.1%)	42 (13.2%)	<0.001
Respiratory distress	91 (11.5%)	33 (10.4%)	0.601
Vomiting	171 (21.7%)	68 (21.5%)	0.971
Diarrhea	89 (11.3%)	38 (12%)	0.739
Rash	37 (4.7%)	46 (14.5%)	<0.001
Restlessness	56 (7.1%)	26 (8.2%)	0.519
Cyanosis	29 (3.7%)	16 (5%)	0.292
Symptom duration before admission, day, (median, IQR)	2 (1-4)	2 (1-4)	0.885
<b>Vital signs</b>			
Tachycardia	183 (23.2%)	71 (22.4%)	0.841
Tachypnea	190 (24.1%)	52 (16.4%)	0.016
Hypoxia	156 (19.8%)	56 (17.7%)	0.459
Hypotension	2 (0.3%)	0	0.369
<b>Laboratory and imaging tests</b>			
White blood cell count, ×10 <sup>9</sup> /L, (median, IQR)	10.2 (7.7-10.3)	10.0 (7.0-14.2)	0.194
Neutrophil count, ×10 <sup>9</sup> /L, (median, IQR)	3.6 (2.0-6.6)	3.7 (2.0-6.6)	0.962
Lymphocyte count, ×10 <sup>9</sup> /L, (median, IQR)	4.5 (3.1-6.2)	4.3 (2.9-6.1)	0.232
Neutrophil-lymphocyte ratio (NLR), (median, IQR)	0.87 (0.42-1.80)	0.78 (0.37-1.66)	0.016
C-reactive protein level, (median, IQR), mg/L	0.81 (0.31-2.18)	0.88 (0.35-2.76)	0.024
Chest X-ray	557 (70.6%)	191 (60.3%)	0.001
<b>Management</b>			
Inhaler salbutamol	265 (33.6%)	64 (20.2%)	<0.001
Oral antibiotic	234 (29.7%)	77 (24.2%)	0.078
Intravenous antibiotic	227 (28.8%)	75 (23.6%)	0.093
Oseltamivir	314 (39.8%)	85 (26.8%)	<0.001
Oxygen with mask	65 (8.2%)	14 (4.4%)	0.026
High flow nasal cannula	47 (6.0%)	15 (4.7%)	0.423
Non-invasive mechanical ventilation	16 (2.0%)	2 (0.6%)	0.725
Mechanical ventilation	6 (0.8%)	2 (0.6%)	0.818
Hospitalization	271 (34.3%)	89 (28.0%)	0.055
Pediatric intensive care unit admission	19 (2.4%)	6 (1.8%)	0.602
Duration of hospitalization (mean ± SD)	3.09±7.87	2.6±7.79	0.045

IQR; Interquartile range, SD; Standard deviation

**Table 2.***Evaluation of the management of the patients with chronic disease according to multiplex polymerase chain reaction results*

	Viral agent (+) (n=195)	Viral agent (-) (n=81)	p-value
Oral antibiotic, n (%)	56 (28.7%)	20 (24.6%)	0.495
Intravenous antibiotic, n (%)	89 (45.6%)	36 (44.4%)	0.856
Oseltamivir, n (%)	69 (35.4%)	21 (25.9%)	0.127
Pediatric ward admission, n (%)	107 (54.8%)	42 (51.8%)	0.647
Pediatric intensive care unit admission, n (%)	14 (7.2%)	4 (4.9%)	0.492
Duration of hospitalization, (mean ± standard deviation) day	7.83±13.7	7.07±13.6	0.813

**Table 3.***The number of certain pathogen coinfecting with another pathogen*

Pathogen	HPIV1	HPIV3	HPIV4	InfA	InfB	HRV	HMPV	ADV	HBoV	RSVA	RSVB	Enterovirus
HPIV 1		0	0	0	0	4	0	0	0	0	1	0
HPIV 3			0	0	0	6	0	0	0	0	1	0
HPIV 4				0	0	1	0	0	0	0	0	0
Inf A					2	10	0	1	4	9	7	0
Inf B						5	0	0	1	3	1	0
HRV							2	4	5	13	7	1
HMPV								0	0	0	0	0
ADV									1	1	1	0
HBoV										2	3	0
RSV A											2	1
RSV B												0
Enterovirus												

ADV; Adenovirus, HBoV; Human bocavirus, HMPV; Human metapneumovirus, HPIV; Human parainfluenza virus, HRV; Human rhinovirus, InfA; Influenza A virus, InfB; Influenza B virus, RSV; Respiratory syncytial virus

patient's use of antibiotics, hospitalisation, and length of hospital stay. The results suggest that although physicians apply for this assay when possible, they only consider the result of the assay a little when deciding on the patient's antibiotic treatment and hospitalisation. Wishaupt et al.<sup>12</sup> examined the use of multiplex PCR for ARIs in the pediatric population and found that antibiotic use was similarly higher. Also, Oosterheert et al.<sup>19</sup>, who investigated the clinical consequences of viral RT-PCR diagnostic results in a randomized controlled trial involving adults, found results similar to those in our study. Excessive use of antibiotics is not expected. There may be different reasons for this. First, it was not expected that the multiplex PCR results would influence the decision to start antibiotic treatment because these results become available 12 to 24 hours after sampling. Multiplex PCR can be expected to affect the duration of antibiotic use rather than the rate of antibiotic initiation. This data could not be investigated in our study because they were unavailable. Wishaupt et al.<sup>12</sup> also did not detect a change in the duration of antibiotic use. Another reason antibiotic use was higher in the study group may be that doctors do not want to interrupt or change an antibiotic treatment that had been started. Doctors may be making this decision because they are concerned about bacterial superinfections. Also, in the present study, it was observed that more chest X-rays were taken in these patients. Imaging may have caused more antibiotics to start. Considering that the hospitalizations and respiratory support needs of the patients with viral

agents are somewhat high, these patients have a more severe clinical course, and therefore more antibiotics are preferred.

Other possible clinical effects of multiplex-PCR use in patients with respiratory tract infections are hospitalisation and length of hospital stay. No effect was observed in this area either. Similar to the decision to start antibiotics, hospitalization is a situation mainly decided according to the patient's clinical condition and follow-up. The effect of the multiplex PCR test may be on hospital stay and patient isolation. We could not evaluate isolation in our study because of the limited availability of isolated rooms in our emergency department. Oosterheert et al.<sup>19</sup> found no decrease in hospital stay, similar to our study. Andrews et al.<sup>20</sup> compared the effect of routine laboratory-based tests such as culture, serology or batch molecular testing with the FilmArray® RP panel, which is a multiplex PCR test, on the length of stay in adults with respiratory tract infections and found no difference. Isolation of patients according to viral PCR results and thus prevention of infectious diseases is a targeted situation in this regard.<sup>15</sup> In extraordinary situations, such as during the COVID-19 pandemic, this indication is an important reason for using viral PCR.

When we look at the frequency of viral pathogens without considering the month or season, the rhinovirus was detected as the most common viral agent. Appak et al.<sup>6</sup> reported that rhinovirus/enterovirus was the most common agent in their study in which they examined patients diagnosed with viral respiratory infection with the use of multiplex PCR in pediatric patients. Zhang

et al.<sup>21</sup> in their study, examined respiratory tract viruses in children and adults. They found human bocavirus as the most common agent. In our study, the presence of rhinovirus may result from the inclusion of patients with all respiratory tract infections, not just LRTIs. Knowing the frequencies and seasonal distributions of the factors may be necessary for the emergency physician to make preparations before the possible increases in the amount of an agent.

The use of multiplex PCR in chronic disease (i.e., cancer patients, immunodeficiency patients, cystic fibrosis) is an area that needs further study in the literature.<sup>12</sup> The use of multiplex PCR in this group of patients will provide a more accurate flu diagnosis and may be more appropriate for the use of neuraminidase inhibitors.<sup>8</sup> On the other hand, the detection rates have been higher in asymptomatic individuals with a chronic disease than in asymptomatic individuals without such a condition. Therefore, false positivity can be seen.<sup>22</sup> However, it is clear that further studies are needed on the use of multiplex PCR in the treatment of specific groups such as cystic fibrosis, immunodeficiency patients, and cancer patients.<sup>8</sup>

Although multiplex PCR is practically requested, it does not seem to contribute to patient management. However, detecting the viral agent and making the treatment specific to the patient is an important step for precision medicine. Algorithms can be produced to increase the contribution of multiplex PCR to patient care. These algorithms should be in different ways according to the patients who will be followed up and outpatients. Furthermore, in recent studies on this subject, the use of multiplex PCR is being investigated together with other examinations (sputulum, procalcitonin, etc.) is being investigated. The goal is to interpret the results of multiplex PCR together with other tests, especially in patients with chronic disease, to de-escalate or stop antibiotic treatment for the individual patient by minimising exposure to antibiotics and improve targeted use of antibiotics. The duty of the emergency services in this regard is to apply this test in the emergency department within the first 24 hours after the application, so that multiplex PCR can be used especially in the management of hospitalized patients.

### Study Limitations

The present study had several limitations. Children were evaluated at a single center. Additionally, the study was not double-blind and did not have long-term follow-up information. Patients with chronic disease were not divided into subgroups. The duration of antibiotic use could not be evaluated because it was not found in the patient's medical records. The patients were selected only from patients with respiratory tract infections, admitted to the emergency department, and not classified according to their diagnosis.

### Conclusion

Multiplex PCR is a relatively rapid, sensitive and highly specific method that is now routinely used to detect respiratory tract viruses. NWS enables the detection of

the viral agent in the patient admitted to the pediatric emergency department with respiratory symptoms, but it does not seem to be of significant benefit to the clinician on patient management. On the other hand, there are not many studies in the literature on the effect of NWS on patient management, especially in patients with chronic diseases. Studies in which a larger number of patients with chronic diseases are evaluated will make significant contributions to the literature.

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**Conflict of Interest:** There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

**Ethics Committee Approval:** The study was approved by the Institutional Review Board of the Hacettepe University (date: 15.03.2022, decision no: 2022/05-02).

**Financial Disclosure:** The authors have no conflicts of interest to declare.

**Informed Consent:** Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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