

Evaluation of BioFire Filmarray panel for respiratory pathogens: a demographic and clinical analysis in Istanbul, Turkey

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Abstract

We aimed to analyze the distribution of respiratory pathogens (RP) detected by a multiplex PCR-based method (BioFire Diagnostics, USA) among patients with suspected respiratory tract infections (RTI) and to evaluate the demographic, clinical and radiological characteristics of infected individuals. RP were detected in 1621/6376 (25.4%) of the samples in the years 2018-2020. Rhinovirus/enterovirus (RV/EV) were the most commonly detected pathogens (38.1%) followed by influenza A and B viruses (21%) and parainfluenza virus (PIV) (9.5%). Single pathogen was detected in 1361 (84%) and multi pathogens in 260 (16%) of 1621 samples. At least one comorbidity was present in 379 (30.5%) of the patients. Fever was the most common sign followed by cough and dyspnea. Thorax CT was present in 426 of 1243 RP positive patients (34.3%). Any radiological findings was found significantly related for a specific pathogen. No medication was given to 52.9% whereas antibiotics in 35.7% and antivirals in 3.8% of the patients. Film Array panel as a multiplex PCR test is not used rationally in our hospital and results were not dramatically improve management of RTI. A better communication between clinician and microbiologist is required for efficient use of laboratory and rational use of antimicrobials.

Key words: Respiratory tract infections, Film Array respiratory panel, Multiplex PCR, antibiotic, antiviral

Evaluación del panel BioFire Filmarray para patógenos respiratorios: un análisis demográfico y clínico en Estambul, Turquía

Resumen

Nuestro objetivo fue analizar la distribución de patógenos respiratorios (RP) detectados por un método basado en PCR multiplex (BioFire Diagnostics, EE. UU.) entre pacientes con sospecha de infecciones del tracto respiratorio (ITR) y evaluar las características demográficas, clínicas y radiológicas de los individuos infectados. Se detectaron RP en 1621/6376 (25,4%) de las muestras en los años 2018-2020. Los rinovirus/enterovirus (RV/EV) fueron los patógenos detectados con mayor frecuencia (38,1 %), seguidos de los virus de la influenza A y B (21 %) y el virus de la parainfluenza (PIV) (9,5 %). Se detectó un solo patógeno en 1361 (84%) y múltiples patógenos en 260 (16%) de 1621 muestras. Al menos una comorbilidad estaba presente en 379 (30,5%) de los pacientes. La fiebre fue el signo más frecuente seguido de tos y disnea. La TC de tórax estuvo presente en 426 de 1243 pacientes positivos para RP (34,3%). Cualquier hallazgo radiológico se encontró significativamente relacionado con un patógeno específico. No se administró medicación al 52,9%, mientras que al 35,7% se le administraron antibióticos y al 3,8% antivirales. El panel Film Array como una prueba de PCR multiplex no se usa racionalmente en nuestro hospital y los resultados no mejoraron drásticamente el manejo de las ITR. Se requiere una mejor comunicación entre el médico y el microbiólogo para el uso eficiente del laboratorio y el uso racional de los antimicrobianos.

Key words: Infecciones del tracto respiratorio, panel respiratorio Film Array, Multiplex PCR, antibiótico, antiviral

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Introduction

Respiratory tract infections (RTI) are one of the leading causes of morbidity and mortality worldwide. Lower RTIs were reported to be the fourth most common cause of death, causing approximately 3 million deaths in 2019¹. Most deaths occur in children younger than 5 years of age, elderly, and patients with suppressed immune systems.

The majority of RTIs are caused by a viral pathogen. Since the laboratory diagnosis of viral infections is difficult, the diagnosis is largely based on the clinical signs and symptoms of the patients². However, symptoms such as sore throat, nasal discharge, cough, wheezing, shortness of breath, sputum and nasal congestion are not specific for a single pathogen and can also be seen in both viral and bacterial infections³. In addition, atypical microorganisms which are responsible for 30% of pneumonia cases are difficult to isolate in routine culture plates⁴. Factors such as nonspecific symptoms of RTIs and limited use of rapid and sensitive diagnostic tests lead clinicians to start empirical antibiotic therapy. However, unnecessary use of antibiotics not only has a negative impact on health expenses, but also leads to increased antibiotic resistance^{2,5,6}. This situation is especially critical for Turkey, where is known to have the highest daily dose of antibiotic usage for every 1000 people per day in the world⁷.

Diagnostic systems for detecting infectious agents ideally should provide fast, sensitive, specific and reproducible results while minimizing the need for special laboratory equipment and qualified technicians. The introduction of molecular tests has made the detection of a broad spectrum of respiratory tract pathogens fast, sensitive and easy to implement, thus making laboratory diagnosis more effective in clinical patient management⁸. Therefore, multiplex PCR-based respiratory panels are becoming more and more common in microbiology laboratories.

We had two main objectives in this study: first, detailed retrospective analysis of the respiratory tract panel results performed in three years to reveal the positivity rates of viral agents, and their distribution according to different age groups, seasons and in coinfections, second: .to reveal the detailed characteristics of the patients such as demographic features ,whether the patients were followed as outpatients or inpatients, symptoms accompanying the test requests, radiological findings at diagnosis, comorbidities, prognosis and the agents used in the treatment.

Material and Methods

Study population and laboratory analysis

This retrospective study was conducted at Marmara University Pendik Training and Research Hospital in Istanbul, Turkey.

Detection of respiratory pathogens

Nasopharyngeal specimens from patients with suspected RTI were tested with the BioFire FilmArray Respiratory Panel (RP) (BioFire Diagnostics, Inc., Salt Lake City, UT, USA) and results

obtained between January 1, 2018 to October 31, 2020 were analyzed. This Panel is a fully automated multiplexed PCR technique that detects 14 viral agents: adenovirus (AdV), coronaviruses (CoV) (OC43, NL63, 229E, HKU1), influenza A (Flu A) (H1, H1-2009, H3), influenza B (Flu B), human metapneumovirus (hMPV), parainfluenza (PIV) 1–4, human rhinovirus/enterovirus (RV/EV), respiratory syncytial virus (RSV), and 3 bacterial agents; *Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae* and *Bordetella pertussis*.

Nasopharyngeal swab samples were collected using a dacron swab from respiratory tract infection suspected patients and transported to the laboratory in Carry Blair transport medium. Samples were included in the study as soon as they were accepted in the laboratory. The samples were stored at +4 °C until they studied, during test preparation. Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. The purified nucleic acid solution is combined with a preheated master mix for multiplex PCR. The BioFire Software controls the operation of the BioFire Module, collects and analyzes data, and automatically generates a test report at the end of the run. The entire process takes about an hour. Two process controls are included in each pouch: The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. A positive control result indicates that all steps carried out in the BioFire RP pouch were successful. The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful. Both control assays must be positive for the test run to pass.

The BioFire Software evaluates the DNA melting curve for each well of the 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (T_m) of the curve. The T_m value is then compared against the expected T_m range for the assay. For most organisms detected by the BioFire RP, the organism is considered to be detected if a single corresponding assay is positive.

Demographic data and clinical analysis

Patient information such as gender, age, admission date and admission type (outpatient or inpatient) were obtained from the digital laboratory information system. Medical records were reviewed for the clinical features including symptoms and signs (the presence of fever (>37.5°C), cough, dyspnea, sputum, and nasal discharge), immune-competence status, comorbidities, treatments, and post-treatment prognosis. Immune-competence was evaluated based on clinical data such as clinical diagnosis (malignancies, organ transplantation, HIV infection), inpatient service (oncology, hematology, transplant unit), use of immunosuppressive agents (antineoplastic agents or long-term high-dose corticosteroid therapy). Antibiotics and antivirals given to the patients were recorded and efficacy of treatment was evaluated by clinical outcome.

Radiological analysis

Chest computed tomography (CT) results were analyzed for the patients who found to be positive by RP. Image analysis was done using PACS (Picture Archiving and Communication System) workstation (INFINITT Healthcare Co., Ltd). Two radiologists blindly reviewed CT findings for the presence of ground glass opacity, consolidation, pleural effusion, mediastinal lymphadenopathy, bronchiectasis, peribronchial thickening, and tree-in-bud pattern. These were recorded by reaching a consensual diagnosis.

Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2016 (Redmond, WA, USA) and SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Pearson chi-square test or Fisher's exact test was used for comparison of categorical variables. The results were considered statistically significant when the p value was <0.05 .

Results

In this retrospective study, a total of 6376 nasopharyngeal specimens obtained in the period January 1, 2018 and October 31, 2020 were examined. Respiratory pathogens were detected in 25.4% (1621/6376) of the samples (Table 1). Positivity rate was 27.2% for pediatric and 23.5% for adult patients.

Annual distribution of respiratory pathogens is given in Table 2. RV/EV were the most commonly detected pathogens (726, 38.1%) followed by influenza viruses (A and B) (400, 21%) and PIV (181, 9.5%). All the pathogens except influenza viruses and coronaviruses were significantly more common in patients under 18 years of age.

Single pathogen was detected in 1361 of 1621 samples whereas there were two types of pathogens in 240 samples, 3 types of pathogens in 18 samples and 4 pathogens in 2 samples (Table 3). The most common combination was RV/EV and PIV combination (18.3%, 49/260). Multi pathogens were detected in 2.5% (75/3040) of the samples from adults and in 5.5% (185/3336) of samples from pediatric patients ($p < 0.05$).

Demographic and clinical characteristics of 1243 RP positive patients for 2018 and 2019 were given in Table 4. Majority of the patients (59.4%) was younger than 18 years old, the median age was 7 years (IQR: 1-42) and 46.7% were female. At least one comorbidity was present in 379 participants (30.5%). The most common comorbidity was hypertension (99/379). In 348 (28%) patients an immunosuppressive condition existed. Fever was the most common sign followed by cough and dyspnea. Fever most commonly observed in patients with AdV and Flu A (42.6% and 42.1%, respectively) and dyspnea was observed at the highest rate in Flu A and CoV cases (data not shown). Hospitalization was recorded in 653 (52.5%) of patients and 23 died (1.9%).

Monthly distribution of respiratory pathogens for 1243 positive patients for 2018 and 2019 was given in Figures 1 and 2, respectively. RV/EV as the most common pathogens peaked 3-4 for times in a year. PIV peaked in the summer months and remained high in the second half. Influenza A positivity was significantly higher in January and December whereas Influenza Flu B peaked in February in 2018 and in April/May in 2019.

Thorax CT imaging was present in 426 of 1243 patients (34.3%) who was FilmArray RP positive in 2018 and 2019. While 101 (8.1%) of them had normal radiological findings, pathological findings were detected in 325 (26.1%) patients. The most common CT finding was ground glass appearance (in 242 patients, 56.8%). Any radiological findings were found significantly related to a specific pathogen ($p > 0.05$).

Antimicrobial agents were prescribed in 585 of 1243 (47%) RP positive patients (Table 5a). Antibiotics were given in 35.7%, antivirals in 3.8% and antibiotic-antiviral combination in 7.6% of the patients. As seen in Table 5b an antibiotic was prescribed in 73.2% hospitalized patients and 23.8% of outpatients. In 74.6% of outpatients were not given any antimicrobials despite Film Array positivity.

Discussion

Epidemiological surveillance is essential to improve diagnosis, treatment, and prognosis in RTIs. This study examines the relationship between demographic, clinical, and radiological characteristics of patients having respiratory tract symptoms and presents epidemiological data and the seasonal dynamics of respiratory pathogens. The results of Biofire FilmArray Respiratory Panel for the years 2018-2020 and characteristics of the patients tested positive for the years 2018-2019 followed in the Marmara University Pendik Training and Research Hospital are discussed. The overall test positivity rate of 25.4% (1621 of 6376 samples) for 3 years was lower than previous studies that reports 30-80% test positivity⁹⁻¹⁴. We might speculate that patient selection was not appropriate or sampling the material was not optimal since transport of the samples and performing the test in the laboratory were done as indicated by manufacturer in a standardized manner. Persistent low positivity for 3 consecutive years and no improvement for increasing the yield of the test might also be related with inadequate communication between the microbiologists and the clinicians.

For 6376 specimens sent 2018-2020, RV/EV was the most commonly detected pathogens (38.1%) followed by influenza viruses (21%). The percentage of influenza viruses was higher in adults whereas RV/EV was the most common pathogen in children consistent with other studies^{9,13,14}. RSV was detected in 9.3% of children as the 4th common pathogen after RV/EV, PIV, and influenza viruses in our study where as in other studies RSV was the most common pathogen in children^{8,15-17}. In 260 of 6376 (4.1%) samples there were more than one type of pathogen accounting 16% of all positive samples (260/1621). Multi-pathogen detection rates in adults reported as 8.7-

Table 1. The results of the FilmArray RP for 2018-2020

	2018			2019			2020			TOTAL		
	<18 age (n/%)	≥18 age (n/%)	Total (n/%)	<18 age (n/%)	≥18 age (n/%)	Total (n/%)	<18 age (n/%)	≥18 age (n/%)	Total (n/%)	<18 age (n/%)	≥18 age (n/%)	Total (n/%)
Negative	1313 (73.6)	734 (79.1)	2047 (75)	864 (76.4)	997 (76.2)	1861 (76.3)	252 (59.9)	595 (74)	847 (69.1)	2429 (72.8)	2326 (76.5)	4755 (74.6)
Positive	471 (26.4)	194 (20.9)	665 (25)	267 (23.6)	311 (23.8)	578 (23.7)	169 (40.1)	209 (40.1)	378 (30.9)	907 (27.2)	714 (23.5)	1621 (25.4)
Total	1784 (65.8)	928 (34.2)	2712 (100.0)	1131 (46.4)	1308 (53.6)	2439 (100.0)	421 (34.4)	421 (34.4)	1225 (100.0)	3336 (52.0)	3040 (48.0)	6376 (100.0)

15.9% while this rate went up to 27% in pediatric patients¹⁵⁻²¹. RV/EV and PIV was the most common multi pathogens in our group as shown previously^{9,15,19,22}. The clinical significance of multi-pathogenic infections is not clear in terms of disease severity and hospital stay. Immunodeficiency, high viral exposure, or an immature immune system can cause higher levels of viral replication and prolonged viral positivity in children^{11,15,16}. Studies have shown that viral RNA can still be detected positive 4-5 weeks after an RV/EV infection²³, and that the most common virus detected in asymptomatic children is RV/EV²⁴.

In 348 of 1243(28%) positive patients there was an immunosuppressive condition. The distribution of pathogens in immunocompromised patients was not different from other patients. There are many cases showing that hMPV infections are more severe in immunocompromised patients, and that immunosuppression in most of these patients is due to an underlying hematological malignancy²⁵⁻²⁷. When the clinical data of 17 hMPV cases in our immunocompromised patients were analyzed, 8 had hematological malignancies and RTI related deaths were not recorded in any of them.

Table 2. Distribution of respiratory pathogens according to the years

PATHOGENS	2018			2019			2020			Total			P value
	<18 age (n/%)	≥18 age (n/%)	Total (n/%)	<18 age (n/%)	≥18 age (n/%)	Total (n/%)	<18 age (n/%)	≥18 age (n/%)	Total (n/%)	<18 age (n/%)	≥18 age (n/%)	Total (n/%)	
RV/EV	257 (78.8)	69 (21.2)	326 (41.9)	143 (59.1)	99 (40.9)	242 (35.3)	98 (62.0)	60 (38.0)	158 (35.8)	498 (69.0)	228 (31.0)	726 (38.)	p=0.019
Flu A	21 (36.8)	36 (63.2)	57 (7.3)	30 (20.7)	115 (79.3)	145 (21.2)	27 (26.0)	77 (74.0)	104 (23.6)	78 (25.0)	228 (75.0)	306 (16.1)	p<0.001
PIV	97 (88.2)	13 (11.8)	110 (14.1)	38 (62.3)	23 (37.7)	61 (8.9)	5 (50.0)	5 (50.0)	10 (2.3)	140 (77.0)	41 (23.0)	181 (9.5)	p<0.001
RSV	46 (70.8)	19 (29.2)	65 (65)	20 (35.1)	37 (64.9)	57 (8.3)	37 (68.5)	17 (31.5)	54 (12.2)	103 (59.0)	73 (41.0)	176 (9.2)	p=0.044
CoV	25 (54.3)	21 (45.7)	46 (5.9)	24 (34.8)	45 (65.2)	69 (10.1)	8 (29)	22 (71.0)	30 (7.0)	58 (40.0)	88 (60.0)	145 (7.7)	p=0.09
AdV	51 (83.6)	10 (16.4)	61 (7.8)	30 (75.0)	10 (25.0)	40 (5.8)	16 (59.3)	11 (40.7)	27 (6.1)	97 (76.0)	31 (24.0)	128 (6.7)	p=0.27
Flu B	11 (34.4)	21 (65.6)	32 (4.1)	15 (78.9)	4 (21.1)	19 (2.8)	11 (25.6)	32 (74.4)	43 (9.8)	37 (39.0)	57 (61.0)	94 (4.9)	p<0.001
hMPV	15 (62.5)	9 (37.5)	24 (3.1)	20 (57.1)	15 (42.9)	35 (5.1)	3 (27.3)	8 (72.7)	11 (2.5)	38 (54.0)	32 (46.0)	70 (3.7)	p<0.04
<i>B. pertussis</i>	24 (82.8)	5 (17.2)	29 (3.7)	6 (85.7)	1 (14.3)	7 (1.0)	-	-	-	30 (83.0)	6 (17.0)	36 (1.9)	-
<i>M. pneumonide</i>	24 (92.3)	2 (7.7)	26 (3.3)	5 (62.5)	3 (37.5)	8 (1.2)	1 (50.0)	1 (50.0)	2 (0.5)	30 (83.0)	6 (17.0)	36 (1.9)	-
<i>C. pneumonide</i>	1 (50.0)	1 (50.0)	2 (0.3)	1 (62.5)	1 (50.0)	2 (0.3)	1 (100.0)	0 (0.0)	1 (0.2)	3 (60.0)	2 (40.0)	5 (0.3)	-
Total	572 (74.0)	206 (48.0)	778 (100.0)	332 (48.0-9)	353 (52.0)	685 (100.0)	207 (47.0)	233 (53.0)	440 (100.0)	1111 (58.0)	792 (42.0)	1903 (100.0)	-

Adv: adenovirus, CoV: coronavirus, RV/EV: rhino/enterovirus, hMPV: human metapneumovirus, Flu A: influenza A virus, Flu B: influenza B virus, RSV: respiratory syncytial virus, PIV: parainfluenza virus

Table 3. Distribution of microorganisms according to the number of pathogens in positive samples

Positivity	RV/ EV	Flu A	PIV	RSV	CoV	AdV	Flu B	hMPV	<i>B. pertussis</i>	<i>M. pneumoniae</i>	<i>C. pneumoniae</i>	Total (No. of pathogens / No. of specimens)
+1	554	257	119	109	94	71	73	42	20	19	4	1361/1361
+1	160	45	56	63	39	48	17	25	12	14	1	480/240
+3	13	4	7	3	12	9	4	3	4	3	0	54/18
+4	-	-	1	1	1	2	1	2	-	-	-	8/2
Total	727	306	182	175	145	128	94	70	36	36	5	1903/1621

Adv: adenovirus, CoV: coronavirus, RV/EV: rhino/enterovirus, hMPV: human metapneumovirus, Flu A: influenza A virus, Flu B: influenza B virus, RSV: respiratory syncytial virus, PIV: parainfluenza virus

Table 4. Demographic and clinical characteristics of patients for 2018-2019

Demographic and Clinical Characteristics	2018 (n=665)	2019 (n=575)	Total (n=1243)	P value
Gender n (%)				
Female	317 (47.7)	263 (45.5)	580 (46.7)	p=0.445
Male	348 (52.3)	315 (54.5)	663 (53.3)	
Age n (%)				
<18	471 (70.9)	267 (46.2)	738 (59.4)	p<0.001
18-44	92 (13.8)	121 (20.9)	213 (17.1)	
45-64	59 (8.9)	100 (17.3)	159 (12.8)	
≥65	43 (6.4)	90 (15.6)	133 (10.7)	
Age, median (IQR)	3 (0-24)	24 (3-56)	7 (1-42)	p<0.001
Admission type n (%)				
Outpatient	400 (60.2)	253 (43.8)	653 (52.5)	p<0.001
Inpatient	265 (39.8)	325 (56.2)	590 (47.5)	
Comorbidity n (%)				
Yes	187 (28.1)	192 (33.2)	379 (30.5)	p=0.052
No	478 (71.9)	386 (66.8)	864 (69.5)	
Immunosuppression n (%)				
Yes	138 (20.8)	210 (36.3)	348 (28.0)	p<0.001
No	527 (79.2)	368 (63.7)	895 (72.0)	
Symptoms and signs n (%)				
Fever	188 (51.6)	235 (63.2)	423 (57.5)	p=0.25
Cough	197 (54.1)	204 (54.8)	401 (54.5)	
Sputum	75 (20.6)	106 (28.5)	181 (24.6)	
Dyspnea	130 (35.7)	127 (34.1)	257 (35.0)	
Nasal discharge	40 (11.0)	46 (12.4)	86 (11.7)	
Prognosis n (%)				
Death	6 (0.9)	17 (2.9)	23 (1.9)	
Survival	659 (99.1)	561 (97.1)	1220 (98.1)	

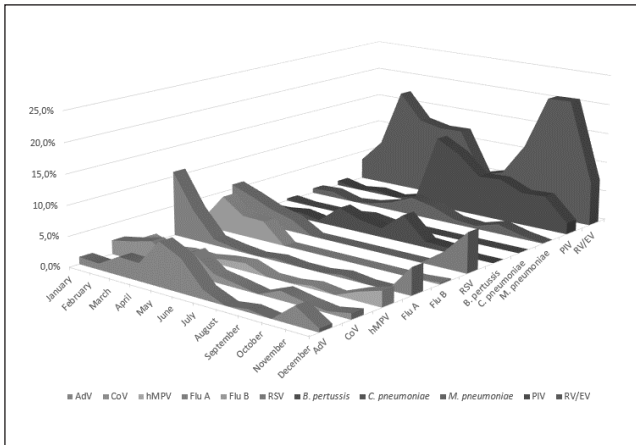


Figure 1. Monthly distribution of respiratory pathogens for 1243 RP positive patients for 2018

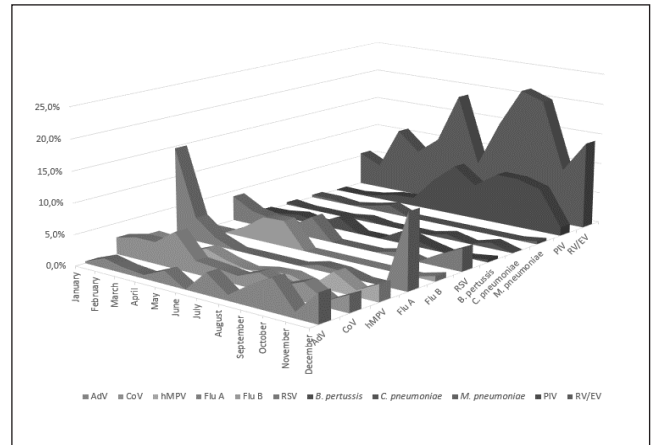


Figure 2. Monthly distribution of respiratory pathogens for 1243 RP positive patients for 2019

Fever and cough were the most common symptoms as indicated previously^{2,12,14}. Thorax CT imaging was present in 426 of 1243 positive patients (34.3%) while 101 (8.1%) of them had normal radiological findings. The most common CT finding was ground glass appearance (56.8%). Any radiological findings was found significantly related for a specific pathogen ($p>0.05$). Studies have already stated that most patients do not show a clear typical radiological pattern in viral RTI, imaging findings alone are insufficient for a definitive diagnosis, and CT can only be useful as an auxiliary test in the diagnosis^{32–34}.

RV/EV positivity remained high for two years, and the highest peaks were reached in October 2018 and September 2019. This data is consistent with the literature reporting that although RV/EV continues to run in all seasons, it peaks in September with the onset of the school season and in the spring^{9,28,29}. The first influenza cases were detected in November in both years, and Flu A was found to be significantly higher in December and January compared to the other months of the year. Flu B, on the other hand, peaked in February in 2018 and April in 2019, and was not detected in the period from June to December. These data indicate that Flu B tends to circulate slightly later than Flu A during the season. Finkelman et al. reported that for countries in the northern hemisphere Flu B peaked approximately 2 weeks after Flu A H1N1

and 4 weeks after A H3N2³⁰. RSV and influenza viruses were coexisted in circulation with the highest frequency in December and January. Similarly, it has been shown that RSV occur simultaneously with influenza viruses or shortly before the influenza season^{14,31}.

The rapidity in test results availability to physicians is a key factor for determining changes in medical practice. Rational use of antibiotic and antivirals, shortening hospital stay, proper isolation procedures related with molecular test results were confirmed in previous studies^{11,35–39}. In our study, a bacterial infection was confirmed in only 50 of 444 (11.2%) RP positive antibiotic receiving patients. Antiviral treatment was given in 47 RP positive patients who 44(93.6%) of them were influenza positive. We may suggest that antiviral usage is more rational than antibiotic usage in our hospital.

As conclusion, our positivity rates are low compared to other studies and the reflection of the Film Array RP results in clinical practice is not at the expected level. This low rate could be related with unnecessary test request without real clinical indications and taking NFS sample require qualified personnel since viral agents can only be detected at the right time (symptomatic), from the right anatomical region (nasopharynx), in a sufficient amount of sample and theoretically these factors might increase the sensitivity of the test.

Table 5a. Antimicrobial prescriptions for 1243 RP positive patients according to the RP type*

	Flu A/B (n=253)	Non-influenza viral pathogens (n=916)	Bacterial pathogens (n=74)	Total (n=1243)
Antibiotics (n. %)	19 (4.3)	393 (88.5)	32 (7.2)	444 (35.7)
Antivirals (n. %)	44 (93.6)	3 (6.4)		47 (3.8)
Antibiotic/antiviral combination (n. %)	76 (80.8)	14 (14.9)	4 (4.3)	94 (7.6)
No antimicrobial (n. %)	114 (17.3)	506 (76.9)	38 (5.8)	658 (52.9)

*Clinical analysis was done for the years for the years 2018-2019

Table 5b. Antimicrobial prescriptions for 1243 RP positive patients according to the administration type*

Antimicrobial prescriptions	Outpatient n=653	Inpatient n=590	Total n=1243
Antibiotics (n. %)	119 _a (26.8)	325 _b (73.2)	444 (35.7)
Antivirals (n. %)	18 _a (38.3)	29 _b (61.7)	47 (3.8)
Antibiotic/antiviral combination (n. %)	25 _a (26.6)	69 _b (73.4)	94 (7.6)
No antimicrobial (n. %)	491 _a (74.6)	167 _b (25.4)	658 (52.9)

a,b: Each subscript letter denotes a subset of Admission type categories whose column proportions do not differ significantly from each other at the .05 level. **Clinical analysis was done for the years for the years 2018-2019

On the other hand, 74.6% of RP positive outpatients were not given any antimicrobials whereas an antibiotic was prescribed in 73.2% hospitalized patients. The analysis of the results showed us that the respiratory panel is not used wisely and although test order is restricted in some situations determined by infectious diseases committee, the hospital automation system does not allow us to control the request strictly. We had meeting with the hospital management and explained that clinicians were not taking into account of the results of respiratory panel for prescribing drugs since there is no legal restriction in that area in Turkey. For hepatitis viruses and HIV, molecular results are mandatory for drug prescription, however this is not the case for respiratory pathogens. In Turkey, all the cost of the patients regarding diagnostic tests and treatment is covered by the government in government hospitals. A better communication between clinician and microbiologist might improve the efficiency of such an expensive test to monitor patients better and to use antimicrobials more rationally.

Ethical disclosures

Declaration of Competing Interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Any of the authors have had industrial connections during the study period

Ethical statement. The study was approved by the Clinical Research Ethics Committee of Marmara University Faculty of Medicine (Approval No. 09.2020.675, June 12th, 2020). All participants have provided written informed consent.

Author contributions. AK, ND,RCS: initiated and coordinated the research. AK, ND, NCC, planned and recruited the study. AK, ND, RCS, NCC: conception and design of the study. AK, ND, MMG, BNK, BA, NCC: acquisition of data. ND, MMG: statistical analysis. AK, ND, RCC, NCC: interpretation of data, drafting the article and revising it

References

- World Health Organization (2020, May 24). *The top 10 causes of death.* <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>.
- Kaku N, Hashiguchi K, Iwanaga Y, Akamatsu N, Matsuda J, Kosai K, et al. Evaluation of FilmArray respiratory panel multiplex polymerase chain reaction assay for detection of pathogens in adult outpatients with acute respiratory tract infection. *J Infect Chemother* 2018 Sep;24(9): 734-738. doi: 10.1016/j.jiac.2018.05.006.
- JuvÉN T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. *Ped Infect Dis J* 2000;19(4).
- Stamm DR, Stankewicz HA. Atypical Bacterial Pneumonia. In: *StatPearls.* StatPearls Publishing; 2020.
- Babady NE. The FilmArray® respiratory panel: an automated, broadly multiplexed molecular test for the rapid and accurate detection of respiratory pathogens. *Expert Rev Mol Diagn* 2013;13(8):779-788. doi:10.1586/14737159.2013.848794
- Huang H-S, Tsai C-L, Chang J, Hsu T-C, Lin S, Lee C-C. Multiplex PCR system for the rapid diagnosis of respiratory virus infection: systematic review and meta-analysis. *Clin Microbiol Infect* 2018;24(10):1055-1063. doi:10.1016/j.cmi.2017.11.018
- Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc Natl Acad Sci U S A*. 2018;115(15):E3463.
- Mengelle C, Mansuy JM, Pierre A, Claudet I, Grouteau E, Micheau P, et al., The use of a multiplex real-time PCR assay for diagnosing acute respiratory viral infections in children attending an emergency unit. *J Clin Virol*. 2014 Nov;61(3):411-7. doi: 10.1016/j.jcv.2014.08.023.
- Litwin CM, Bosley JG. Seasonality and prevalence of respiratory pathogens detected by multiplex PCR at a tertiary care medical center. *Arch Virol* 2014;159(1):65-72. doi:10.1007/s00705-013-1794-4
- Brittain-Long R, Andersson L-M, Olofsson S, Lindh M, Westin J. Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reaction assay. *Scand J Infect Dis* 2012;44(1):9-17. doi:10.3109/00365548.2011.598876
- Busson L, Bartiaux M, Brahim S, Konopnicki D, Dauby N, Gérard M, et al. Contribution of the FilmArray Respiratory Panel in the management of adult and pediatric patients attending the emergency room during 2015-2016 influenza epidemics: An interventional study. *Int J Infect Dis* 2019 Jun;83:32-39. doi: 10.1016/j.ijid.2019.03.027.
- Yang S, Li H, Tang Y, Yu F, Ma C, Zhang H, et al. Multiplex Tests for Respiratory Tract Infections: The Direct Utility of the FilmArray Respiratory Panel in Emergency Department. *Can Respir J* 2020 Jul 25;2020:6014563. doi: 10.1155/2020/6014563.
- Echavarría M, Marcone DN, Querci M, Seoane A, Ypas M, Videla C, et al. Clinical impact of rapid molecular detection of respiratory pathogens in patients with acute respiratory infection. *J Clin Virol* 2018 Nov;108:90-95. doi: 10.1016/j.jcv.2018.09.009.
- Ciotti M, Maurici M, Santoro V, Coppola L, Sarmati L, De Carolis G, et al. Viruses of Respiratory Tract: an Observational Retrospective Study on Hospitalized Patients in Rome, Italy. *Microorganisms*. 2020;8(4):501. doi: 10.3390/microorganisms8040501.
- Li J, Tao Y, Tang M, Du B, Xia Y, Mo X, et al. Rapid detection of respiratory organisms with the FilmArray respiratory panel in a large children's hospital in China. *BMC Infect Dis* 2018 Oct 11;18(1):510. doi: 10.1186/s12879-018-3429-6.
- Bhat N, Tokarz R, Jain K, Haq S, Weatherholtz R, Chandran A, et al. A prospective study of agents associated with acute respiratory infection among young American Indian children. *Pediatr Infect Dis J* 2013 Aug;32(8):e324-33. doi: 10.1097/INF.0b013e31828ff4bc.
- Aygün FD. Evaluation of epidemiological and clinical features of respiratory viruses among hospitalized children. *Turk Pediatri Ars* Published online 2020. doi:10.14744/TurkPediatriArs.2020.39114
- Mahony JB. Detection of respiratory viruses by molecular methods. *Clin Microbiol Rev* 2008;21(4):716-747. doi: 10.1128/CMR.00037-07
- Bierbaum S, Königfeld N, Besazza N, Blessing K, Rücker G, Kontny U, et al. Performance of a novel microarray multiplex PCR for the detection of 23 respiratory pathogens (SYMP-ARI study). *Eur J Clin Microbiol Infect Dis* 2012 Oct;31(10):2851-61. doi: 10.1007/s10096-012-1639-1.
- Olofsson S, Brittain-Long R, Andersson LM, Westin J, Lindh M. PCR for detection of respiratory viruses: seasonal variations of virus infections. *Expert Rev Anti Infect Ther*.2014;9(8):615-626. doi:10.1586/eri.11.75
- Fairchok MP, Martin ET, Chambers S, Kuyppers J, Behrens M, Braun LE, et al.

- Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010 Sep;49(1):16-20. doi: 10.1016/j.jcv.2010.06.013.
22. Visseaux B, Collin G, Ichou H, Charpentier C, Bendhafer S, Dumitrescu M, et al. Usefulness of multiplex PCR methods and respiratory viruses' distribution in children below 15 years old according to age, seasons and clinical units in France: A 3 years retrospective study. *PLoS One* 2017 Feb 24;12(2):e0172809. doi: 10.1371/journal.pone.0172809.
 23. Jartti T, Lehtinen P, Vuorinen T, Koskenvuo M, Ruuskanen O. Persistence of rhinovirus and enterovirus RNA after acute respiratory illness in children. *J Med Virol* 2004;72(4):695-699. doi:10.1002/jmv.20027
 24. Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkäranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. *J Med Virol* 2002;66(3):417-420. doi:10.1002/jmv.2161
 25. Walker E, Ison MG. Respiratory viral infections among hospitalized adults: experience of a single tertiary healthcare hospital. *Influenza and Other Respir Viruses* 2014;8(3):282-292. doi:10.1111/irv.12237
 26. Huck B, Egger M, Bertz H, Peyerl-Hoffman G, Kern WV, Neumann-Haefelin D, et al. Human metapneumovirus infection in a hematopoietic stem cell transplant recipient with relapsed multiple myeloma and rapidly progressing lung cancer. *J Clin Microbiol* 2006 Jun;44(6):2300-3. doi: 10.1128/JCM.00152-06.
 27. Englund JA, Boeckh M, Kuypers J, Nichols WG, Hackman RC, Morrow RA, et al. Brief communication: fatal human metapneumovirus infection in stem-cell transplant recipients. *Ann Intern Med* 2006 Mar 7;144(5):344-9. doi: 10.7326/0003-4819-144-5-200603070-00010.
 28. Monto AS. Occurrence of respiratory virus: time, place and person. *Pediatr Infect Dis J* 2004;23(1 Suppl):S58-64. doi:10.1097/01.inf.0000108193.91607.34
 29. Lee WM, Lemanske RF Jr, Evans MD, Vang F, Pappas T, Gangnon R, et al. Human rhinovirus species and season of infection determine illness severity. *Am J Respir Crit Care Med* 2012 Nov 1;186(9):886-91. doi: 10.1164/rccm.201202-0330OC.
 30. Finkelman BS, Viboud C, Koelle K, Ferrari MJ, Bharti N, Grenfell BT. Global Patterns in Seasonal Activity of Influenza A/H3N2, A/H1N1, and B from 1997 to 2005: Viral Coexistence and Latitudinal Gradients. *PLOS ONE* 2007;2(12):e1296. doi:10.1371/journal.pone.0001296
 31. Al-Romaihi HE, Smatti MK, Ganesan N, Nadeem S, Farag E, Coyle PV, et al. Epidemiology of respiratory infections among adults in Qatar (2012-2017). *PLoS One* 2019 Jun 13;14(6):e0218097. doi: 10.1371/journal.pone.0218097.
 32. Koo HJ, Lim S, Choe J, Choi S-H, Sung H, Do K-H. Radiographic and CT Features of Viral Pneumonia. *RadioGraphics* 2018;38(3):719-739. doi:10.1148/rg.2018170048
 33. Kanne JP, Godwin JD, Franquet T, Escuissato DL, Müller NL. Viral Pneumonia After Hematopoietic Stem Cell Transplantation: High-Resolution CT Findings. *J Thorac Imaging* 2007;22(3):292-299. doi:10.1097/RTI.0b013e31805467f4
 34. Franquet T. Imaging of Pulmonary Viral Pneumonia. *Radiology*. 2011;260(1):18-39. doi:10.1148/radiol.11092149
 35. Keske Ş, Ergönül Ö, Tutucu F, Karaaslan D, Palaoglu E, Can F. The rapid diagnosis of viral respiratory tract infections and its impact on antimicrobial stewardship programs. *Eur J Clin Microbiol Infect Dis* 2018;37(4):779-783. doi:10.1007/s10096-017-3174-6
 36. Brendish NJ, Malachira AK, Clark TW. Molecular point-of-care testing for respiratory viruses versus routine clinical care in adults with acute respiratory illness presenting to secondary care: a pragmatic randomised controlled trial protocol (ResPOC). *BMC Infect Dis* 2017;17. doi:10.1186/s12879-017-2219-x
 37. Brendish NJ, Malachira AK, Armstrong L, Houghton R, Aitken S, Nyimbili E, et al. Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial. *Lancet Respir Med* 2017 May;5(5):401-411. doi: 10.1016/S2213-2600(17)30120-0.
 38. Shengchen D, Gu X, Fan G, Sun R, Wang Y, Yu D, et al. Evaluation of a molecular point-of-care testing for viral and atypical pathogens on intravenous antibiotic duration in hospitalized adults with lower respiratory tract infection: a randomized clinical trial. *Clin Microbiol Infect*. 2019 Nov;25(11):1415-1421. doi: 10.1016/j.cmi.2019.06.012.
 39. Xu M, Qin X, Astion ML, Rutledge JC, Simpson J, Jerome KR, et al. Implementation of filmarray respiratory viral panel in a core laboratory improves testing turnaround time and patient care. *Am J Clin Pathol* 2013 Jan;139(1):118-23. doi: 10.1309/AJCPH7X3NLYZPHBW.