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ORIGINAL ARTICLE

Changes in the Distribution of Respiratory Pathogens After the COVID-19 Pandemic

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ABSTRACT

Background: **Diverse quarantine measures against the coronavirus disease 2019 (COVID-19) pandemic have affected the distribution patterns of respiratory pathogens. This study analyzed changes in the incidence and distribution of major respiratory pathogens during the COVID-19 pandemic and after the endemic declaration. In addition, the study aimed to provide a basis for future community-centered infectious disease response strategies by identifying co-infection patterns and whether pathogens re-emerge after mitigation.**
Methods: **In this retrospective study, the results of 105,619 respiratory pathogen tests, including FilmArray® Respiratory Panel (BioFire Diagnostics) assay (FilmArray RP), influenza tests, and COVID-19 tests, were collected at a Korean university hospital during the COVID-19 pandemic (Period I: March 2021 through April 2023) and endemic (Period II: May 2023 through February 2025). The changes in the distribution of major pathogens, age and gender characteristics, seasonality, and co-infection patterns were compared between the two periods.**
Results: **The overall positivity rate for the FilmArray RP significantly increased from 64.4% in Period I to 71.1% in Period II ($p < 0.001$). In the age-specific analysis, age group 3 (4 months to 2 years) had the highest positivity rate in Period II (82.7%). Human rhinovirus/enterovirus was the most frequently detected pathogen, whereas respiratory syncytial virus cases increased in number, but decreased in proportion to all positive cases in Period II. The prevalence of adenoviruses and *Mycoplasma pneumoniae* has significantly increased since the pandemic. Human metapneumovirus has shifted its seasonality from autumn to summer and spring, whereas parainfluenza virus 3 has shifted from autumn to spring and summer. Among all positive cases, co-infections significantly increased to 17.6% and 22.3% during Periods I and II, respectively ($p < 0.001$).**
Conclusions: **This study revealed significant changes in the incidence, seasonality, age characteristics, and co-infection patterns of respiratory pathogens after the mitigation of COVID-19. These changes result from the re-emergence of pathogens that were suppressed during the pandemic, immune vaccination, and the resumption of social activities, suggesting the need to establish an age- and pathogen-specific surveillance system for future infectious disease responses.**
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INTRODUCTION

Since its outbreak in December 2019 in Wuhan, China, coronavirus disease 2019 (COVID-19) has spread rapidly worldwide and has been declared a pandemic by the World Health Organization [1]. COVID-19 is a highly infectious respiratory disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has caused a profound crisis in human health [2].
In response to the pandemic, countries have implemented various non-pharmaceutical public health interventions (NPIs), including mask-wearing, social distancing, increased hand hygiene, travel restrictions, and school closures [3,4]. These measures curbed SARS-CoV-2 transmission and affected the distribution and prevalence of other respiratory pathogens [5,6]. During the pandemic, substantial reductions in the incidence of influenza A virus (Flu A), influenza B virus (Flu B), respiratory syncytial virus (RSV), and human rhinovirus/enterovirus (Rhino/Entero), and a delay in the seasonal peak have been observed in several countries, including the United States, countries in Europe, China, and Japan [7-12].
Acute respiratory infections (ARIs) are infectious diseases that cause a major disease burden worldwide, with high prevalence in children and older populations. Respiratory viruses, including Rhino/Entero, RSV, adenovirus (AdV), human metapneumovirus (HMPV), parainfluenza virus (PIV), flu, coronavirus (CoV), and human bocavirus, are the leading infectious agents of ARIs, accounting for approximately 70% [13].
In Korea, changes in pathogen distribution between pre-pandemic and pandemic periods have been reported in local hospitals, including Daegu [6,14]. Social distancing policies such as school closures have contributed significantly to the reduction of respiratory infectious disease outbreaks, especially in pediatric age groups [9]. Lifestyle habits also changed during the pandemic with decreased exercise, weight gain, and dietary changes [15,16]. These changes may affect the transmission of infectious diseases as well as personal immunity. In addition, improved mask-wearing practices effectively inhibit the spread of various respiratory pathogens, including SARS-CoV-2 [17].
In a retrospective analysis from a university hospital in South Korea, HMPV was undetectable during the pandemic and rapidly re-emerged in the second half of 2022 [18], whereas PIV showed atypical behavior, with outbreaks occurring outside of the traditional epidemic seasons [19]. These phenomena suggest that pathogens that have been suppressed since the lifting of NPIs may re-emerge outside the seasonal cycle.
Recent advances in molecular diagnostic technologies have enabled the rapid and accurate detection of various respiratory pathogens using multiplex PCR tests such as the FilmArray® Respiratory Panel (BioFire Diagnostics, Salt Lake City, UT, USA) assay (FilmArray RP). The introduction of these technologies has reduced unnecessary antibiotic use and improved diagnostic efficiency and speed of decision-making in clinical settings [20-22].
This study aimed to analyze changes in the occurrence and distribution characteristics of major respiratory pathogens during the COVID-19 pandemic and after the endemic declaration. In addition, it sought to provide a basis for future community-centered infectious disease response strategies by identifying co-infection patterns and their re-emergence after mitigation.

MATERIALS AND METHODS

Specimens

The study included 105,619 patients who underwent respiratory pathogen tests, including FilmArray RP (n = 5,621), flu antigen and gene amplification tests (flu Ag/PCR) (n = 15,561), and COVID-19 antigen and gene amplification tests (COVID-19 Ag/PCR) (n = 84,437), from March 2021 through February 2025 in a Korean university hospital.
This retrospective study was conducted to compare the prevalence of major respiratory pathogens, their distribution by age and gender, changes in seasonal distribution characteristics, and co-infections during the COVID-19 pandemic (Period I: March 2021 through April 2023) and post-pandemic periods (Period II: May 2023 through February 2025).

FilmArray® respiratory panel assay

The FilmArray RP was performed using the FilmArray® Respiratory 2.1 plus Panel (BioFire Diagnostics). Nasopharyngeal, nasal, and oropharyngeal samples were injected into a disposable pouch, and cell lysis, nucleic acid extraction, primary PCR, secondary real-time PCR, and melting curve analysis were performed automatically according to the manufacturer's instructions.
A total of 23 respiratory pathogens, including AdV, CoV 229E, CoV HKU1, CoV NL63, CoV OC43, HMPV, Rhino/Entero, Flu A (including Flu A H1, Flu A H1-2009, and Flu A H3), Flu B, Middle East respiratory syndrome coronavirus, PIV1, PIV2, PIV3, PIV4, RSV, SARS-CoV-2, *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae,* can be detected simultaneously.

Influenza and SARS-CoV-2 antigen test

A fluorescence immunoassay-based test using the Sofia™ Flu A+B FIA (Quidel Co., San Diego, CA, USA), which detected Flu A and B antigens qualitatively, was performed according to the manufacturer's instructions.

A fluorescence immunoassay-based test using STANDARD F COVID-19 Ag FIA (SD Biosensor, Inc., Cheongju, Republic of Korea) was performed according to the manufacturer's instructions to qualitatively detect the SARS-CoV-2 antigen.

Influenza and SARS-CoV-2 PCR tests

Real-time RT-PCR tests targeting the *E*, *N*, and *RdRp* genes of SARS-CoV-2 were performed according to the manufacturer's instructions using the STANDARD™ M nCoV Real-Time Detection Kit (SD Biosensor, Inc.) and Xpert® Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA).

SARS-CoV-2 and Flu A/B were detected simultaneously by multiplex real-time PCR of a single nasal specimen using the cobas® Liat System (Roche Molecular Systems, Inc., Pleasanton, CA, USA).

Comparison of respiratory pathogens between the two periods

The positivity rate and distribution of respiratory pathogens by age group and gender by period were compared to analyze demographic characteristics. A total of six age groups were categorized: age group 1 (up to 28 days), age group 2 (1 - 3 months), age group 3 (4 months to 2 years), age group 4 (3 - 6 years), age group 5 (7 - 18 years), and age group 6 (19 years and older).

The major respiratory pathogens, including Rhino/Entero, RSV, AdV, *M. pneumoniae*, HMPV, PIV3, Flu A, Flu B, and SARS-CoV-2, were analyzed for monthly and seasonal incidence and distribution patterns by period. The frequency of co-infections by period and pathogen combination patterns by age group, according to the FilmArray RP results, were analyzed.

Statistical analyses

The results of each test were analyzed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA), and statistical analysis was performed using SPSS® Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA).

The chi-squared test was performed to determine the correlation between variables according to season, gender, and age group, and the Kolmogorov–Smirnov test was used for normality.

For all statistical analyses, a p-value of < 0.05 was considered significant.

RESULTS

Positivity rates of the respiratory pathogens according to age group and gender

The number of tests performed was 68,507 and 37,112 in Periods I and II, respectively. The positivity rates by age group and gender according to the test items are shown in Tables 1 and 2, respectively.

The positivity rates for FilmArray RP were 64.4% and 71.1% in Periods I and II, respectively, with a significant increase in Period II ($p < 0.001$) (Table 1). The positivity rate by age group was the highest in age group 4 at 77.4% in Period I, and 82.7% in age group 3 in Period II. Age group 5 showed the largest and most significant increase in the positivity rate at 44.1% and 64.4% in Periods I and II, respectively ($p < 0.001$) (Table 1). Gender analysis showed that the positivity rate increased significantly in Period II compared to Period I for both males and females ($p < 0.001$) (Table 2).

For flu antigen and PCR tests, the positivity rate decreased from 15.3% (565/3,681) in Period I to 13.9% (1,656/11,880) in Period II; however, the difference was not significant. In the combined analysis of flu results from the FilmArray RP and antigen and PCR tests, the overall positivity rate increased slightly in Period II, from 10.1% (603/5,966) to 11.9% (1,805/15,216) in Periods I and II, respectively. In the analysis by age group, Flu A significantly increased in Period II in age groups 2 ($p = 0.018$) and 3 ($p = 0.001$), whereas Flu B significantly increased in age group 5 ($p < 0.001$) and decreased in age group 6 ($p = 0.027$) (Table 1).

For the COVID-19 antigen and PCR tests, the positivity rates were 20.9% and 9.7% in Periods I and II, respectively, with a significant decrease in Period II ($p < 0.001$). An analysis combining SARS-CoV-2 results from FilmArray RP with COVID-19 antigen and PCR test results also showed a sharp decrease in positivity rates to 20.3% and 8.5% in Periods I and II, respectively (Table 1).

Distribution patterns of major respiratory pathogens according to age group

The total number of positive cases of major respiratory pathogens detected by FilmArray RP was 1,755 and 2,649 in Periods I and II, respectively. Age groups 3 and 4 were the major positive age groups (Table 3).

Rhino/Entero was the most frequently detected pathogen in both periods, accounting for 40.1% and 40.7% in Periods I and II, respectively.

Although the number of RSV-positive cases increased in Period II, its proportion among the major respiratory pathogens decreased from 23.6% in Period I to 18.2% in Period II ($p < 0.001$). The primary age groups for RSV positivity were 3 and 2.

AdV accounted for 6.5% and 14.9% in Periods I and II, respectively, with a significant increase in Period II ($p = 0.002$), and the highest detection rates were observed in age groups 3 and 4.

Mycoplasma pneumoniae was not detected in Period I; however, 270 cases were detected in Period II, accounting for 10.2% of the positive cases, primarily in age groups 5 and 4.

HMPV accounted for 4.4% and 5.1% of cases in Periods I and II, respectively.

PIV3 accounted for 17.2% and 4.9% of the cases in Periods I and II, respectively, with a significant decrease in Period II ($p < 0.001$).

Flu A increased from 2.1% in Period I to 4.4% in Period II; however, this increase was not significant. Flu B in-

Table 1. Positivity rates by age group and period according to test item.

Age group	Age distribution	Period	FilmArray® Respiratory Panel assay				Influenza test							COVID-19 test				Total N
							Influenza A			Influenza B								
			positive n (%)	p-value	negative n (%)	total N	positive n (%)	p-value	negative n (%)	positive n (%)	p-value	negative n (%)	total N	positive n (%)	p-value	negative n (%)	total N	
1	≤ 28 days	I	34 (20.5)	0.025	132 (79.5)	166	0 (0.0)	0.434	107 (100.0)	0 (0.0)	-	107 (100.0)	107	121 (19.1)	0.000	512 (80.9)	633	906
		II	53 (31.4)		116 (68.6)	169	1 (1.2)		81 (98.8)	0 (0.0)		82 (100.0)	82	9 (6.1)		139 (93.9)	148	399
2	1 - 3 months	I	277 (62.7)	0.013	165 (37.3)	442	3 (5.4)	0.018	53 (94.6)	2 (3.6)	0.676	54 (96.4)	56	170 (19.4)	0.202	708 (80.6)	878	1,376
		II	538 (69.8)		233 (30.2)	771	33 (18.8)		143 (81.3)	5 (2.8)		171 (97.2)	176	123 (22.2)		431 (77.8)	554	1,501
3	4 months - 2 years	I	695 (76.2)	0.001	217 (23.8)	912	32 (6.6)	0.001	454 (93.4)	22 (4.5)	0.306	464 (95.5)	486	445 (17.3)	0.004	2,120 (82.7)	2,565	3,963
		II	710 (82.7)		149 (17.3)	859	117 (12.4)		829 (87.6)	32 (3.4)		914 (96.6)	946	229 (21.5)		835 (78.5)	1,064	2,869
4	3 - 6 years	I	309 (77.4)	0.484	90 (22.6)	399	125 (27.4)	0.894	332 (72.6)	17 (3.7)	0.468	440 (96.3)	457	176 (14.6)	0.000	1,033 (85.4)	1,209	2,065
		II	507 (79.3)		132 (20.7)	639	208 (27.9)		538 (72.1)	35 (4.7)		711 (95.3)	746	27 (4.7)		549 (95.3)	576	1,961
5	7 - 18 years	I	145 (44.1)	0.000	184 (55.9)	329	119 (35.0)	0.499	221 (65.0)	13 (3.8)	0.000	327 (96.2)	340	276 (16.9)	0.000	1,354 (83.1)	1,630	2,299
		II	502 (64.4)		278 (35.6)	780	288 (33.0)		586 (67.0)	95 (10.9)		779 (89.1)	874	48 (5.8)		786 (94.2)	834	2,488
6	≥ 19 years	I	12 (32.4)	0.058	25 (67.6)	37	169 (7.6)	0.621	2,066 (92.4)	63 (2.8)	0.027	2,172 (97.2)	2,235	11,870 (21.3)	0.000	43,756 (78.7)	55,626	57,898
		II	61 (51.7)		57 (48.3)	118	658 (7.3)		8,398 (92.7)	184 (2.0)		8,872 (98.0)	9,056	1,692 (9.0)		17,028 (91.0)	18,720	27,894
Total N (%)		I	1,472 (64.4)	0.000	813 (35.6)	2,285	448 (12.2)	0.048	3,233 (87.8)	117 (3.2)	0.509	3,564 (96.8)	3,681	13,058 (20.9)	0.000	49,483 (79.1)	62,541	68,507
		II	2,371 (71.1)		965 (28.9)	3,336	1,305 (11.0)		10,575 (89.0)	351 (3.0)		11,529 (97.0)	11,880	2,128 (9.7)		19,768 (90.3)	21,896	37,112

n: number of samples, N: total number of samples. Underlined numbers indicate a p-value of < 0.05.

Table 2. Positivity rates by gender and period according to test item.

Gender	Period	FilmArray® Respiratory Panel assay				Influenza test							COVID-19 test				Total N
						Influenza A			Influenza B								
		positive n (%)	p-value	negative n (%)	total N	positive n (%)	p-value	negative n (%)	positive n (%)	p-value	negative n (%)	total N	positive n (%)	p-value	negative n (%)	total N	
Male	I	815 (63.8)	0.000	462 (36.2)	1,277	247 (12.2)	0.019	1,775 (87.8)	58 (2.9)	0.656	1,964 (97.1)	2,022	6,276 (21.1)	0.000	23,503 (78.9)	29,779	33,078
	II	1,310 (70.5)		549 (29.5)	1,859	661 (10.3)		5,731 (89.7)	198 (3.1)		6,194 (96.9)	6,392	1,067 (9.3)		10,357 (90.7)	11,424	19,675
Female	I	657 (65.2)	0.000	351 (34.8)	1,008	201 (12.1)	0.665	1,458 (87.9)	59 (3.6)	0.116	1,600 (96.4)	1,659	6,782 (20.7)	0.000	25,980 (79.3)	32,762	35,429
	II	1,061 (71.8)		416 (28.2)	1,477	644 (11.7)		4,844 (88.3)	153 (2.8)		5,335 (97.2)	5,488	1,061 (10.1)		9,411 (89.9)	10,472	17,437
Total	I	1,472 (64.4)	0.000	813 (35.6)	2,285	448 (12.2)	0.048	3,233 (87.8)	117 (3.2)	0.509	3,564 (96.8)	3,681	13,058 (20.9)	0.000	49,483 (79.1)	62,541	68,507
	II	2,371 (71.1)		965 (28.9)	3,336	1,305 (11.0)		10,575 (89.0)	351 (3.0)		11,529 (97.0)	11,880	2,128 (9.7)		19,768 (90.3)	21,896	37,112

n: number of samples, N: total number of samples. Underlined numbers indicate a p-value of < 0.05.

Table 3. Frequency of detection of major respiratory pathogens by age group and period with FilmArray® Respiratory Panel assay.

Age group	P case	Rhino/ Entero		RSV		AdV		*M. pneumoniae*		HMPV		PIV3		Flu A		Flu B		SARS-CoV-2		Total	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	n	9	11	14	29	2	2	0	0	1	1	4	3	0	1	0	0	4	0	34	47
	(%)	(1.3)	(1.0)	(3.4)	(6.0)	(1.8)	(0.5)	(0.0)	(0.0)	(1.3)	(0.7)	(1.3)	(2.3)	(0.0)	(0.9)	(0.0)	(0.0)	(3.8)	(0.0)	(1.9)	(1.8)
2	n	92	183	118	192	5	29	0	1	6	17	43	40	9	18	1	3	32	1	306	484
	(%)	(13.1)	(17.0)	(28.5)	(39.8)	(4.4)	(7.3)	(0.0)	(0.4)	(7.7)	(12.5)	(14.2)	(31.0)	(25.0)	(15.5)	(50.0)	(9.1)	(30.5)	(10.0)	(17.4)	(18.3)
3	n	371	388	193	153	68	156	0	25	38	59	173	45	6	24	0	2	35	3	884	855
	(%)	(52.7)	(36.0)	(46.6)	(31.7)	(59.6)	(39.4)	(0.0)	(9.3)	(48.7)	(43.4)	(57.3)	(34.9)	(16.7)	(20.7)	(0.0)	(6.1)	(33.3)	(30.0)	(50.4)	(32.3)
4	n	172	290	66	65	26	140	0	57	28	36	60	27	5	26	1	4	12	5	370	650
	(%)	(24.4)	(26.9)	(15.9)	(13.5)	(22.8)	(35.4)	(0.0)	(21.1)	(35.9)	(26.5)	(19.9)	(20.9)	(13.9)	(22.4)	(50.0)	(12.1)	(11.4)	(50.0)	(21.1)	(24.5)
5	n	58	189	23	39	12	68	0	179	5	19	20	12	15	36	0	24	18	1	151	567
	(%)	(8.2)	(17.5)	(5.6)	(8.1)	(10.5)	(17.2)	(0.0)	(66.3)	(6.4)	(14.0)	(6.6)	(9.3)	(41.7)	(31.0)	(0.0)	(72.7)	(17.1)	(10.0)	(8.6)	(21.4)
6	n	2	16	0	4	1	1	0	8	0	4	2	2	1	11	0	0	4	0	10	46
	(%)	(0.3)	(1.5)	(0.0)	(0.8)	(0.9)	(0.3)	(0.0)	(3.0)	(0.0)	(2.9)	(0.7)	(1.6)	(2.8)	(9.5)	(0.0)	(0.0)	(3.8)	(0.0)	(0.6)	(1.7)
Total	N	704	1,077	414	482	114	396	0	270	78	136	302	129	36	116	2	33	105	10	1,755	2,649
	(%)	(40.1)	(40.7)	(23.6)	(18.2)	(6.5)	(14.9)	(0.0)	(10.2)	(4.4)	(5.1)	(17.2)	(4.9)	(2.1)	(4.4)	(0.1)	(1.2)	(6.0)	(0.4)	(100.0)	(100.0)
p-value		0.000		0.000		0.002		-		0.166		0.000		0.370		-		0.041		0.000	

P: positive, n: number of samples, N: total number of samples, Rhino/Entero: human rhinovirus or enterovirus, RSV: respiratory syncytial virus, AdV: adenovirus, HMPV: human metapneumovirus, PIV3: parainfluenza virus 3, Flu: influenza, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2. Underlined numbers indicate a p-value of < 0.05.

creased from 0.1% to 1.2%.

The prevalence of SARS-CoV-2 significantly decreased from 6.0% in Period I to 0.4% in Period II (p = 0.041).

Seasonal distribution patterns of major respiratory pathogens

The monthly and seasonal distribution patterns of the major respiratory pathogens are listed in Table 4.

Rhino/Entero was distributed throughout all seasons in Period I, with the highest prevalence in spring (35.2%). In Period II, it was also detected year-round, but was more prevalent in autumn (31.1%) and summer (29.6%) than in winter and spring.

RSV showed a similar seasonal distribution in winter and spring in both Periods I and II; however, the distribution changed in summer and autumn in Period II, with the summer proportion increasing significantly from 2.2% in Period I to 15.6% (p < 0.001), and the autumn proportion decreasing from 25.6% to 13.1% (p < 0.001).

AdV was more prevalent in winter and spring in Period I but accounted for 40.4% and 35.9% in autumn and summer in Period II, respectively, with particularly high prevalence rates in August and September.

Mycoplasma pneumoniae was not detected in Period I; however, it was prevalent in summer and autumn in Period II, accounting for 41.1% and 38.9% of the positive cases, respectively.

HMPV was concentrated in autumn (79.5%) in Period I. However, summer and spring accounted for 40.4% and 35.3%, respectively, in Period II.

PIV3 was predominantly distributed in autumn (57.9%) and spring (27.2%) in Period I, but was more prevalent in spring (58.9%) and summer (38.0%) in Period II.

The combination of FilmArray RP and antigen and PCR tests indicated that Flu A had the highest distribution in winter in Period I, with 79.8% (386/484) detected, and 66.4% (944/1,421) in winter in Period II. During both periods, Flu A showed a clear seasonal bias, predominantly with winter epidemics.

Based on the combination of FilmArray RP and antigen and PCR tests, Flu B accounted for a high proportion of cases in winter and spring in Period I, at 50.4% (60/119) and 32.8% (39/119), respectively. In Period II, 57.3% (220/384) of the cases occurred in winter, and both periods were predominantly characterized by winter outbreaks.

The combination of FilmArray RP and antigen and PCR tests indicated that SARS-CoV-2 had the highest positivity rate of 34.3% (4,519/13,163) in winter in Period I, followed by autumn (23.6%, 3,111/13,163), spring (21.5%, 2,832/13,163), and summer (20.5%, 2,701/13,163). In contrast, in Period II, the highest positivity rate was 48.6% (1,039/2,138) in summer, whereas autumn (22.3%, 477/2,138) and winter (18.1%, 388/2,138) showed relatively low distributions. In particular,

Table 4. Frequency of detection of major respiratory pathogens by season and period with FilmArray® Respiratory Panel assay.

Season	Month	P case	Rhino/ Entero		RSV		AdV		*M. pneumoniae*		HMPV		PIV3		Flu A		Flu B		SARS-CoV-2		Total N (%)	
			I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Spring	3	n	66	30	49	14	6	1	0	4	2	4	26	1	5	0	1	1	5	1	160	56
		(%)	(9.4)	(2.8)	(11.8)	(2.9)	(5.3)	(0.3)	(0.0)	(1.5)	(2.6)	(2.9)	(8.6)	(0.8)	(13.9)	(0.0)	(50.0)	(3.0)	(4.8)	(10.0)	(9.1)	(2.1)
	4	n	126	47	62	7	25	3	0	2	6	13	55	9	6	0	1	4	14	1	295	86
		(%)	(17.9)	(4.4)	(15.0)	(1.5)	(21.9)	(0.8)	(0.0)	(0.7)	(7.7)	(9.6)	(18.2)	(7.0)	(16.7)	(0.0)	(50.0)	(12.1)	(13.3)	(10.0)	(16.8)	(3.2)
	5	n	56	178	1	96	5	41	0	4	0	31	1	66	0	10	0	0	9	0	72	426
		(%)	(8.0)	(16.5)	(0.2)	(19.9)	(4.4)	(10.4)	(0.0)	(1.5)	(0.0)	(22.8)	(0.3)	(51.2)	(0.0)	(8.6)	(0.0)	(0.0)	(8.6)	(0.0)	(4.1)	(16.1)
	Total N		248	255	112	117	36	45	0	10	8	48	82	76	11	10	2	5	28	2	527	568
	(%)		(35.2)	(23.7)	(27.1)	(24.3)	(31.6)	(11.4)	(0.0)	(3.7)	(10.3)	(35.3)	(27.2)	(58.9)	(30.6)	(8.6)	(100.0)	(15.2)	(26.7)	(20.0)	(30.0)	(21.4)
Summer	6	n	62	92	0	45	9	24	0	13	0	25	5	26	0	6	0	3	5	0	81	234
		(%)	(8.8)	(8.5)	(0.0)	(9.3)	(7.9)	(6.1)	(0.0)	(4.8)	(0.0)	(18.4)	(1.7)	(20.2)	(0.0)	(5.2)	(0.0)	(9.1)	(4.8)	(0.0)	(4.6)	(8.8)
	7	n	61	117	1	17	5	36	0	40	1	24	1	15	0	3	0	1	4	0	73	253
		(%)	(8.7)	(10.9)	(0.2)	(3.5)	(4.4)	(9.1)	(0.0)	(14.8)	(1.3)	(17.6)	(0.3)	(11.6)	(0.0)	(2.6)	(0.0)	(3.0)	(3.8)	(0.0)	(4.2)	(9.6)
	8	n	22	110	8	13	4	82	0	58	4	6	0	8	1	5	0	0	9	0	48	282
		(%)	(3.1)	(10.2)	(1.9)	(2.7)	(3.5)	(20.7)	(0.0)	(21.5)	(5.1)	(4.4)	(0.0)	(6.2)	(2.8)	(4.3)	(0.0)	(0.0)	(8.6)	(0.0)	(2.7)	(10.6)
	Total N		145	319	9	75	18	142	0	111	5	55	6	49	1	14	0	4	18	0	202	769
	(%)		(20.6)	(29.6)	(2.2)	(15.6)	(15.8)	(35.9)	(0.0)	(41.1)	(6.4)	(40.4)	(2.0)	(38.0)	(2.8)	(12.1)	(0.0)	(12.1)	(17.1)	(0.0)	(11.5)	(29.0)
Autumn	9	n	48	136	41	19	4	95	0	38	26	2	10	4	0	6	0	0	11	0	140	300
		(%)	(6.8)	(12.6)	(9.9)	(3.9)	(3.5)	(24.0)	(0.0)	(14.1)	(33.3)	(1.5)	(3.3)	(3.1)	(0.0)	(5.2)	(0.0)	(0.0)	(10.5)	(0.0)	(8.0)	(11.3)
	10	n	75	104	44	11	11	48	0	37	25	2	114	0	2	11	0	1	7	2	278	216
		(%)	(10.7)	(9.7)	(10.6)	(2.3)	(9.6)	(12.1)	(0.0)	(13.7)	(32.1)	(1.5)	(37.7)	(0.0)	(5.6)	(9.5)	(0.0)	(3.0)	(6.7)	(20.0)	(15.8)	(8.2)
	11	n	68	95	21	33	9	17	0	30	11	2	51	0	1	9	0	0	7	0	168	186
		(%)	(9.7)	(8.8)	(5.1)	(6.8)	(7.9)	(4.3)	(0.0)	(11.1)	(14.1)	(1.5)	(16.9)	(0.0)	(2.8)	(7.8)	(0.0)	(0.0)	(6.7)	(0.0)	(9.6)	(7.0)
	Total N		191	335	106	63	24	160	0	105	62	6	175	4	3	26	0	1	25	2	586	702
	(%)		(27.1)	(31.1)	(25.6)	(13.1)	(21.1)	(40.4)	(0.0)	(38.9)	(79.5)	(4.4)	(57.9)	(3.1)	(8.3)	(22.4)	(0.0)	(3.0)	(23.8)	(20.0)	(33.4)	(26.5)
Winter	12	n	40	80	31	102	7	21	0	20	0	9	15	0	18	29	0	7	16	2	127	270
		(%)	(5.7)	(7.4)	(7.5)	(21.2)	(6.1)	(5.3)	(0.0)	(7.4)	(0.0)	(6.6)	(5.0)	(0.0)	(50.0)	(25.0)	(0.0)	(21.2)	(15.2)	(20.0)	(7.2)	(10.2)
	1	n	37	52	78	82	19	21	0	13	2	8	13	0	3	30	0	13	11	3	163	222
		(%)	(5.3)	(4.8)	(18.8)	(17.0)	(16.7)	(5.3)	(0.0)	(4.8)	(2.6)	(5.9)	(4.3)	(0.0)	(8.3)	(25.9)	(0.0)	(39.4)	(10.5)	(30.0)	(9.3)	(8.4)
	2	n	43	36	78	43	10	7	0	11	1	10	11	0	0	7	0	3	7	1	150	118
		(%)	(6.1)	(3.3)	(18.8)	(8.9)	(8.8)	(1.8)	(0.0)	(4.1)	(1.3)	(7.4)	(3.6)	(0.0)	(0.0)	(6.0)	(0.0)	(9.1)	(6.7)	(10.0)	(8.5)	(4.5)
	Total N		120	168	187	227	36	49	0	44	3	27	39	0	21	66	0	23	34	6	440	610
	(%)		(17.0)	(15.6)	(45.2)	(47.1)	(31.6)	(12.4)	(0.0)	(16.3)	(3.8)	(19.9)	(12.9)	(0.0)	(58.3)	(56.9)	(0.0)	(69.7)	(32.4)	(60.0)	(25.1)	(23.0)
Total N			704	1,077	414	482	114	396	0	270	78	136	302	129	36	116	2	33	105	10	1,755	2,649
(%)			(40.1)	(40.7)	(23.6)	(18.2)	(6.5)	(14.9)	(0.0)	(10.2)	(4.4)	(5.1)	(17.2)	(4.9)	(2.1)	(4.4)	(0.1)	(1.2)	(6.0)	(0.4)	(100.0)	(100.0)

P: positive, n: number of samples, N: total number of samples, Rhino/Entero: human rhinovirus or enterovirus, RSV: respiratory syncytial virus, AdV: adenovirus, HMPV: human metapneumovirus, PIV3: parainfluenza virus 3, Flu: influenza, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

Table 5. Distribution of respiratory pathogen counts by age group in positive cases with FilmArray® Respiratory Panel assay results.

Age group	Number of pathogens n (%)						
	1	2	3	4	5	6	Total N
1	78 (89.7)	8 (9.2)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	87
2	631 (77.4)	163 (20.0)	19 (2.3)	2 (0.2)	0 (0.0)	0 (0.0)	815
3	895 (63.7)	413 (29.4)	75 (5.3)	21 (1.5)	1 (0.1)	0 (0.0)	1,405
4	544 (66.7)	207 (25.4)	50 (6.1)	6 (0.7)	7 (0.9)	2 (0.2)	816
5	482 (74.5)	140 (21.6)	21 (3.2)	3 (0.5)	1 (0.2)	0 (0.0)	647
6	67 (91.8)	5 (6.8)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	73
Total N (%)	2,697 (70.2)	936 (24.4)	167 (4.3)	32 (0.8)	9 (0.2)	2 (0.1)	3,843

n: number of samples, N: total number of samples.

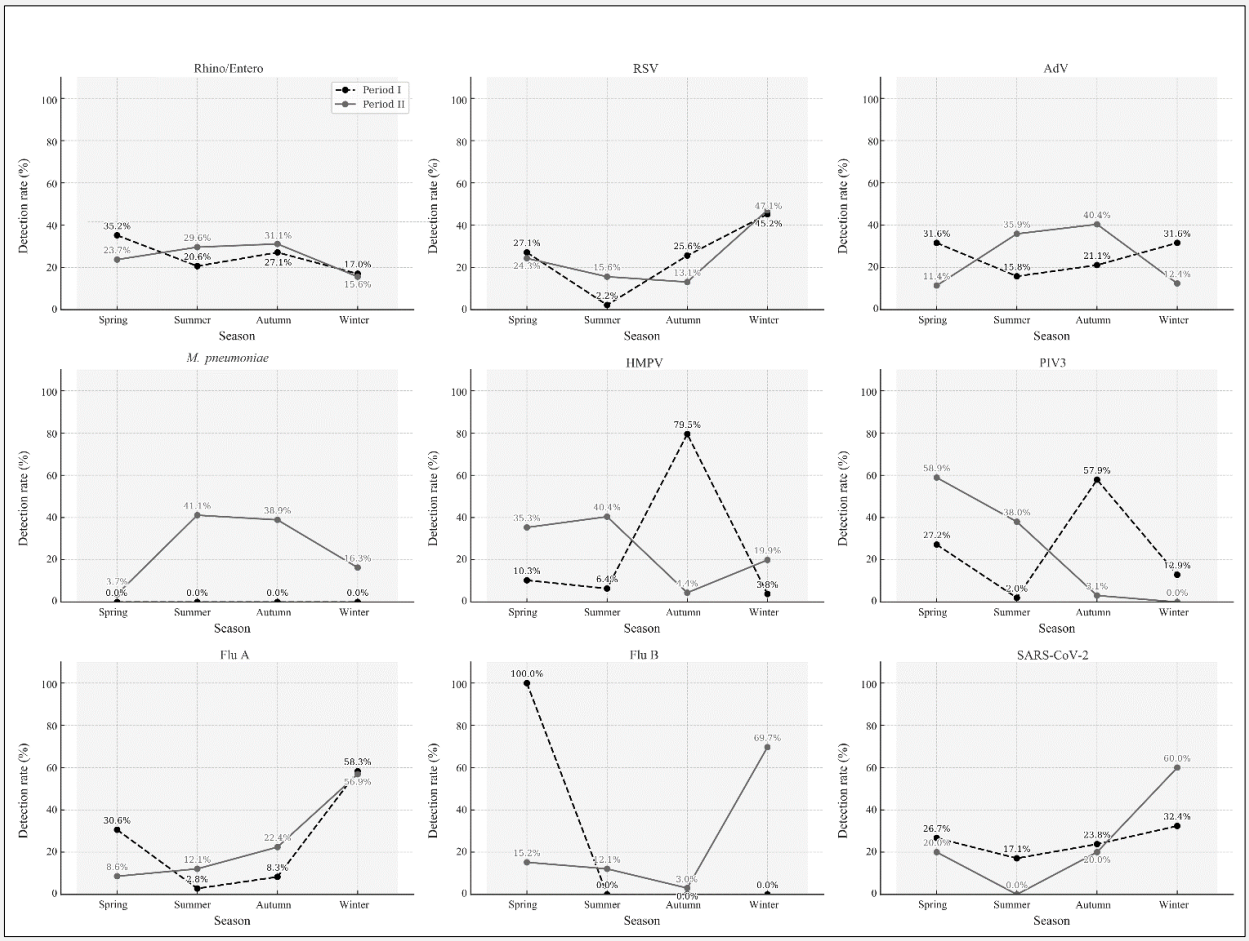

Figure 1. Frequency of detection of major respiratory pathogens by season and period with FilmArray® Respiratory Panel assay. The graph indicates tendency shift in detection of major respiratory pathogens based on season and period.

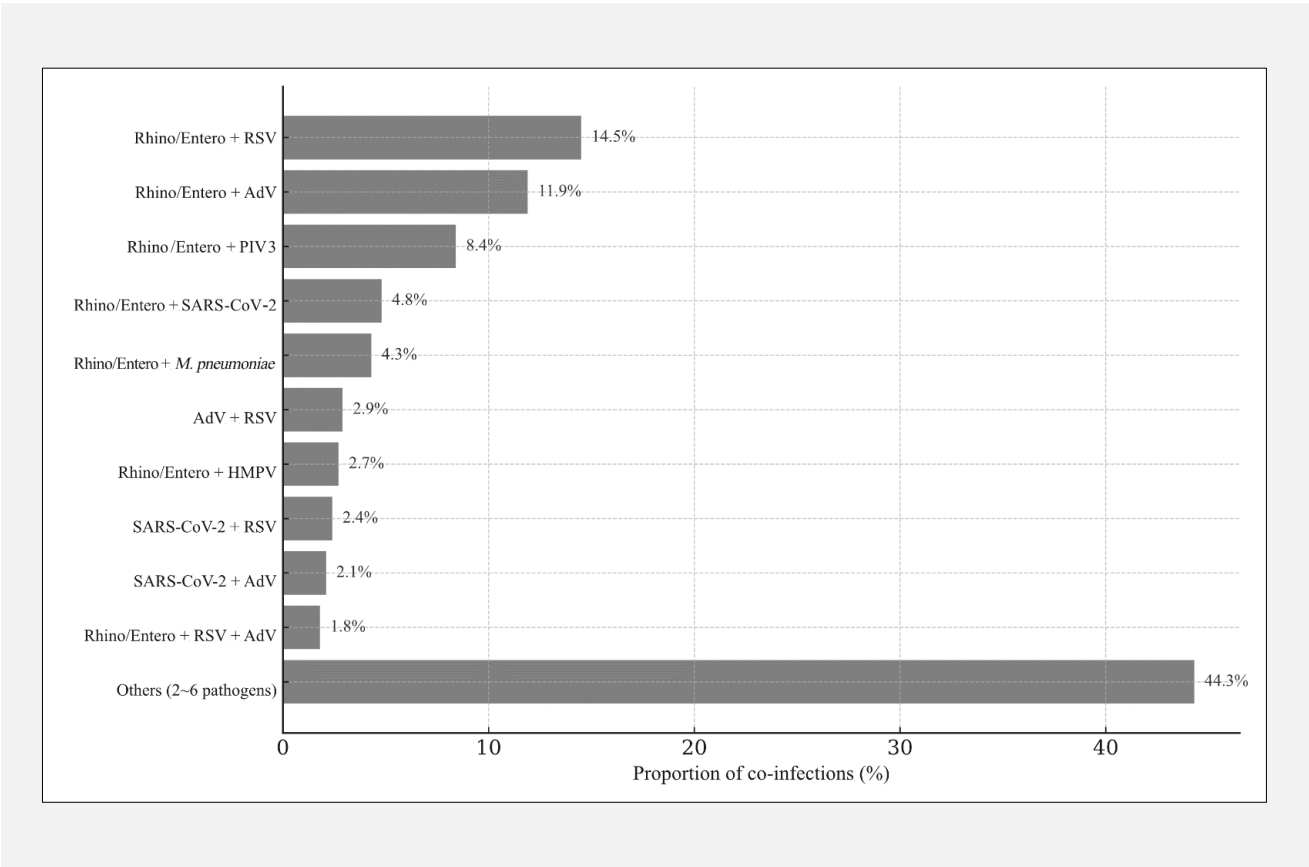

Figure 2. Distribution of co-infections in FilmArray® Respiratory Panel assay results. The graph indicates the combination of pathogens in all co-infections cases.

in Period II, the positivity rate increased significantly in summer compared to that in Period I, showing a change in the seasonal pattern of the epidemic.

The seasonal distribution of major respiratory pathogens was graphed by period to determine the differences between the two periods (Figure 1), with AdV, *M. pneumoniae*, HMPV, PIV3, and Flu B showing distinct differences.

Co-infection patterns

Among all positive cases, co-infections by two or more respiratory pathogens were 17.6% (402/2,285) and 22.3% (744/3,336) in Periods I and II, respectively, with a significant increase in Period II ($p < 0.001$). Infections caused by two pathogens accounted for 81.7% (936/1,146) and those caused by three pathogens accounted for 14.6% (167/1,146).

Co-infections with two pathogens were the highest in age group 3 (29.4%) (Table 5).

In the seasonal analysis, co-infections with two or more pathogens were relatively evenly distributed across all four seasons, with no seasonal peaks.

Out of the 3,843 total positives, out of which 2,697 were single-pathogen detections, a combination of pathogens was detected in 1,146 cases, with the most common pathogen combination being Rhino/Entero and RSV (14.5% of all co-infections). This was followed by a combination of Rhino/Entero and AdV (11.9%), Rhino/Entero and PIV3 (8.4%), and Rhino/Entero and SARS-CoV-2 (4.8%) (Figure 2). Among cases where three or more pathogens were detected simultaneously, the combination of Rhino/Entero, RSV, and AdV was the most common ($n = 21$).

Co-infection with two or more pathogens was significantly higher in Period II (31.4%, 744/2,371) than in Period I (27.3%, 402/1,472) ($p = 0.008$). The most prevalent combination in Period I was Rhino/Entero and RSV (79 cases, 19.7%), followed by Rhino/Entero and PIV3 with 71 cases (17.7%). In Period II, the combination of Rhino/Entero and AdV was the most common (112 cases, 15.1%), followed by Rhino/Entero and RSV (87 cases, 11.7%).

When analyzing changes between periods according to pathogen combination, the co-infection of Rhino/Entero and RSV was most frequently detected in both periods. However, the proportion of patients with two or more co-infections decreased from 19.7% in Period I to 11.7% in Period II. In contrast, the combination of Rhi-

no/Entero and AdV was only 2.1% in Period I but increased to 15.1% in Period II, showing a distinct pattern of change between periods.

DISCUSSION

This study analyzed the distribution of respiratory pathogens during the COVID-19 pandemic and after the government's endemic declaration, and compared the distribution characteristics of respiratory pathogens by age group and gender, seasonal changes in incidence, and co-infections. This study aimed to determine whether and how suppressed pathogens re-emerged after mitigation. Comprehensive quarantine measures such as wearing masks, social distancing, and increased personal hygiene have significantly curbed the spread of respiratory pathogens during the pandemic [3-6].

In a study based in Daegu, South Korea, pathogen detection rates in suspected respiratory tract infections were significantly lower during the pandemic period (March 2020 through February 2021) than during the pre-pandemic period (January 2019 through December 2019) [6]. This study analyzed the post-pandemic period (May 2023 through February 2025) and observed the re-spread and re-emergence of several pathogens, showing a change from the results during the pandemic. Specifically, the overall positivity rate of FilmArray RP increased significantly from Periods I and II to 64.4% and 71.1%, respectively ($p < 0.001$). Similarly, a study conducted in China reported a significant increase in positivity rates from 27.63% and 24.38% in 2021 and 2022, respectively, during the COVID-19 pandemic to 34.62% in 2023 after restrictions were eased [23]. This finding can be attributed to the relaxation of various NPIs implemented during the pandemic, such as mask-wearing, social distancing, increased hand hygiene, travel restrictions, and institutional closures, which increased the opportunities for exposure to pathogens.

A study in China observed age-specific differences in positivity rates for major respiratory viruses, such as RSV, PIV3, PIV1, Flu A, Flu B, and AdV, after the pandemic, with RSV, PIV3, and PIV1 showing the highest positivity rates in infants and preschoolers. Additionally, most pathogens show a decreasing trend in positivity rates with increasing age [24]. These results are similar to those of the current study, which found the highest positivity rates in age group 3. However, these results differ from those of a Chinese study that showed that the largest increase in positivity was observed in age group 5, which included school-age children. These differences may be attributed to differences in mitigation policies between countries, the timing of the reopening of educational institutions, and differences in immunity by age.

The COVID-19 positivity rates were 20.9% and 9.7% in Periods I and II, respectively, with a significant decrease in Period II ($p < 0.001$). This result is likely due to the increased vaccination rates, immunity acquired after natural infection, and public health measures implemented during the pandemic. FilmArray RP for respiratory pathogens was significantly less positive in all age groups during the pandemic than before the pandemic, and HMPV and PIV3 were detected at low rates before the pandemic but not during the pandemic (March 2020 through February 2021) [6]. In addition, before the pandemic, major viruses such as RSV and PIV showed distinct seasonality in another Korean study; however, during the pandemic, virus detection decreased because of the effect of NPIs, and the typical seasonal epidemic pattern disappeared [8]. In contrast, in this study, the overall positivity rate of respiratory pathogens increased again after the pandemic with the relaxation of control measures, and the re-emergence of major respiratory pathogens, such as AdV and *M. pneumoniae*, which were suppressed, was confirmed.

Rhino/Entero was the most frequently detected pathogen, both before and after the pandemic, likely because it was less affected by NPIs. Korean [6] and Japanese studies [11] also reported the consistent detection of Rhino/Entero during the pandemic. In addition, 270 new cases of *M. pneumoniae* (10.2%), which were not detected during the pandemic, were identified in Period II. Further, the prevalence of AdV increased significantly, from 6.5% in Period I to 14.9% in Period II ($p = 0.002$). These changes have also been reported in studies from Germany [25] and China [26] and may result from the resumption of cyclical pathogen epidemics with the resumption of social activities after the pandemic suppression.

The number of RSV detections increased in Period II but decreased in proportion to all major respiratory pathogens ($p < 0.001$). The predominant age groups were 2 and 3, and the pattern of infections remained infant- and child-centered, likely because of the simultaneous re-emergence of various pathogens, which reduced their relative detection.

PIV3 accounted for 17.2% of the major respiratory pathogens in Period I but significantly decreased to 4.9% in Period II ($p < 0.001$). This finding suggests that the epidemic was suppressed by strong control measures early in the pandemic, temporarily re-emerged at a time not associated with the usual seasonality of PIV, and returned to low levels after the pandemic. PIV3 and HMPV were not detected during the pandemic and were categorized as typical epidemic-suppressing pathogens in a Korean study [6]. Another Korean study [19] reported a temporary outbreak of PIV in 2021 during the pandemic at a Korean university hospital at an out-of-season time, suggesting the irregularity of viral re-emergence and the possibility of seasonal disruption.

In terms of seasonal distribution, Rhino/Entero occurred evenly throughout the year, whereas RSV showed an increase in summer detection rates to 2.2% and 15.6% in Periods I and II, respectively ($p < 0.001$). In both periods, the center of the seasonal epidemic remained in winter, with the highest positivity rates; however, a broadening of the seasonal distribution was observed af-

ter the pandemic, with an increase in summer detection rates. This finding suggests that the seasonal distribution of RSV has changed since the pandemic. These changes were also reported in an 8-year retrospective study from Jalisco, Mexico [27], which found that RSV had a predictable winter-centered epidemic pattern before the pandemic; however, after the pandemic, the timing of the epidemic shifted and the number of infections increased. In the current study, RSV had the highest detection rate in winter during both periods; however, a significant increase in summer detection rates was observed after the pandemic. This finding suggests that geographic characteristics, such as national and regional climatic conditions and the level of public health intervention, may have contributed to the variability in epidemic timing. PIV3 also showed a shift in the peak season from autumn to spring and summer, consistent with a previous domestic study [19]. In contrast, Flu A and Flu B maintained their seasonality, with a tendency to concentrate in winter after the pandemic. The combination of FilmArray RP and antigen and PCR tests indicated that SARS-CoV-2 showed a sharp decrease in the positivity rate from 20.3% (13,163/64,826) in Period I to 8.5% (2,138/25,232) in Period II, similar to trends in previous studies [2,5]. In particular, when examining seasonal patterns, a clear shift was observed in the epidemic from winter to summer, which could be interpreted as a result of immune acquisition influenced by expanded vaccination and cumulative infections.

Co-infection analysis showed that the proportions of co-infection in Periods I and II among all positive cases were 17.6% and 22.3%, respectively, with a significant increase in Period II ($p < 0.001$). The combination of Rhino/Entero and RSV accounted for 79 (19.7%) and 87 (11.7%) cases in Periods I and II, respectively, with a relative decrease in the proportion in Period II; however, no significant difference was observed between the periods. This study analyzed the post-pandemic period (May 2023 through February 2025) and observed the re-spread and re-emergence of several pathogens, showing a change from the results during the pandemic. Specifically, the overall positivity rate of FilmArray RP increased significantly from Periods I (64.4%) and II (71.1%) ($p < 0.001$). The combination of Rhino/Entero and AdV differed between periods, with only 24 cases (2.1%) in Period I and 112 cases (15.1%) in Period II. The highest proportion of co-infection was in age group 3, and among all positive cases, 936 (81.7%) out of the 1,146 co-infection cases, excluding single pathogen detection, were caused by two pathogens. These findings were similar to those of a study conducted in Shijiazhuang, China. This study included the period during the pandemic (2021 - 2022) and up to 2023, shortly after the endemic transition, and found that 165 out of the 2,095 patients were co-infected with two pathogens and 13 with three pathogens, with two pathogens being the most common among the co-infection cases. Furthermore, the rate of co-infection in 2023 was significantly higher than that in 2021 and 2022 ($p < 0.05$), which was related to the increased co-introduction of pathogens owing to post-pandemic quarantine mitigation [23]. However, the primary pathogen combinations differed, with RSV and AdV frequently detected alongside Rhino/Entero in this study. In contrast, the combination of RSV and *M. pneumoniae* was the most common in a Chinese study, with RSV accounting for a lower proportion of the overall detections. These differences may have been influenced by the fact that the study covered the later part of the pandemic (2021 - 2022) when the RSV epidemic was suppressed, suggesting that infection patterns may differ between regions depending on the nature of the prevalent pathogens, differences in the age composition of the target population, and access to testing.

This study builds on recent advances in molecular diagnostics, particularly the introduction of multiplex PCR tests, such as FilmArray RP, which allow for more rapid and accurate detection of various respiratory pathogens. These technologies have greatly improved diagnostic efficiency and speed during the pandemic and have enabled the surveillance of co-infection with respiratory pathogens.

Prior to the COVID-19 pandemic, major respiratory viruses had relatively consistent seasonal outbreak cycles. However, during the pandemic, these typical seasonal outbreaks were suppressed or eliminated owing to the implementation of various health policies. In other words, pathogens such as RSV, HMPV, and PIV3 were almost undetectable during the early part of the pandemic (March 2020 through February 2021) in a previous study [6]. However, in this study, they reappeared during the pandemic period (March 2021 through April 2023). Outbreaks during this time were atypical of those observed before the pandemic, occurring at different times from traditional seasonal cycles or having irregular patterns, suggesting that altered social and environmental factors during the pandemic influenced pathogen prevalence.

In contrast, in the post-pandemic period, suppressed respiratory pathogens re-emerged at various time points, showing a distinct pattern from that during the previous epidemic. This finding suggests that it is not a simple resurgence of the epidemic but rather a reorganization of the existing epidemic structure owing to the combined effects of the immune hiatus accumulated during the pandemic and the resumption of social activities. The fact that the same pathogen exhibited different epidemiologic patterns in different periods suggests that traditional seasonal forecasting alone may have limitations in future respiratory disease surveillance and emphasizes the need to comprehensively consider the effect of policy changes and changes in the immune environment of population groups on the structure of pathogen epidemics.

Previous studies have focused on differences between the pre-pandemic and pandemic periods; however, this study is significant in that it compares changes in respiratory pathogen distributions during and after the pan-

demic, providing insights into how cumulative social, environmental, and epidemiologic factors influence pathogen transmission and epidemic characteristics throughout the pandemic. However, as this study was based on data from a single region, Daegu, and the results of a specific molecular diagnostic test, its nationwide generalizability is limited. A more sophisticated analysis of the distribution of respiratory pathogens is required in future multi-center, national-level studies reflecting regional characteristics.

CONCLUSION

This study identified several changes in the distribution of respiratory pathogens. In the post-COVID-19 pandemic environment, various respiratory pathogens are re-emerging, some with seasonal and age-specific characteristics different from those previously observed. In particular, the re-emergence of pathogens in pediatric and school-age groups has crucial implications for future infectious disease response strategies and highlights the need for precise surveillance that reflects age-specific pathogen characteristics. In addition, atypical seasonal changes and an increased frequency of co-infections suggest the need for continued monitoring and further research.

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The authors have no conflicts of interest to declare.

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