Article

EpidemiologicalCharacterizationofRespiratoryPathogens Using the Multiplex PCR FilmArray™ Respiratory Panel

Abstract: Variouspathogenscancauseupperrespiratorytractinfections,presentingchallengesin accurate diagnosis due to similar symptomatology.Therefore, rapid and precise diagnostic tests arecrucial for effective treatment planning.Traditional culture-based methods for diagnosis are limited by their reliance on skilled personnel and lengthy processing times.In contrast, multiplex poly- merase chain reaction (PCR) techniques offer enhanced accuracy and speed in identifying respiratorypathogens.Inthisstudy,weaimedtoassesstheefficacyoftheFilmArray™RespiratoryPanel(RP),a multiplex PCR test capable of simultaneously screening 20 pathogens. This retrospective analysis wasconductedatDankookUniversityHospital,SouthKorea,betweenJanuary2018andDecember 2022. Samples from patients with upper respiratory tract infections were analyzed. Results revealed adenovirusasthemostprevalentpathogen(18.9%),followedbyinfluenzavirusA(16.5%),among others. Notably, a 22.5% co-infection rate was observed. The FilmArray™ RP method successfully identified 20 pathogens within 2 h, facilitating prompt treatment decisions and mitigating unnecessary antibiotic prescriptions. This study underscores the utility of multiplex PCR in respiratory pathogen identification, offering valuable insights for epidemiological surveillance and diagnosis.

Keywords:diagnostictest;FilmArray™RP;multiplexPCR;upperrespiratorytractinfections; virus diseases

1. Introduction

Upper respiratory tract infections, commonly associated with symptoms such as sore throat, fever, runny nose, and cough, are prevalent worldwide, necessitating medical intervention for a significant number of affected individuals.The escalating need for healthcare services has led to substantial financial implications due to the high medical costs incurred [1,2].Additionally, these infections significantly contribute to the global disease burden, with an estimated 3.5 million deaths worldwide in 2008 attributable to upper respiratory tract infections [3,4].The pervasive nature of these infections underscorestheurgencyofaddressingtheirimpactonpublichealthsystemsandimplementing effective prevention and management strategies. Upper respiratory tract infections, caused byvariouspathogens suchasbacteria andviruses,presentadiagnosticchallenge owing to their overlapping symptoms.This similarity often leads to indiscriminate antibiotic prescriptions, fostering the emergence of antibiotic-resistant strains [5].Distinguishing between bacterial and viral infections is crucial for effective treatment.However, relying solely on symptoms for diagnosis may not suffice.Implementing diagnostic tools and guidelines can aid in accurate identification, curbing unnecessary antibiotic use, and combatingantimicrobialresistance. Researchindicatesthatasignificantportionofpediatric patients (approximately 21%) receive antibiotic prescriptions; of these, 44% of patients presentwithupperrespiratorytractinfections, indicatingapervasiveissueofantibiotic

over-prescriptioninhealthcare[6,7]. Addressingthisconcernrequiresconcertedefforts tominimizeunnecessaryantibioticuseandadopttreatmentstrategiesguidedbyprecise diagnostic evaluations.Implementing accurate diagnostic tests can assist in discerning the need for antibiotics, thus promoting judicious prescribing practices and mitigating the development of antibiotic resistance.

Severaldiagnostictestsareavailablefortheidentificationofupperrespiratorypathogens, each with ~~their~~ itsown set of advantages and limitations.Antigen tests, for instance, offer rapidresultsbutareplaguedbylowsensitivity[8,9].Ontheotherhand,traditionalmeth-- odssuchasconventionalvirusculturehighersensitivitybutentaillongerprocessing times,potentiallydelayingtreatmentdecisions[10].Multiplexpolymerasechainreaction (PCR)testshaveemergedasavaluabletoolinhospitalsforrapidlyandaccuratelyidenti- fyingrespiratorypathogens.Amongthese,theFilmArray™RespiratoryPanel(RP)stands out.ItisanFDA-approveddiagnostictoolandiscapableofdetectingover20respiratory pathogenswithin2h. However,thismethodrequiresspecializedfacilitiesandskilledperson- nelfortestingandresultinterpretation.Despiteitsefficacy,limitedstudieshaveevaluated its utility in identifying respiratory pathogens [11].We employed the FilmArray™ RP to epidemiologically characterize upper respiratory tract infections, assessing its suitability for suchresearchendeavors.Ourinvestigationaimedtofurnishfoundationaldatacrucialfor informinghealthcarepolicies,includingtheidentificationofinfectionpatterns,formulationof vaccinationstrategies,andcontainmentofpathogenspreadwithincommunities.Spanning from2018to2022,ourstudyperiodencompassedbothpre-andpost-outbreakphasesof thecoronavirusdisease2019(COVID-19)pandemicprecipitatedbysevereacuterespiratory syndromecoronavirus2(SARS-CoV-2). Assuch,ourstudycouldofferinvaluableinsights into the evolving landscape of upper respiratory tract infections amidst the COVID-19 crisis. By shedding light on the impact of the pandemic on respiratory infection patterns, our study couldnotonlyenrichourunderstandingofdiseasedynamicsbutalsoprovideaframework for devising proactive measures to mitigate future outbreaks.In essence, our findings could serveasacornerstoneforevidence-basedhealthcarestrategiesaimedatsafeguardingpublic healthinthefaceofinfectiousdiseasethreats.

2. Materialsand Methods

2.1. Samples

Nasopharyngeal swab (NPS) samples, totaling 300 µL in volume, were collectedfor infection testing at Dankook University (Cheonan, Republic of Korea) over a period spanningfrom1January2018to31December2022. Thesesamplesunderwentanalysis withoutcentrifugation.Specifically,onlyrefrigeratedandfrozensamplesadheringtothe conditions outlined in the manufacturer's manual were included in this study.

2.2. PathogenIdentificationwiththeFilmArray™RP

TheNPSsamplesunderwentrigoroustestinginaccordancewiththemanufacturer's manualfortheFilmArray™RP(BioFireDiagnostics,SaltLakeCity,UT,USA).Ensuringad- herencetostringentsafetyprotocols,alltestsweremeticulouslyconductedwithinabiosafety cabinet, with operators equipped with appropriate personal protective equipment.The sampleprocessingcommencedwiththeinjectionofsamplesintothekitusingahydration solutionandsamplebuffer,followedbyinsertionintothedesignatedequipment. Thetesting system followed a sequential workflow, beginning with nucleic acid extraction facilitated by samplebufferandzirconiumbeads,followedbyreversetranscription,auto-nestedmultiplex PCR,andconcludingwithmeltingcurveanalysis.Notably,theinitialPCRphaseinvolveda highlymultiplexedPCR,followedbyanindividualPCRstepincorporatingacyaninedyefor enhancedsensitivityandspecificity. TheFilmArray™2.1softwarewasusedforanalyzingthe DNAmeltingcurveforeverywellwithinthePCR2array. Uponobservingthepresenceof PCRproductsinawellandconfirmingthemeltingprofilealigningwithPCRproducts,the softwarewasusedtocalculatethemeltingtemperature(Tm)ofthecurve.Ifthecalculated Tmfellwithinthespecifiedrangedesignatedforanalysis,theresultwasdeemed"Detected".

Conversely,ifthesoftwaredeterminedthatthecurvedidnotalignwithinthepredetermined melting range, the outcome was labeled as “Not Detected”.

The FilmArray™ RP boasts the capability to detect a comprehensive range of pathogens, encompassingfourbacteriaand19viruses.Amongthebacterialtargetsare*Bordetellaper-tussis* (detection of ptxP), *Bordetella parapertussis*, *Chlamydia pneumoniae* (previously named *Chlamydophilapneumoniae*),and*Mycoplasmapneumoniae*. Additionally,thepanelincludes detection for various viruses such as adenovirus (AdV), coronavirus 229E (CoV-229E), coronavirus HKU1 (CoV-HKU1), coronavirus NL63 (CoV-NL63), coronavirus OC43 (CoV-OC43),human metapneumovirus (hMPV), human rhinovirus/enterovirus (HRV/EV),influenzavirusA(FluA),influenzavirusAH1(FluAH1),influenzavirusAH1-2009(FluA H1-2009), influenza virus A H3 (FluA H3), influenza virus B (FluB), parainfluenza virus1 (PIV1), parainfluenza virus 2 (PIV2), parainfluenza virus 3 (PIV3), parainfluenza virus 4 (PIV4), respiratory syncytial virus (RSV), Middle East respiratory syndrome coronavirus (MERS-CoV),andSARS-CoV-2.

Inourtestingprocess,wescreenedfor16viruses(AdV,CoV-229E,CoV-HKU1,CoV-NL63,CoV-OC43,hMPV,HRV/EV,FluA,FluB,PIV1,PIV2,PIV3,PIV4,RSV,MERS-CoV, SARS-CoV-2) and four bacteria (*Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydia pneumoniae*,and*Mycoplasmapneumoniae*)withintheNPSsamples.Eachtestingpouchwas equipped with two positive controls, ensuring the reliability and accuracy of our results.The first positive control, known as the RNA Process Control, specifically targeted the transcription of RNA from the yeast *Schizosaccharomyces pombe*.A positive outcome fromthis control indicated the successful execution of all steps performed within the pouch. Similarly, the second positive control detected a dried DNA target present in the well of the array, along with the corresponding primers. A positive result from this control confirmed the successful amplification of PCR2.To validate the overall test results, both control outcomes were required to be positive. In the event that either control failed, the test was repeatedusinganewkittomaintaintheintegrityandaccuracyofthetestingprocess.

3. Results

A total of 6367 respiratory samples underwent analysis, revealing 1538 positive cases. Amongthesepositivecases,1744pathogenswereidentified;theseincluded1351instancesof singleinfection,169doubleinfections,17tripleinfections,andasolitarycaseofquadruple infection(Table1).Amongthe20pathogensdetectableusingtheFilmArray™RP,15were identifiedinthisstudy,exclusivelycomprisingviruses(AdV,CoV-229E,CoV-HKU1,CoV-NL63,CoV-OC43,FluA,FluB,hMPV,HRV/EV,PIV1,PIV2,PIV3,PIV4RSV,andSARS-CoV-2). Theprevalenceofpathogensinthisstudywasintheorderof AdV(18.9%),FluA(16.5%), PIV3(12.3%),HRV/EV(10.4%),andhMPV(9.7%); thesecollectivelyconstitutedoverhalf ofthetotalpathogensdetected.Especially,dataforSARS-CoV-2wasincludedstartingfrom 1October2022.Outofatotalof73samplestestedsincetheinceptionofthestudy,28tested positive.Among these positive samples, twenty-two exhibited a single infection, while fivesamplesshoweddoubleinfection,andonesampledisplayedtripleinfection(Table2).

Table1. PositivityratesofFilmArray™RespiratoryPanelpathogens.

Parameter	Positivity	
	NumberofSamples	%of Total
Allsamples(*N*=6367)		
Negativesamples	4.829	75.8%
Positivesamples	1538	24.1%
Single detections	1351	21.2%
Co-infections	187	2.9%
	Co-infections(*n*=187)	
Doubleinfection	169	90.3%
Triple infection	17	9.1%
Quadrupleinfection	1	0.6%

Table 2. Detection and co-infection rates for each pathogen.

	Detected	Detection Rate (%)	Co-Infection	Co-Infection Rate (%)
AdV	329	18.9	93	28.3
CoV-229E	40	2.3	16	40.0
CoV-HKU1	5	0.3	3	60.0
CoV-NL63	68	3.9	26	38.2
CoV-OC43	101	5.8	31	30.7
SARS-CoV-2	28	1.6	6	21.4
hMPV	170	9.7	29	17.1
HRV/EV	181	10.4	57	31.5
FluA	289	16.6	38	13.1
FluB	107	6.1	15	14.0
PIV1	77	4.4	15	19.5
PIV2	29	1.7	4	13.8
PIV3	214	12.3	29	13.6
PIV4	9	0.5	5	55.6
RSV	97	5.6	26	26.8
Total	1744	100.0	393	22.5

AdV, adenovirus; CoV-229E, coronavirus 229E; CoV-HKU1, coronavirus HKU1; CoV-NL63, coronavirus NL63;CoV-OC43, coronavirus OC43; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; hMPV, human metapneumovirus; HRV/EV, human rhinovirus/enterovirus; FluA, influenza virus A; FluB, influenza virus B;PIV1, parainfluenza virus 1; PIV2, parainfluenza virus 2; PIV3, parainfluenza virus 3; PIV4, parainfluenza virus 4;RSV, respiratory syncytial virus.

Notably, the 0–10 age group exhibited the highest distribution of pathogens, accounting for 72.3% of the total age group positivity rate. AdV emerged as the most frequently detected pathogen within the 0–10 age group, comprising 17.0% of cases. Furthermore, within the 0–10 age group, AdV, PIV3, HRV/EV, and RSV constituted 90.3%, 83.6%, 95%, and 97.9% of total pathogens identified, respectively, signifying elevated positivity rates among children. Conversely, unlike other pathogens, FluA and FluB exhibited higher positivity rates in the 51–90 age group, representing 56.4% and 53.3% of the distribution, respectively (Figure 1). This variance in age group distribution underscores the differential susceptibility and manifestation of respiratory pathogens across various age demographics, highlighting the need for age-specific preventive and management strategies.

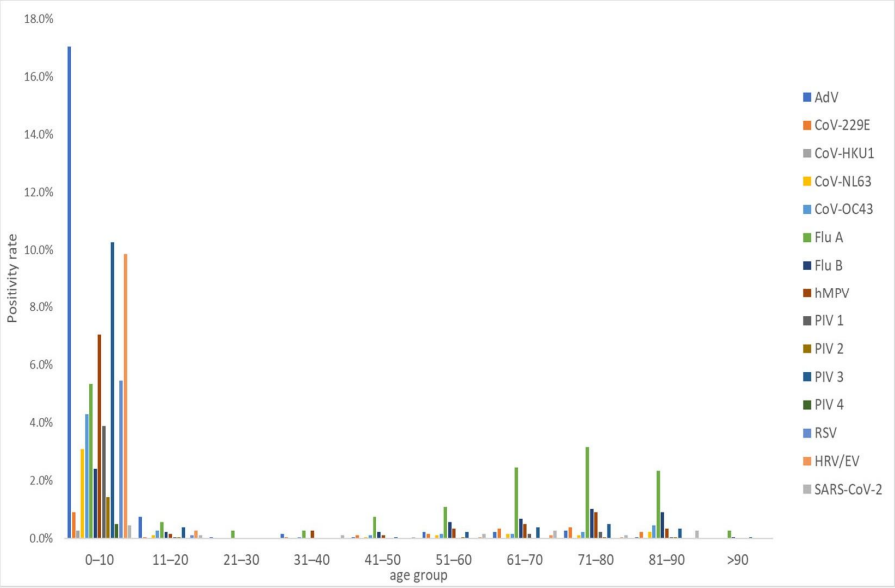

Figure 1. FilmArray™ Respiratory Panel (RP) positive detection of 1744 pathogens by age group collected over five years at Dankook University Hospital in Cheonan, Republic of Korea.

Thehighestnumberofpathogenswasidentifiedin December,representing18.2%ofall detected pathogens.Notably, a significant portion, accounting for 44.1% of the pathogens, was detected between December and February.During December and February, FluA andFluBexhibitedhighpositivityratesof87.2%and71.0%,respectively.Conversely,the positivity rate of PIV3 was notably lower at 51.4% from March to May.Throughout the year, pathogens such as AdV, hMPV, PIV1, PIV3, and HRV/EV showed relatively even distribution patterns (Figure 2).

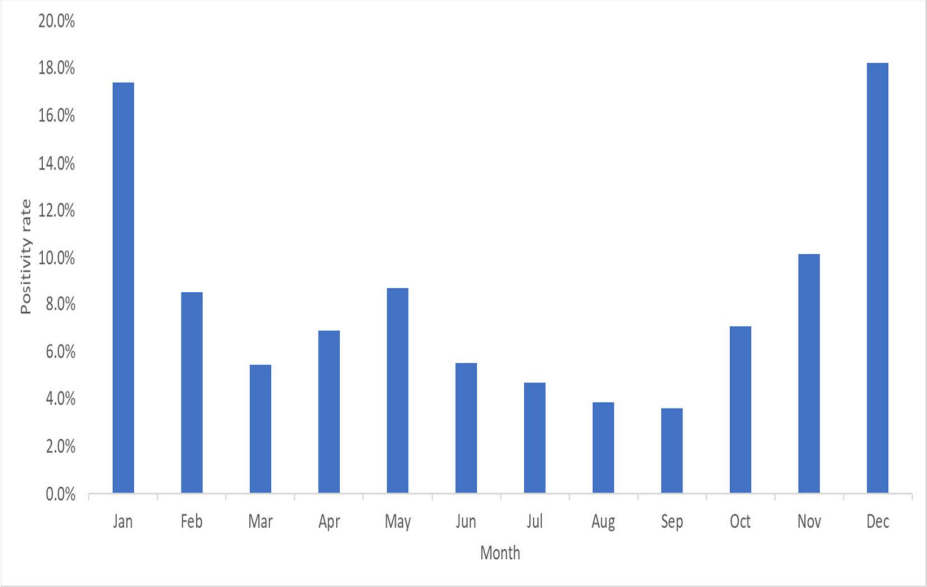

Figure2. Monthly FilmArray™ Respiratory Panel (RP) positivity rate of 1744 pathogens detected over five years at Dankook University Hospital in Cheonan, Republic of Korea.

Atotalof187casesofco-infectionwereidentified, with393outofthetotal1744pathogensshowing a co-infection rate of 22.5%.Among the pathogens, CoV-HKU1 exhibited the highestco-infectionrateat60%(threeoutoffive),followedbyPIV4at55.6%(fiveoutof nine),CoV-229Eat40%(sixteenoutofforty),andCoV-NL63at38.2%(twenty-sixoutof sixty-eight). Conversely,FluBdisplayedthelowestco-infectionrateat8.8%,followedby FluA at 13.1% and PIV3 at 13.6%.Regarding double infections by pathogen type, AdV and HRV/EV, as well as HRV/EV and RSV double infections, accounted for the highest proportion at 8.3% (14 out of 169).Co-infections involving AdV, HRV/EV, and PIV3 represented the largest proportion of triple co-infections at 17.6% (three out of seventeen). Additionally, one case of quadruple infection was noted, involving AdV, CoV-NL63, CoV- OC43, and HRV/EV (Table 3).

Table3. Distributionofco-infectionsofrespiratorypathogens.

SingleInfection		**DoubleInfection**			**TripleInfection**		
AdV	236(17.5)	AdV	CoV-229E	2(1.2)	AdV&CoV-NL63	CoV-229E	1(5.9)
CoV-229E	24(1.8)		CoV-OL63	4(2.4)		CoV-OC43	1(5.9)
CoV-HKU1	2(0.1)		CoV-OC43	11(6.5)		PIV3	1(5.9)
CoV-NL63	42(3.1)		hMPV	13(7.7)		HRV/EV	1(5.9)
CoV-OC43	70(5.2)		HRV/EV	14(8.3)	AdV&HRV/EV	PIV1	1(5.9)
SARS-CoV-2	22(1.6)		FluA	10(5.9)		RSV	2(11.8)
HRV/EV	141(10.4)		FluB	2(1.2)		CoV-OC43	1(5.9)
RV/EV	124(9.2)		PIV1	2(1.2)		PIV3	3(17.6)

Table3.*Cont.*

SingleInfection		DoubleInfection			TripleInfection		
FluA	251(18.6)		PIV2	1(0.6)	AdV&CoV-OC43	CoV-HKU1	1(5.9)
FluB	92(6.8)		PIV3	13(7.7)		PIV3	1(5.9)
PIV1	62(4.6)		PIV4	1(0.6)	AdV&RSV	SARS-CoV-2	1(5.9)
PIV2	25(1.9)		RSV	5(3.0)	CoV-NL63&PIV1	PIV3	1(5.9)
PIV3	185(13.7)	CoV-229E	CoV-OL63	2(1.2)	CoV-NL63&HRV/EV	RSV	1(5.9)
PIV4	4(0.3)		FluA	8(4.7)	CoV-OC43&hMPV	FluA	1(5.9)
RSV	71(5.3)		FluB	3(1.8)		total	17(100)
Total	1351(100)	CoV-HKU1	hMPV	1(0.6)			
			FluA	1(0.6)			
		CoV-OL63	CoV-OC43	2(1.2)			
			hMPV	2(1.2)			
			HRV/EV	1(0.6)			
			FluA	4(2.4)			
			FluB	1(0.6)			
			PIV1	1(0.6)			
			PIV3	2(1.2)			
		C.OC43	HRV/EV	1(0.6)			
			FluA	4(2.4)			
			FluB	3(1.8)			
			PIV3	3(1.8)			
			RSV	1(0.6)			
		SARS	hMPV	1(0.6)			
			HRV/EV	2(1.2)			
			FluA	1(0.6)			
			RSV	1(0.6)			
		MV	HRV/EV	4(2.4)			
			FluB	1(0.6)			
			PIV1	2(1.2)			
			PIV2	1(0.6)			
			PIV3	3(1.8)			
		HRV/EV	FluA	1(0.6)			
			PIV1	5(3.0)			
			PIV2	1(0.6)			
			PIV3	4(2.4)			
			RSV	14(8.3)			
		FluA	FluB	5(3.0)			
			PIV1	1(0.6)			
			PIV2	1(0.6)			
			PIV4	1(0.6)			
		PIV1	PIV3	1(0.6)			
			RSV	1(0.6)			
			Total	169(100)			

AdV, adenovirus; CoV-229E, coronavirus 229E; CoV-HKU1, coronavirus HKU1; CoV-NL63, coronavirus NL63;CoV-OC43, coronavirus OC43; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; hMPV, humanmetapneumovirus; HRV/EV, human rhinovirus/enterovirus; FluA, influenza virus A; FluB, influenza virus B;PIV1, parainfluenza virus 1; PIV2, parainfluenza virus 2; PIV3, parainfluenza virus 3; PIV4, parainfluenza virus 4;RSV, respiratory syncytial virus.

4. Discussion

Acuterespiratoryinfectionisaprevalentdisease,accountingforapproximately20–40% of outpatient cases and 12–35% of inpatient cases in general hospitals. Among these, upper respiratoryinfections,includingnasopharyngitis,pharyngitis,andtonsillitis,accountfor 87.5%ofallrespiratoryinfections[12]. Bothadultsandchildrenshowahigherinfection rate with viral respiratory pathogens than with bacterial respiratory pathogens; however, childrenaccountformorethan80%ofallrespiratoryinfectioncases[13,14].Consistent with previous findings, all 1744 (100%) of the FilmArray™ RP-positive pathogens detected in this study were viruses.With respect to the distribution of the pathogens, we found

that AdV had the highest positivity rate (18.9%), followed by FluA (16.5%) and PIV3 (12.3%). Previous studies focusing on viral upper respiratory infections have reported the highest positivity rates for HRV/EV and RSV and the lowest positivity rate for FluA [15,16]. However, these results may vary depending on regional and climatic factors, necessitating further research.

In our analysis, we observed 187 instances of co-infection, leading to the detection of 393 pathogens. Among these cases, one hundred and sixty-nine involved double infections, seventeeninvolvedtripleinfections,andoneinvolvedaquadrupleinfection,reflecting anoverallco-infectionrateof22.5%.Comparableco-infectionrateshavebeenreported inpreviousstudiesutilizingtheBioFireFilmArray™RVandQIAstat-DxRespiratory Panels, which also documented rates of approximately 20%, mirroring the findings of our investigation [17,18]. However, this rate notably exceeded that reported in a prior study using the FilmArray™ Meningitis/Encephalitis panel [19]. Nevertheless, it remained lower thantheratereportedinanotherstudyemployingtheFilmArray™GIpanel[20].

Incasesofco-infection,AdV(*n*=93)emergedasthemostfrequentlyobservedpathogen, followed by HRV/EV (*n*= 57) and FluA (*n*= 38).Particularly noteworthy were the high co-infection rates of CoV-HKU1 and PIV4, which stood at 60% and 55.6%, respectively, indicatingapropensityforco-infectionwithotherpathogens.Comparatively,inprevious studies on respiratory viral infections, HRV/EV surfaced as the most common infectious pathogen,trailedbyFluBandAdV[21].Consistentwithourfindings,anotherearlierstudy also reported multiple co-infecting pathogens, primarily HRV/EV, followed by RSV and AdV.Furthermore,elevatedco-infectionrateswerenotedforCoV-HKU1andPIV4[22].

In the above study, seventeen samples exhibited triple infections, while one sample exhibited quadruple infections, with none exceeding quadruple infections. Samples with tripleinfectionsorhigheraccountedfor1.0%ofthetotalpositivesamples.Inapriorstudyon arelatedtopic,tripleorhigherduplicateinfectionswereobservedatarateofapproximately 1.3%, which was comparable to the ~~triple~~ triple-double infection rate in this study [23].Amongthe eighteen samples with triple infections or higher, AdV was detected in fifteen cases (83.3%), HRV/EVs in ten cases (55.6%), Cov-NL63 in seven cases (38.9%), CoV-OC43 in six cases (33.3%), and PIV-3 in six cases (33.3%), in that order. Notably, one out of five positive samples (20.0%) for Cov-HKU1 and seven out of sixty-eight positive samples (10.3%) for CoV-NL63 exhibited triple or more duplicate infections.Furthermore, SARS-CoV-2 was detectedinsamplesdisplayingduplicateinfections,suchasAdVandRSV.

The presence of multiple respiratory Infections carries diverse clinical implications, including prolonged pathogenic infections, extended hospitalization durations, and heightened severity of respiratory-related illnesses [24,25].However, conflicting findings have also been reported, suggesting that respiratory co-infections may not significantly influence theprevalenceorsevereityofthedisease[26,27]. Consequently,thecorrelationbetweenmultiple respiratory pathogen infections and the prevalence and severity of the disease remains unclear, underscoring the necessity for comprehensive investigations into co-infections involving various respiratory pathogens.

In this study, a substantial portion of positive pathogens (1121 cases, constituting 64.3%) were detected in the 1–5 age group, underscoring notably high positivity rates among younger patients. Specifically, within the 1–5 age group, pathogens such as AdV, PIV3, RSV, and HRV/EV displayed positivity rates of 79.3%, 79.4%, 96.9%, and 81.8%, respectively,indicativeofrobustdetectionratesinthisdemographic. Conversely,among older adults (>49 years), FluA and FluB exhibited positivity rates of 58.1% and 53.3%, respectively, suggesting heightened detection rates in this age group. Our findings align with those of previous studies highlighting elevated positivity rates among young patients (aged < 5 years) and robust detection rates for AdV, PIV3, RSV, and HRV/EV [17,28]. However,incontrasttoourobservations,thesestudiesnotedlowdetectionratesforFluA and FluB among older patients but relatively elevated rates among younger individuals.

Inthisstudy,769pathogensweredetectedbetweenDecemberandFebruary,corresponding toadetectionrateof44.1%. Bycontrast,only245pathogensweredetectedbetweenJune

and August, with a detection rate of 14.0%.In the summer of June-August, 230 samples (16.0%)outofatotalof1443sampleswerepositiveofwhichAdVwasthemostcommon, with78(33.9%).ThiswasfollowedbyPIV3with69(30.0%)andHRV/EVwith41(17.8%).In thewinterofDecember–February,657samples(34.5%)outofatotalof1906sampleswere positive of which FluA was the most common, with 252 (38.4%).After that, 90 AdV (13.7%) and76FluB(11.6%)werediferentfromthesummer.Thesefindingsindicatedahigher detectionrateduringwinterthanduringsummer.Similarly,previousstudiesreportedhigher respiratory pathogen detection rates in summer than in winter [29,30].Research on the age and timing of respiratory pathogen infections can provide fundamental data for implementing-
ingvarioushealthpolicies.Inparticular,suchdatacanhelptodeterminetheappropriate ageandtimingofvaccinationsaswellasaidintheunderstandingoftheepidemiologyof respiratory viruses.Therefore, continuous research and monitoring are necessary to utilize thisinformationasabasisforpublichealthpolicies.

This present study had several limitations. Firstly, as it was conducted using samples solely obtained from a single university hospital in Cheonan rather than from a range of institutions, the consideration of climatic and regional characteristics may be constrained. Additionally, since the majority of patients visiting the hospital for examinations are residents of the local community, there might be limitations in generalizing the findings to reflectuniversaltrendsinrespiratoryinfections.Inaddition,clinicalinformationrelated todiseaseseverity,symptoms,anddiagnosiswasnotincluded,makingitchallenging to assess treatment outcomes and prognosis.Among the 16 viruses and four bacterial species that could be detected using the FilmArray™ RP, MERS-CoV was not detected.Despite these limitations, in this study, we analyzed 6367 samples according to pathogens, age groups, ~~time~~ periods, and co-infections, allowing us to understand the patterns of respiratory pathogen infections. In particular, analyzing respiratory infections according to age and time can provide valuable clinical data for understanding epidemiological patterns and establishing vaccination strategies.Moreover, the analysis of co-infection rates by specific pathogens can help in the identification of common co-infection pathogens, which canaidintreatmentplanningandtheadministrationofmedications.

Traditionally, microbial culture methods serve as the foundational tests for identifying pathogens responsible for infectious diseases.It is recognized that bacterial and viral culture tests demand specialized facilities and highly trained personnel, more so than molecularbiologicaltests. Bacterialculturetypicallyspansfrom24hourstto5days[31]. The confirmationprocessforthecultureofrespiratoryviralpathogens,whichistheprimary focusofthisstudy,canextendoverseveraldaystoseveralweeks[32].Incontrast,theFil-
mArray™ RP offers a rapid and efficient alternative, detecting respiratory pathogens within2 h. This swift turnaround time facilitates timely decision-making regarding treatment. The expeditedresultsprovidedbytheFilmArray™RPallowforashiftfromempiricalantibi-
otics, such as amoxicillin, clavulanic acid, macrolides, and doxycycline, to more targeted antibiotic prescriptions.This transition enables more precise and effective administrationof antibiotics [33].Furthermore, the ability to test for 20 pathogens in a single sample streamlinesthegenerationandutilizationofregional,age-specific,andseasonaldatafor respiratory pathogen surveillance, vaccination planning, and identification of high-risk populations across different communities. The significance of rapid and accurate diagnostic testshasgainedincreasingrecognition,promptingtheadoptionofvariousmultiplexPCR diagnostic methods, including the FilmArray™ RP [34]. Epidemiological research on upper respiratorytractinfectionsnecessitatestheutilizationofdiversemultiplexPCRmethods tocomprehensivelyunderstandthedynamicsofpathogenspreadandinfectionpatterns. Thisunderscoresthepivotalroleofadvanceddiagnostictechnologiesinshapingeffective public health strategies and interventions.

InstitutionalReviewBoardStatement: Thestudyprotocolwasapprovedbythe InstitutionalReviewBoard of Dankook University (IRB File No. 2023–01–013) on 14 February 2023.

InformedConsentStatement: Thisstudywasaretrospectivedata-
onlystudy;therefore,theneedforobtaininginformedconsentfromthepatientswaswaivedbytheDankookUni versityIRB.

DataAvailabilityStatement: The datasets used and analyzed during this current study are availablefrom the corresponding author upon reasonable request.

References

1. Fendrick, A.M.; Monto, A.S.; Nightengale, B.; Sarnes, M. The economic burden of non–influenza-related viral respiratory tract infectionintheUnitedStates. *Arch.Intern.Med.***2003**,*163*,487–494. [CrossRef]
2. Pillet,S.;Lardeux,M.;Dina,J.;Grattard,F.;Verhoeven,P.;LeGoff,J.;Vabret,A.;Pozzetto,B.Comparativeevaluationofsix commercializedmultiplexPCRkitsforthediagnosisofrespiratoryinfections.*PLoSONE***2013**,*8*,e72174.[CrossRef][PubMed]
3. Pitrez,P.M.;Pitrez,J.L.Acuteupperrespiratorytractinfections:Outpatientdiagnosisandtreatment.*J.Pediatr.***2003**,*79*,S77–S86. [CrossRef]
4. Ruopp, M.; Chiswell, K.; Thaden, J.T.; Merchant, K.; Tsalik, E.L. Respiratory tract infection clinical trials from 2007 to 2012. A systematicreviewofClinicalTrials.gov. *Ann.Am.Thorac.Soc.***2015**,*12*,1852–1863. [CrossRef][PubMed]
5. Kuchar,E.;Mis´kiewicz,K.;Nitsch-Osuch,A.;Szenborn,L.Pathophysiologyofclinicalsymptomsinacuteviralrespiratorytract infections. *Adv. Exp. Med. Biol.* **2015**,*857*,25–38.
6. Hersh,A.L.;Shapiro,D.J.;Pavia,A.T.;Shah,S.S.AntibioticprescribinginambulatorypediatricsintheUnitedStates.*Pediatrics* **2011**,*128*,1053–1061.[CrossRef][PubMed]
7. Nyquist,A.-C.;Gonzales,R.;Steiner,J.F.;Sande,M.A. Antibiotic prescribing for children with colds,upper respiratory tract infections,andbronchitis. *JAMA***1998**,*279*,875–877. [CrossRef][PubMed]
8. CentersforDiseaseControlandPrevention. Evaluationof11commerciallyavailablerapidinfluenzadiagnostictests—United States, 2011–2012. *MMWR Morb. Mortal. Wkly. Rep.* **2012**, *61*, 873–876.
9. Salez,N.;Nougairede,A.;Ninove,L.;Zandotti,C.;DeLamballerie,X.;Charrel,R.N.XpertFluforpoint-of-carediagnosisof humaninfluenzainindustrializedcountries. *ExpertRev.Mol.Diagn.***2014**,*14*,411–418. [CrossRef][PubMed]
10. Hammond, S.P.; Gagne, L.S.; Stock, S.R.; Marty, F.M.; Gelman, R.S.; Marasco, W.A.; Poritz, M.A.; Baden, L.R. Respiratory virus detection in immunocompromised patients with FilmArray Respiratory Panel compared to conventional methods.*J. Clin. Microbiol.***2012**,*50*,3216–3221. [CrossRef][PubMed]
11. Kaku,N.;Hashiguchi,K.;Iwanaga,Y.;Akamatsu,N.;Matsuda,J.;Kosai,K.;Uno,N.;Morinaga,Y.;Kitazaki,T.;Hasegawa,H.; etal. EvaluationofFilmArrayRespiratoryPanelmultiplexpolymerasechainreactionassayfordetectionofpathogensinadult outpatientswithacuterespiratorytractinfection.*J.Infect.Chemother.***2018**,*24*,734–738.[CrossRef][PubMed]
12. Jain,N.;Lodha,R.;Kabra,S.K.Upperrespiratorytractinfections.*IndianJ.Pediatr.***2001**,*68*,1135–1138.[CrossRef][PubMed]
13. Ren, L.; Gonzalez, R.; Wang, Z.; Xiang, Z.; Wang, Y.; Zhou, H.; Li, J.; Xiao, Y.; Yang, Q.; Zhang, J.; et al. Prevalence of human respiratory viruses in adults with acute respiratory tract infections in Beijing, 2005–2007.*Clin. Microbiol. Infect.* **2009**, *15*, 1146–1153.[CrossRef] [PubMed]
14. Zhang,G.;Hu,Y.;Wang,H.;Zhang,L.;Bao,Y.;Zhou,X.Highincidenceofmultipleviralinfectionsidentifiedinupperrespiratory tractinfectedchildrenunderthreeyearsofageinShanghai,China. *PLoSONE***2012**,*7*,e44568. [CrossRef][PubMed]
15. Leber,A.L.;Everhart,K.;Daly,J.A.;Hopper,A.;Harrington,A.;Schreckenberger,P.;McKinley,K.;Jones,M.;Holmberg, K.; Kensinger, B. Multicenter evaluation of BioFireFilmArray Respiratory Panel 2 for detection of viruses and bacteria in nasopharyngealswabsamples. *J.Clin.Microbiol.***2018**,*56*,e01945-17. [CrossRef]
16. Li, J.; Tao, Y.; Tang, M.; Du, B.; Xia, Y.; Mo, X.; Cao, Q. Rapid detection of respiratory organisms with the FilmArray Respiratory Panel in a large Children's Hospital in China. *BMCInfect. Dis.* **2018**, *18*, 510. [CrossRef] [PubMed]
17. Leber,A.L.;Lisby,J.G.;Hansen,G.;Relich,R.F.;Schneider,U.V.;Granato,P.;Young,S.;Pareja,J.;Hannet,I.Multicenterevaluation oftheQIAstat-DxRespiratoryPanelfordetectionofvirusesandbacteriainnasopharyngealswabspecimens.*J.Clin.Microbiol.* **2020**, *58*, e00155-20. [CrossRef] [PubMed]

18. Loeffelholz, M.J.; Pong, D.L.; Pyles, R.B.; Xiong, Y.; Miller, A.L.; Bufton, K.K.; Chonmaitree, T. Comparison of the FilmArrayRespiratoryPanelandProdessereal-timePCRassaysfordetectionofrespiratorypathogens. *J.Clin. Microbiol.* **2011**,*49*,4083–4088.[CrossRef] [PubMed]
19. Leber, A.L.; Everhart, K.; Balada-Llasat, J.M.; Cullison, J.; Daly, J.; Holt, S.; Lephart, P.; Salimnia, H.; Schreckenberger, P.C.; DesJarlais,S.;etal.MulticenterevaluationofBioFireFilmArraymeningitis/encephalitispanelfordetectionofbacteria,viruses, andyeastincerebrospinalfluidspecimens. *J.Clin.Microbiol.***2016**,*54*,2251–2261. [CrossRef][PubMed]
20. Piralla, A.; Lunghi, G.; Ardissino, G.; Girello, A.; Premoli, M.; Bava, E.; Arghittu, M.; Colombo, M.R.; Cognetto, A.; Bono, P.; et al. FilmArray™ GI panel performance for the diagnosis of acute gastroenteritis or hemorragic diarrhea. *BMC Microbiol.* **2017**, *17*, 111.[CrossRef] [PubMed]
21. Busson, L.; Bartiaux, M.; Brahim, S.; Konopnicki, D.; Dauby, N.; Gérard, M.; De Backer, P.; Van Vaerenbergh, K.; Mahadeb, B.; Mekkaoui, L.; et al.Contribution of the FilmArray Respiratory Panel in the management of adult and pediatric patients attendingtheemergencyroomduring2015–2016influenzaepidemics: Aninterventionalstudy. *Int. J.Infect. Dis.* **2019**,*83*,32–39. [CrossRef][PubMed]
22. Parcˇina, M.; Schneider, U.V.; Visseaux, B.; Jozic´, R.; Hannet, I.; Lisby, J.G. Multicenter evaluation of the QIAstat Respiratory Panel—Anewrapidhighlymultiplexed PCRbasedassayfordiagnosisofacuterespiratorytractinfections.*PLoSONE***2020**,*15*, e0230183. [CrossRef]
23. Sanghavi,S.K.;Bullotta,A.;Husain,S.;Rinaldo,C.R.Clinicalevaluationofmultiplexreal-timePCRpanelsforrapiddetectionof respiratoryviralinfections. *J.Med.Virol.***2012**,*84*,162–169. [CrossRef][PubMed]
24. Chauhan, J.C.; Slamon, N.B. The impact of multiple viral respiratory infections on outcomes for critically ill children. *Pediatr. Crit. Care Med.* **2017**, *18*, e333–e338. [CrossRef] [PubMed]
25. Richard,N.;Komurian-Pradel,F.;Javouhey,E.;Perret,M.;Rajoharison,A.;Bagnaud,A.;Billaud,G.;Vernet,G.;Lina,B.;Floret,D.;et al. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis.*Pediatr. Infect. Dis. J.* **2008**, *27*, 213–217. [CrossRef]
26. Scotta, M.C.; Chakr, V.C.; de Moura, A.; Becker, R.G.; de Souza, A.P.D.; Jones, M.H.; Pinto, L.A.; Sarria, E.E.; Pitrez, P.M.; Stein,R.T.;etal. Respiratoryviralcoinfectionanddiseaseseverityinchildren: Asystematicreviewandmeta-analysis. *J.Clin. Virol.* **2016**, *80*, 45–56. [CrossRef] [PubMed]
27. Wishaupt, J.O.; vander Ploeg, T.; de Groot,R.; Versteegh, F.G.; Hartwig, N.G. Single-and multiple viral respiratoryinfections in children:Diseaseandmanagementcannotberelatedtoaspecificpathogen.*BMCInfect.Dis.***2017**,*17*,62.[CrossRef][PubMed]
28. Yang, S.; Li, H.; Tang, Y.; Yu, F.; Ma, C.; Zhang, H.; Pang, L.; Zhao, H.; Wang, L. Multiplex tests for respiratory tract infections:Thedirect utility of the FilmArray Respiratory Panel in emergency department.*Can.Respir.J.* **2020**, *2020*, 6014563.[CrossRef]
29. Lamrani Hanchi, A.; Guennouni, M.; Rachidi, M.; Benhoumich, T.; Bennani, H.; Bourrous, M.; Maoulainine, F.M.R.; Younous, S.; Bouskraoui,M.;Soraa,N.Epidemiologyofrespiratorypathogensinchildrenwithsevereacuterespiratoryinfectionandimpact ofthemultiplexPCRFilmArrayRespiratoryPanel: A2-yearstudy. *Int.J.Microbiol.***2021**,*2021*,2276261. [CrossRef][PubMed]
30. Visseaux,B.;Burdet,C.;Voiriot,G.;Lescure,F.X.;Chougar,T.;Brugière,O.;Crestani,B.;Casalino,E.;Charpentier,C.;Descamps, D.; et al. Prevalence of respiratory viruses among adults, by season, age, respiratory tract region and type of medical unit in Paris,France,from2011to2016.*PLoSONE***2017**,*12*,e0180888.[CrossRef][PubMed]
31. Lagier,J.C.;Edouard,S.;Pagnier,I.;Mediannikov,O.;Drancourt,M.;Raoult,D.CurrentandPastStrategiesforBacterialCulture inClinicalMicrobiology. *Clin. Microbiol. Rev.* **2015**,*28*,208–236. [CrossRef][PubMed]
32. Talbot,H.K.;Falsey,A.R.Thediagnosisofviralrespiratorydiseaseinolderadults.*Clin.Infect.Dis.***2010**,*50*,747–751.[CrossRef] [PubMed]
33. Panasiuk,L.;Lukas,W.;Paprzycki,P.;Verheij,T.;Godycki-C´wirko,M.;Chlabicz,S.Antibioticsinthetreatmentofupper respiratorytractinfectionsinPoland.Isthereanyimprovement?*J.Clin.Pharm.Ther.***2010**,*35*,665–669.[CrossRef][PubMed]
34. Chan, M.; Koo, S.H.; Jiang, B.; Lim, P.Q.; Tan, T.Y. Comparison of the BioFireFilmArray Respiratory Panel, SeegeneAnyplexII RV16,andArgeneforthedetectionofrespiratoryviruses. *J.Clin. Virol.* **2018**,*106*,13–17. [CrossRef][PubMed]