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Molecular Characterization of Respiratory Viral Infections and Co-Infections in Pediatric Patients Using FilmArray™ Multiplex PCR in a Tertiary-Care Hospital, South India

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Abstract

Respiratory tract infections (RTIs) are a major cause of pediatric morbidity worldwide, with viruses, such as respiratory syncytial virus, rhinovirus/enterovirus, influenza virus, adenovirus, and parainfluenza virus, contribute significantly to hospitalizations. Conventional diagnostic methods often require prolonged processing times, which can delay clinical decision-making. Rapid molecular platforms, such as the FilmArray™ Panels, offer timely and comprehensive pathogen identification. This study aimed to evaluate the prevalence, seasonal trends, and co-infection patterns of respiratory pathogens among hospitalized children in South India using FilmArray™ Respiratory Panel. This study analyzed data from children under 10 years of age admitted with RTIs in a single pediatric-care hospital between August 2023 and March 2025. All the patients underwent testing with the FilmArray™ Respiratory Panel using nasopharyngeal swabs in Viral transport medium. Demographic characteristics, pathogen distribution, and Inflammatory markers – such as C-reactive protein (CRP) and neutrophil counts – were reviewed. Among the 248 children studied, rhinovirus/Enterovirus (26.63%), respiratory syncytial virus (14%), and adenovirus (13.55%) were the most frequently detected respiratory pathogens, followed by Mycoplasma pneumoniae (11.2%) and other viral agents included in the panel. A notable co-infection rate (28.6%) was identified, indicating concurrent circulation of multiple viruses. A subset of children showed elevated CRP levels and neutrophilia, suggesting a stronger inflammatory response. This study highlights the significant burden of respiratory syncytial virus, Rhinovirus/Enterovirus, and other viral RTIs among pediatric children in South India. Molecular Characterization using Film Array Respiratory Panel demonstrated high utility in rapid and accurate pathogen identification, enabling improved clinical decision-making and strengthening antibiotic stewardship decisions, thereby improving patient outcomes.

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INTRODUCTION

Respiratory viral infections are among the most common illnesses affecting newborns and children, largely due to their developing immune systems and frequent exposure to peers in households, schools, and daycare settings [1–3]. A wide range of viruses are seen, including respiratory syncytial virus, Influenza viruses, rhinoviruses, parainfluenza viruses, adenoviruses, and, more

recently, coronaviruses such as SARS-CoV-2 [2–5]. Among these, the bacterium Mycoplasma pneumoniae is particularly notable, accounting for 10–40% of community-acquired pneumonia cases that require hospitalization in children [6]. These pathogens primarily target the upper and lower respiratory tracts, producing symptoms that may vary from mild manifestations – such as cough, fever, nasal congestion, and rhinorrhea – to more severe conditions, including bronchiolitis, pneumonia, wheezing, and respiratory distress [1, 2]. Infants, young children, and those with underlying chronic illnesses or compromised immunity are particularly susceptible to severe diseases [1, 2]. Transmission commonly occurs through respiratory droplets, close contact with infected individuals, or contact with contaminated surfaces, facilitating rapid spread within group environments.

Several molecular diagnostic modalities are available for identifying upper respiratory pathogens, each with distinct advantages and constraints [7]. Rapid antigen tests provide quick results but suffer from relatively low sensitivity, limiting their reliability in clinical decision-making [8]. In contrast, traditional methods – such as conventional viral culture – offer higher sensitivity but require prolonged processing times, which may delay timely treatment interventions. Multiplex polymerase chain reaction (PCR) assays have become increasingly valuable tools in hospital settings, enabling the rapid and accurate detection of multiple respiratory pathogens simultaneously [7–9]. Among these, FilmArray™ Respiratory Panel (RP) is particularly notable. This FDA-approved platform can identify more than 20 respiratory pathogens (16 viruses +4 bacteria) within approximately one hour, offering both diagnosis and speed of detection [9, 10]. Despite its strong diagnostic capabilities, there remains a scarcity of studies evaluating the FilmArray™ RP specifically for its utility in detecting respiratory pathogens within diverse clinical settings [9–11].

India is a country with great geographical and climatic differences. The seasonality of respiratory infections in India differs markedly from that of temperate regions [12, 13]. Despite this, only a limited number of studies have examined the prevalence of specific respiratory viruses and their seasonal patterns across the country. Post the covid waves, how the pandemic influenced respiratory infection patterns in children is very little explored and credible data on respiratory infections from the southern region remains limited and inadequately explored. There are very few studies in northern and southern India that showed the trend of respiratory infections among children. The present study provides an insight into the pathogens associated with RTI among children in the city of Bengaluru, Karnataka. This allows for a better understanding of planning and response to outbreaks of respiratory infections, such as influenza, at the state and regional level. Our study improves understanding of disease trends and supports the development of proactive measures to prevent future outbreaks.

In this study, we employed the FilmArray™ RP to epidemiologically characterize upper RTIs in children. This study aimed to evaluate the diagnostic performance and clinical application of the FilmArray Respiratory Panel (RP) for identifying respiratory pathogens, and to characterize the seasonal distribution and prevalence of these organisms among pediatric patients in a major children's Aster hospital in Bengaluru, South India.

MATERIALS AND METHODS

Study Population and Sample Collection

This study was conducted in children's belonging to the age group of 0 months to 10 years for the period of August 2023–March 2025. Nasopharyngeal swab (NPS) was collected in viral transport medium under sterile conditions with proper personal protective equipment (PPE), for infection testing at Aster Hospital. Clinically suspected cases having symptoms admitted in hospital as well as OPD (outpatient department) were included in this study. Relevant demographic and clinical details (e.g., age, sex, seasonality, blood profile) were reviewed.

FilmArray RP

The FilmArray™ Respiratory Panel (RP) (BioFire® Diagnostics, Salt Lake City, UT, USA) is a fully automated multiplex PCR assay designed to detect a broad spectrum of respiratory pathogens, including

four bacteria – *Bordetella parapertussis (BoPP)*, *Bordetella pertussis(BoP)*, *Chlamydia pneumoniae (Chy)*, and *Mycoplasma pneumoniae(Myco)* – and 19 viruses such as adenovirus(AdV), human metapneumovirus (hMPV), human rhinovirus/enterovirus (HRV/EV), respiratory syncytial virus (RSV), parainfluenza viruses 1–4, influenza A and B with their subtypes (FluA H1, FluA H1-2009, FluA H3), common human coronaviruses (CoV-229E, CoV-HKU1, CoV-NL63, CoV-OC43), and SARS-CoV-2**.** All testing was performed under strict biosafety measures wearing appropriate PPE. The Film array pouch system contains all dried reagents needed for automated nucleic acid extraction, PCR amplification, and detection. The RNA Process Control detects transcription of an RNA target from *Schizosaccharomyces pombe*, confirming proper execution of all assay steps, whereas the DNA Control verifies successful amplification in the second PCR stage. A valid test required both controls to be positive; if either failed, the assay was repeated using a new pouch. Results for each target were qualitatively reported as "detected" or "not detected" based on endpoint melting-curve analysis. The complete diagnostic process requires approximately one hour per sample Among the 20 pathogens detectable using the Film Array™ RP, all were checked in this study, including 4 bacteria

Statistical Analysis

Positive percentage agreement and negative percentage agreement were assessed for Film Array RP assay with respect to Microsoft excel.

Ethics Statement

This article does not contain any experimental studies on human participants or animals performed by any of the authors. It was a retrospective study and only case records of patients were accessed, and the identity or privacy of the patients were not revealed in the study.

RESULTS AND DISCUSSION

A total of 248 respiratory samples having RTIs underwent analysis in the department of molecular biology, Aster Hospital. The study was divided into 3 groups based on the age group which consisted of 135 males and 113 females (Table 1) revealing 214 (86.2%) positive and 34 negative cases (13.7%). Among these positive cases, 143 instances of single infection (57.6%), 61 double infections, and 10 triple infections were reported (Table 2). Among these 16 (7.47%) samples displayed bacterial and viral co-infection. The distribution of positive cases according to age, sex of the patients is shown in Table 3, male children had higher incidence of RTIs compared to female children. The prevalence of pathogens in this study was in the order of HRV/EV (26.6%) RSV (14.49%), AdV (13.5 %), Myco (11.2%) and followed by other viruses collectively constituted over half of the total pathogens detected (Figure 1).

Table 1. Demographic details of the patients recruited in the study.

Age Group in years	Total	Female	Male
0–1 years	86	38	48
1–2 years	32	15	17
2–10 years	130	60	70
		113 (45.56%)	135 (54.4%)

Note: N = 248.

Table 2. Positivity rates of FilmArray™ respiratory panel pathogens.

	Positivity	
Parameters	**No of samples**	**%Total**
N = 248 samples		
Negative	34	13.70967742
Positive samples	214	86.29032258
Single Infection	143	57.66129032
Co-infection	71	28.62903226
No Co-infections:71		
Double infections	61	84.72222222
Triple infections	10	13.88888889

Table 3. Demographic distribution of samples pertaining to age and sex.

Age group in years	Total	Positive %	Female positivity	Male positivity
0–1 year	86	61 (70.9%)	31 (52.3%)	30 (47.6%)
1–2 years	32	32 (100%)	12 (27.65%)	20 (42.5%)
2–10 years	130	120 (92.3%)	55 (37.16%)	65 (43.91%)

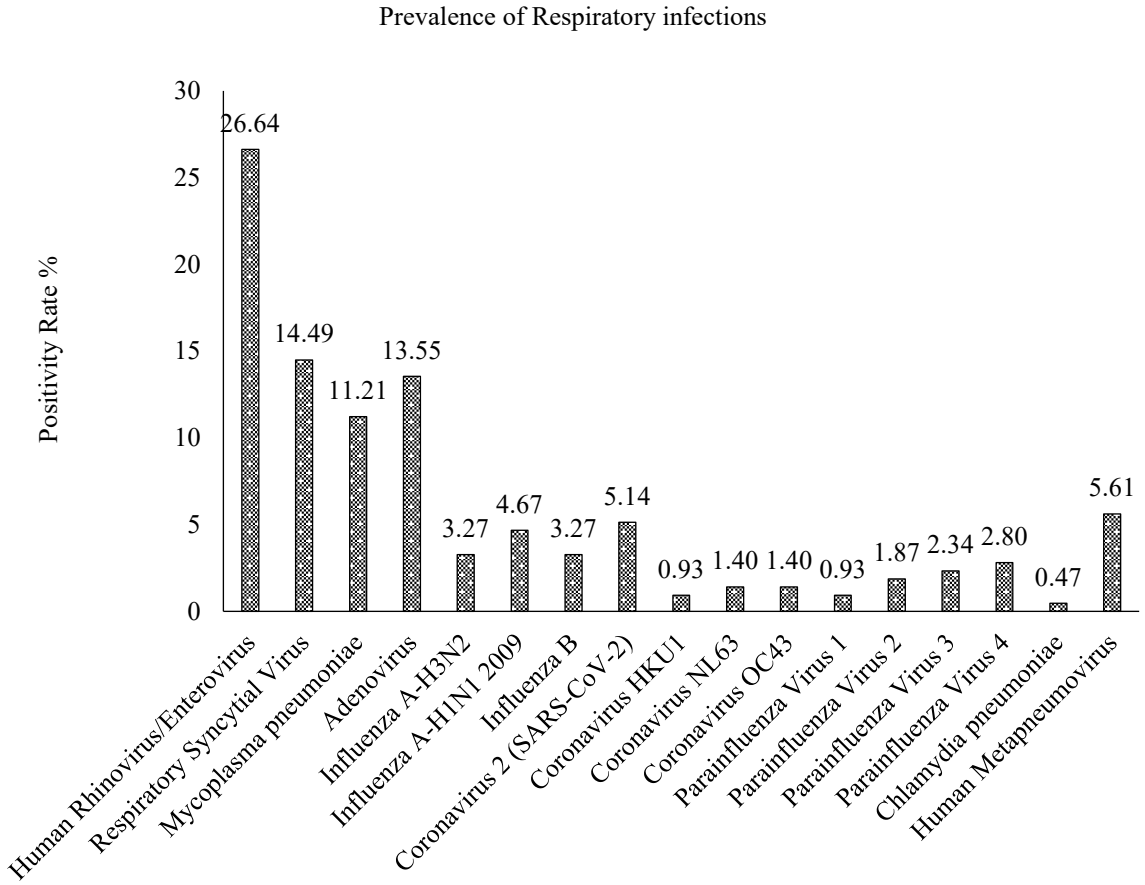

Figure 1. Prevalence of respiratory organisms detected by FilmArray™ RP assay.

Among the age groups, 2–10 group exhibited the highest distribution of pathogens, accounting for the total age group positivity rate. HRV/EV emerged as the most frequently detected pathogen in all age groups comprising of 0–1 group (22.09%), 1–2 group (43.75%), and 2–10 group (30%) signifying elevated positivity rates. Furthermore, within the 0–1 age group, RSV (23.2%) emerged as the most detected followed by hMPV, SARS-CoV-2, CoV-NL63, CoVOC43, HKU1, Chy, Myco, PIV1, PIV4 constituted the total pathogens identified, respectively (Figure 2). In Group (2–10) AdV (14.61%) and Myco (13.8%) showed elevated positive rates followed by other viruses. In the present study, SARS-CoV-2 was identified in 11 cases and showed to be equal among each age groups. Age-stratified analysis identified distinct patterns: (1–2 year) age group showed minimal infections, followed by (0–1) and (1–10) age group demonstrated predominance of HR/EV, AdV, RSV, and Myco infections. This variance in age group distribution underscores the differential susceptibility and clinical manifestation of respiratory pathogens vary significantly across age groups, with infants, young children, adolescents, adults, and the elderly each exhibiting distinct vulnerability patterns, symptom profiles, and disease severity. Figure 2 shows the distribution of each causative agent in relation to age.

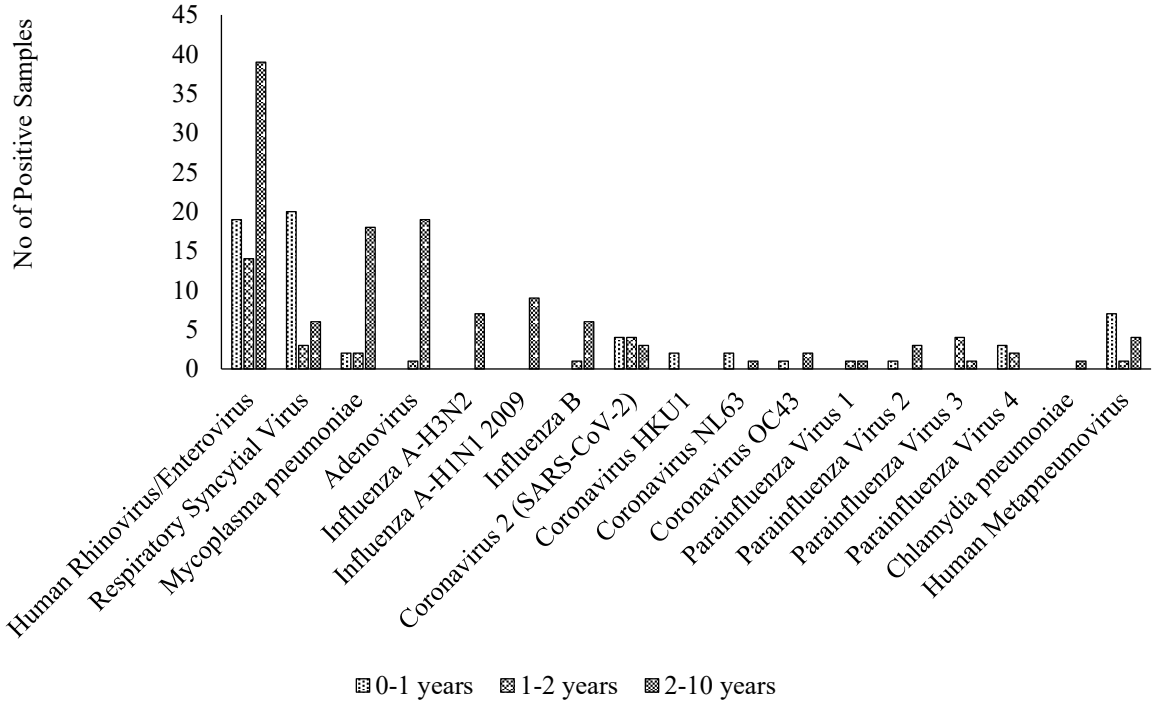

Figure 2. Prevalence of respiratory infections with different age groups.

The highest number of pathogens was identified during Monsoon (37.85 %) (Figure 3 and Table 4) followed by post monsoon/Winter (54.2%) and Summer (7.9%) in both years, representing of all detected pathogens. During Post-Monsoon/Winter HRV/EV, Adv, RSV, Myco exhibited high positive rates, respectively. Conversely, the positive rate of other viruses was notably lower overall in the year. Throughout the year, pathogens – such as SARS-COV2, hMPV, PIV1, PIV3, and HRV/EV – showed relatively even distribution patterns (Figure 3).

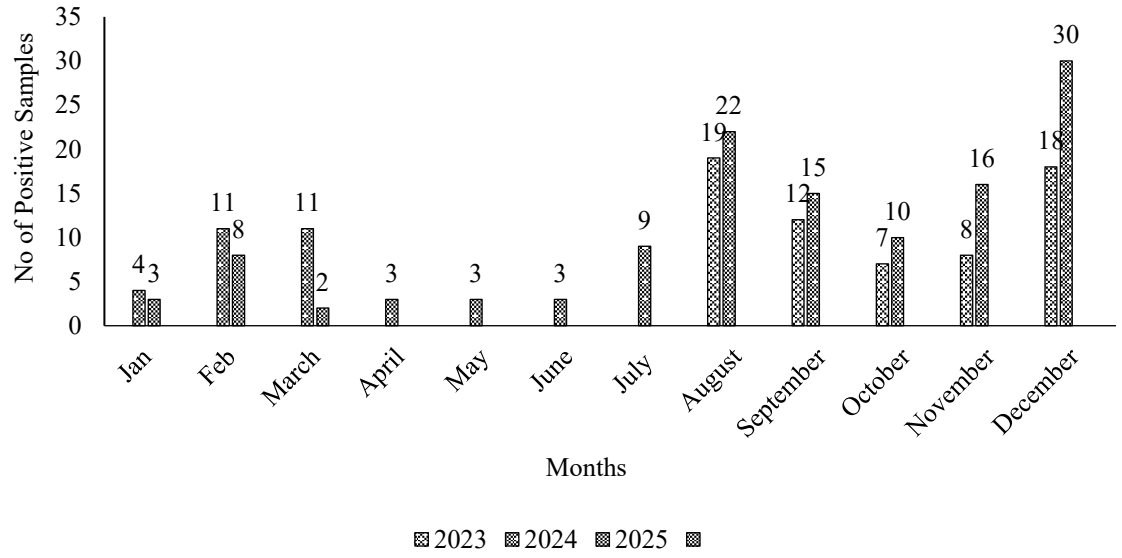

Figure 3. Monthly distribution of positive samples from Aug 2023 to March 2025.

Among the 248 samples total of 71 cases of co-infection were identified. In these 61 were found to be dual infections out of which HRV/EV/RSV exhibited the highest co-infection rate at 6.5%. Intriguingly, 10 patients had coinfections with three pathogens (4.6%). However, we also found 10 coinfections of viral and bacterial combination of Myco with other viruses such as HRV/EV, hMPV, PIV3, CoV-NL63, AdV, and Chy. These findings suggest that HRV's persistent year-round circulation may facilitate synergistic infections with seasonally variable pathogens. Tables 4 and 5 summarize the coinfected-positive samples.

Table 4. Distribution of positive samples based on seasonality.

Annual seasons in Bengaluru	No of positives (total – 214)
Summer (March–May)	17 (7.9%)
Monsoon (June–September)	81 (37.85%)
Post-Monsoon (October–November)	44 (20.56 %)
Winter (December–February)	72 (33.64%)

Table 5. Detection and co-infection rates for each pathogen.

Organisms detected	Total no of samples	Detection rate	Co-infection	Co-infection rate
Human Rhinovirus/Enterovirus	57	26.63551402	30	52.63157895
Respiratory Syncytial Virus	31	14.48598131	22	70.96774194
Mycoplasma pneumoniae	24	11.21495327	16	66.66666667
Adenovirus	29	13.55140187	20	68.96551724
Influenza A-H3N2	7	3.271028037	3	42.85714286
Influenza A-H1N1 2009	10	4.672897196	2	20
Influenza B	7	3.271028037	2	28.57142857
Coronavirus 2 (SARS-CoV-2)	11	5.140186916	5	45.45454545
Coronavirus HKU1	2	0.934579439	1	50
Coronavirus NL63	3	1.401869159	2	66.66666667
Coronavirus OC43	3	1.401869159	1	33.33333333
Parainfluenza Virus 1	2	0.934579439	2	100
Parainfluenza Virus 2	4	1.869158879	3	75
Parainfluenza Virus 3	5	2.336448598	5	100
Parainfluenza Virus 4	6	2.803738318	3	50
Chlamydia pneumoniae	1	0.46728972	1	100
Human Metapneumovirus	12	5.607476636	9	75
	214	100		

Elevated neutrophils, lymphocytes, and C-reactive protein (CRP) together suggest an active inflammatory or infectious process. So, we also investigated the blood profile of 214 infected children (Table 6). Among the 214 elevated serum CRP levels were observed across 21.75% cases. Out of 214 infections 29.62% had neutrophilia (total = 64), 10.18% with neutropenia (total = 22), 27.31% patients with lymphocytosis (total = 59) and 21.75% with lymphocytopenia (total = 18). Interpreting these markers together, along with clinical symptoms and other investigations, helps clinicians determine the type and severity of the underlying condition.

DISCUSSION

Acute respiratory infections are a prevalent disease and the most common reason for hospitalization in children, placing a significant burden on pediatric healthcare services [14, 15]. Upper respiratory infections – such as nasopharyngitis, pharyngitis, and tonsillitis – constitute nearly 90.5% of all respiratory cases. Although both adults and children are more frequently affected by viral rather than bacterial respiratory pathogens, particularly in those younger than 10 years of age, owing to their

immature immune systems and frequent exposure in daycare centers and schools [14–16]. However, these results may vary depending on a multitude of factors, such as age, sex, climatic and regional factors, necessitating further research. The diagnosis of viruses causing respiratory infections has always been a challenge due to limitations of conventional diagnostic methods, which often lack sensitivity, specificity, or timely results.

Table 5. Summary of coinfection-positive samples.

Single infection		Dual infection		Triple infection	
HRV/EV	42	HRV/EV/RSV	16	AdV/RSV/HRV/EV	2
RSV	31	HRV/EV/hMPV	5	SARS-CoV-2/RSV/HRV/EV	1
AdV	10	HRV/EV/PIV1	1	SARS-CoV-2/RSV/hMPV	1
Myco	18	AdV/HRV/EV	7	HRV/EV/PIV3/Myco	1
hMPV	5	SARS-CoV-2/RSV	2	AdV/Chy/Myco	1
FluA-H1N1-2009	10	HRV/EV/PIV4	4	HRV/EV/PIV2/RSV	1
SARS-CoV-2	6	RSV/hMPV	3	PIV1/PIV2/fluA-H1N1-2009	1
fluA-H3N2	7	HRV/EV/Myco	9	CoVOC43/Myco/Adv	1
FluB	6	RSV/FluB	1	SARS-CoV-2/PIV3/HRV/EV	1
CoV-NL63	2	RSV/PIV4	1		
HKU1	1	hMPV/myco	2		
CoVOC43	2	CoV-NL63/Myco	1		
PIV1	0	AdV/FluB	1		
PIV2	0	AdV/CoV-NL63	1		
PIV3	0	AdV/Chy	1		
PIV4	3	PIV3/Myco	1		
		AdV/fluA-H3N2	1		
		AdV/fluA-H1N1-2009	1		
		PIV2/RSV	1		
		AdV/PIV3	1		
		HKU1/HRV/EV	1		

Table 6. Distribution of CRP, Neutrophilia, Lymphocyte counts in respiratory infections.

	Neutrophils (n = 214)		Lymphocytes (n = 214)		HIGH CRP
	Neutrophila	***Neutropenia***	***Lymphocytosis***	***Lymphcoytenpenia***	
Total cases	64	22	59	18	47
%	29.90654206	10.28037383	27.57009346	8.411214953	21.96261682

In recognition of these shortcomings, numerous multiplexed diagnostic panels have been developed, validated, and implemented in diagnostic laboratories over the past few years, enabling simultaneous detection of multiple pathogens. These platforms offer the advantage of fully automated PCR-based testing with a short turnaround time, enabling the simultaneous detection of multiple common respiratory viral and bacterial agents. BioFire FilmArray Respiratory Panel (FRP) is a rapid molecular multiplex test designed for the qualitative detection of nucleic acid targets of viruses and bacteria in nasopharyngeal swab specimens.

In our study, we observed viruses are the main causative agents of ARI in children <10 years with suspected ARI (89%) of all reported cases. HRV/EV, RSV, AdV, HMPV, SARS-COV-2 viral being the most prevalent. In this study, a substantial portion of positive pathogens (120 cases, constituting 56.7%) were detected in the 2–10 age group, highlighting significantly higher positivity rates among younger patients. Our findings aligned with those of previous studies highlight elevated positivity rates among young patients (aged <10 years). Specifically, within the 0–1 age group, pathogens – such as

RSV, HRV/EV and hMPV (61 cases, consisting of 28.5%) – displayed high positivity rates followed by other viruses, indicative of robust detection rates in this demographic. Age-specific analysis demonstrated that children aged 2–10 years accounted for the highest proportion of positive cases (56.7%), whereas infants younger than one year had the highest prevalence of RSV, HRV/EV, and hMPV infections. Previous investigations have similarly reported increased detection rates of RSV and rhinovirus among infants because of immature immunity and greater susceptibility to respiratory viral infections [17–19]. In contrast, influenza A and influenza B were detected less frequently among infants, consistent with observations from several recent surveillance studies [20, 21]. A slight male predominance (53.7%) was observed in our study, which is consistent with previous reports describing a higher incidence and severity of respiratory tract infections among male children [15, 19]. The underlying reasons remain unclear but may involve sex-related differences in immune responses, hormonal influences, and behavioral factors.

Respiratory pathogen co-infections were identified in 71 patients, corresponding to an overall co-infection rate of 33.1%. Dual infections accounted for 61 cases, while triple infections were observed in 10 cases. HRV/EV was the pathogen most frequently involved in co-infections, and SARS-CoV-2 was detected together with adenovirus, HRV/EV, hMPV, PIV3, and RSV. Similar co-infection rates have been reported using multiplex PCR platforms including the BioFire FilmArray™ Respiratory Panel, with rates ranging between 20% and 35% [17, 18, 23]. Although several studies have associated respiratory co-infections with prolonged hospitalization and increased disease severity, other investigators have found no significant effect on clinical outcomes, suggesting that the clinical significance of viral co-infections remains controversial [18, 23].

Bengaluru, Karnataka in India has a pleasant, tropical savanna climate with 3 seasons. The summer lasting from March–May followed by the monsoon/Post Monsoon season from June to November. Winter spans from December to February, when the weather is mostly dry and cool. Respiratory infections in India typically peak in the monsoon season. Seasonal analysis demonstrated that respiratory infections occurred predominantly during the monsoon (June–November; 58.4%) and winter (December–February; 33.6%) seasons, whereas only 8.4% of infections were detected during summer. Similar seasonal patterns have been described in India, where RSV, influenza viruses, and rhinoviruses circulate predominantly during periods of increased rainfall and humidity [19, 25]. These observations support previous reports indicating that climatic factors play an important role in respiratory virus transmission. Laboratory investigations revealed elevated C-reactive protein (CRP) concentrations and neutrophilia in children infected with viral, bacteria, and mixed respiratory pathogens. Recent studies have demonstrated that inflammatory markers alone cannot reliably distinguish viral from bacterial respiratory infections and should, therefore, be interpreted in conjunction with multiplex PCR findings and clinical assessment [26].

The principal strength of this study is the comprehensive characterization of respiratory pathogens using the BioFire FilmArray™ Respiratory Panel in a tertiary pediatric hospital in South India during the post-COVID-19 period. The study provides valuable regional epidemiological data regarding pathogen prevalence, age distribution, co-infections, and seasonal trends, thereby contributing to the limited published literature from southern India. Nevertheless, this study has several limitations. First, it was conducted at a single tertiary care hospital, which may limit the generalizability of the findings. Second, the observational design precluded assessment of causality between pathogen detection and disease severity. Third, the relatively small sample size limited subgroup analyses. Finally, asymptomatic controls and community surveillance data were not included, preventing comparison between hospital-based and community-acquired respiratory infections The findings emphasize the utility of multiplex PCR as a sensitive and rapid diagnostic tool for pathogen detection in respiratory infections, facilitating timely and targeted therapeutic interventions. Moreover, the integration of molecular diagnostics with clinical and laboratory parameters enhances the understanding of pneumonia etiology, epidemiology, and aids in the appropriate management of infected patients. Research on the age and timing of respiratory pathogen infections can provide fundamental data for

implementing various health policies. Such data can help to determine the appropriate age and timing of vaccinations as well as aid in the understanding of the epidemiology of respiratory viruses. Therefore, continuous research and monitoring are necessary to utilize this information as a basis for public health policies.

CONCLUSION

In conclusion, the BioFire FilmArray™ Respiratory Multiplex panel demonstrated excellent utility as a rapid and sensitive diagnostic tool for respiratory pathogen detection in hospitalized children. Viral pathogens, particularly HRV/EV and RSV, predominated, while respiratory pathogen co-infections were common. Respiratory infections showed a marked seasonal increase during the monsoon and winter months, highlighting the influence of climatic factors on pathogen circulation in southern India. Integration of multiplex molecular diagnostics with clinical and laboratory findings facilitates accurate diagnosis, supports antimicrobial stewardship, improves patient management, and strengthens infection prevention strategies. Continued molecular surveillance is essential to monitor changing epidemiological patterns, guide vaccination strategies, and inform evidence-based public health policies for pediatric respiratory infections in India.

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