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Original Research Article

Rapid multiplex polymerase chain reaction for the detection of respiratory pathogens in pediatric patients under 5 years of age: a single center study

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ABSTRACT

Background: Acute respiratory tract infections (ARTIs) are leading causes of morbidity and mortality in children under 5 years, with similar clinical presentations making pathogen identification challenging. The objective of this study is to rapidly detect the causative organism of acute upper respiratory tract infection using the FilmArray respiratory panel 2.1 plus (FARP).
Methods: A cross-sectional prospective study was conducted at the Bangladesh Institute of Tropical and Infectious Diseases from September 2024 to February 2025. Nasopharyngeal swab specimens were collected from 60 pediatric patients (≤5 years) presenting with ARTI symptoms within 7 days of onset. Samples were analyzed using FARP 2.1 plus, capable of detecting 19 viruses and 4 atypical bacteria within 45 minutes.
Results: The cohort comprised 35 males (58.33%) and 25 females (41.67%) with a mean age of 20±17 months. The diagnostic yield was 95% (57/60). Human rhinovirus/enterovirus was most prevalent (53.3%), followed by respiratory syncytial virus (43.3%) and adenovirus (33.3%). mixed infections predominated, occurring in 55% of cases, with rhinovirus/enterovirus and RSV being the most common combination (21.2%). Age-stratified analysis revealed higher rhinovirus/enterovirus prevalence in infants <1 year (68.1%) compared to children 1-5 years (44.7%).
Conclusions: FilmArray demonstrated excellent diagnostic performance with high pathogen detection rates and revealed complex coinfection patterns in pediatric ARTI. The predominance of mixed infections and age-specific pathogen distributions emphasizes the importance of comprehensive molecular diagnostics for optimal clinical management and infection control strategies in pediatric respiratory medicine.

Keywords: Acute respiratory tract infections, FilmArray respiratory panel, Real-time RT-PCR, Respiratory pathogens, Nasopharyngeal swab

INTRODUCTION

Acute respiratory tract infections (ARTIs) are common illnesses that can infect individuals of any age. It causes significant morbidity and mortality globally, particularly in children under five. According to estimates from the World Health Organization (WHO), acute respiratory infections cause between 1.9 and 2.2 million childhood fatalities each year, with southeast Asia and Africa accounting for 70% of these cases. ARTIs were the third most common cause of mortality worldwide in 2019.[1,2] Fungi, viruses, and bacteria are the etiological agents of ARIs. Children with ARIs are most commonly found to have respiratory syncytial virus (RSV), influenza viruses type A and B, human parainfluenza viruses type 1, 2, and 3 (HPIV-1, HPIV-2, and HPIV-3), adenoviruses (ADV), and human metapneumoviruses (hMPV).[3,4] Respiratory tract infections (RTIs) are largely caused by atypical

bacteria in addition to viruses in children. 10-40% of children hospitalized with community-acquired pneumonia are infected with *Mycoplasma pneumoniae* (*M. pneumoniae*), one of the most prevalent atypical bacteria.[5] Choosing the right therapy depends on early diagnosis of this pathogen. However, regardless of the causative agents, ARTIs can have very similar clinical symptoms, making diagnosis and treatment difficult. Because clinical presentation is the only basis for decision-making, this presents a challenge for doctors in deciding on the best course of treatment. Significant global concerns are presented by the rise of drug-resistant microorganisms and the paucity of clinical data. To effectively prevent and treat ARTIs, information on the causative agents, enhanced diagnostic methods, and the range of drug sensitivity/resistance is essential. Without this knowledge, there is a chance that antimicrobials would be used inappropriately and irrationally, which fuels the growth of microorganisms that are resistant to several drugs.[6,7] Clinicians can determine whether additional diagnostic tests or antibiotic or antiviral medication are required by accurately identifying the infections. Additionally, this information helps with hospitalization decisions and infection control strategies.[8,9] Currently, culture and immunological techniques are widely used diagnostic procedures for identifying bacterial or viral respiratory infections.[10] However, although the gold standard for virus detection is cell culture, which is a time-consuming and tedious procedure that can produce erroneous results in the early stages of the disease.[11] Serological tests have the advantage of being faster, although its sensitivity and specificity may be low.[12] However, it has been demonstrated that multiplex real-time reverse transcriptase PCR (RT-PCR) is more sensitive than conventional antigen detection techniques, bacterial culture, and respiratory virus culture.[13,14] This specific test makes it easier to determine the most frequent causal agents of ARTIs by enabling the amplification of several viruses or bacteria in a single reaction.[15] Nevertheless, this molecular test necessitates distinct areas and is technically complex. To reduce the possibility of contamination and consequent false positive results, for example, multiple locations are required for sample preparation, reagent formulation, reaction setup, and amplification.[16] Therefore, the need for quicker, more accurate, and easier-to-use assays that can identify several respiratory pathogens at once is urgent. Many labs throughout the world have embraced FilmArray multiplex PCR, which was created by (Biofire diagnostics, a Utah, USA-based company that is owned by bioMérieux) to meet this demand. This molecular system consists of multiplex nested PCR, automated nucleic acid extraction, reverse transcription as a first step, and is followed by melting curve analysis.[17]

The FilmArray respiratory panel (FARP) is both approved by the FDA and CE IVD-marked. The current version of FARP 2.1 plus can detect 19 viral and 4 atypical respiratory organisms**,** respectively.

The organisms detected by FARP 2.1 plus includes adenovirus, coronavirus 229E (CoV-229E), coronavirus HKU1 (CoV-HKU1), coronavirus NL63 (CoV-NL63), coronavirus OC43 (CoV-OC43), human metapneumovirus (hMPV), human rhinovirus/enterovirus (HRV/EV), influenza A (FluA), influenza B (FluB), parainfluenza virus 1 (para1), parainfluenza virus 2 (para2), parainfluenza virus 3 (para3), parainfluenza virus 4 (para4), respiratory syncytial virus (RSV), middle east respiratory syndrome (MERS-CoV), *Bordetella pertussis* (detection of ptxP), *Bordetella parapertussis* (detection of IS1001), *Chlamydia pneumonia* and *Mycoplasma pneumonia*.

The testing process is conducted within a closed system, requiring 5 minutes of direct involvement and 45 minutes of instrumentation time. There is limited data available on the application of the FARP in detecting ARTI pathogens in symptomatic pediatric patients in Bangladesh.

Therefore, this study aims to rapidly detect the causative organism of acute upper respiratory tract infection by FARP 2.1 plus in outpatients visiting Bangladesh Institute of Tropical and Infectious Diseases (BITID) with suspected respiratory tract infections.

METHODS

Study design

Nasopharyngeal swab samples from 60 individuals suspected of having acute respiratory infections were included in this cross-sectional investigation. Participants were recruited from BITID outpatient department between September 2024 and 20 February 2025. For children, parental or guardian consent is obtained. The BITID institutional review board (IRB) granted ethical approval for the study protocol.

Inclusion criteria

Individuals of ≤5 years of age who had one or more of the following symptoms within seven days of the onset of an ARTI were included: cough, fever, rhinitis, sore throat, nasal congestion, sneezing, headache, wheezing, throat discomfort, muscle ache, chest tightness, or shortness of breath.

Exclusion criteria

Individuals who presented with serious infections like pneumonia, had incomplete consent papers, or had unsuccessful nasopharyngeal swab collection were not allowed to continue in the study.

Specimen collection

Nasopharyngeal swabs (NPS) were collected according to standard procedures. After being carefully inserted into the nose to a depth of approximately one to one and a half

centimeter, a sterile cotton-fiber swab was rotated against the anterior nasal mucosa for three seconds. The NPS sample was immediately placed in viral transport media (VTM). Following collection, specimens were handled in accordance with the guidelines provided by the manufacturer.[18]

Laboratory analysis

Pathogen detection using FilmArray technology

The FARP 2.1 plus, which can identify 4 atypical bacteria and 19 viruses, was used for examining respiratory samples. Following the manufacturer's instructions, 500 µl of sample buffer was combined with 300 µl of viral transport media (VTM) containing the NPS specimen for each test, which was then injected into the testing pouch's sample port. In a biosafety cabinet, all specimens were processed while wearing the proper PPE. A barcode scanner was used to scan the barcode on the testing pouch, and as soon as it was in the device, the automatic analysis procedure started. Automated nucleic acid extraction, reverse transcription, and first-stage multiplexed PCR are used in the FARP 2.1 plus panel. Individual nested second-stage real-time PCR is then carried out on a microarray chip. Each target was examined in triplicate, and results were produced in about 45 minutes per run. Melting curve data was used for automated result analysis using Biofire/Biomérieux's FilmArray torch software (version 3.1.317.0). In a legitimate run, every target was marked as either "detected" or "not detected." Two internal controls are included in the FARP 2.1 plus: one for RNA processing and extra controls for every stage inside the testing pouch. The software automatically generated an 'Invalid' result for all panel analytes in the event that either internal control failed.

Statistical analysis

IBM corporation's statistical package for the social sciences (SPSS) version 23.0 was used to analyze the data. Descriptive statistics presented categorical data as frequencies and percentages, while continuous variables were expressed as means ± standard deviation. Fisher's exact test was used to compare pathogen rates across age groups for categorical variables. The threshold for statistical significance was set at $p<0.05$.

RESULTS

Demographic characteristics and clinical presentation of study participants

This study included 60 patients with ARTIs. There was a slight male predominance in the cohort, with 25 females (41.67%) and 35 males (58.33%). The standard deviation was ±17, and the mean age was 20 months. 22 patients (36.67%) were younger than 1 year, and 38 patients (63.33%) were between 1 and 5 years old. Respiratory symptoms predominated in the clinical presentation; cough was the most common symptom, affecting 58 patients (96.67%), closely followed by fever in 56 patients (93.33%). Forty patients (66.67%) had runny noses, and eighteen patients (30%) had nasal congestion. Less common symptoms included headache (5%), malaise (3.3%), sore throat (1.7%), and conjunctivitis (1.7%) (Table 1).

Table 1: Demographic and clinical characteristics of ARTI cases.

Variables	Number (%)
Sex	
Male	35 (58.33)
Female	25 (41.67)
Age (months)	
Mean	20±17
Median	16
Age groups (years)	
<1	22 (36.67)
1-5	38 (63.33)
Clinical features	
Symptomatic	
Cough	58 (96.67)
Fever	56 (93.33)
Runny nose	40 (66.67)
Nasal congestion	18 (30)
Headache	03 (5)
Malaise	02 (3.3)
Sore throat	01 (1.7)
Conjunctivitis	01 (1.7)

Pathogen detection and positivity rates

With 57 out of 60 samples (95%) testing positive for at least one respiratory pathogen, the Biofire FARP 2.1 plus showed a high diagnostic yield. Of the positive cases, 34 (56.67%) were male, and 23 (38.33%) were female. 36 positive cases (60%) in the 1-5-year age group and 21 positive cases (35%) in children under 1 (Table 2).

Table 2: Positivity rate of ARTI cases using Biofire FARP 2.1 plus.

Samples tested (n=60)	Number (%)
Positive	57 (95)
Sex	
Male	34 (56.67)
Female	23 (38.33)
Age group (years)	
<1	21 (35)
1-5	36 (60)

Overall pathogen spectrum

A wide range of respiratory pathogens were identified in our study (Figure 1). The most common pathogen found in

32 samples (53.3%) was human rhinovirus/enterovirus. Respiratory syncytial virus was found in 26 samples (43.3%), and adenovirus was found in 20 samples (33.3%). Human parainfluenza virus 4 was found in 4 samples (6.7%), whereas human metapneumovirus was found in 11 samples (18.3%). The prevalence of bacterial pathogens was lower; only one sample (1.7%) had *Mycoplasma pneumoniae* and four samples (6.7%) had *Chlamydia pneumoniae*. SARS-CoV-2 (1.7%, 1 sample) and other coronaviruses, such as HKU1 (5%, 3 samples) and NL63 (1.7%, 1 sample), were less commonly found. Two samples (3.3%) had Influenza A, and two samples (3.3%) each had human parainfluenza viruses 2 and 3.

Single infection versus coinfection patterns

Coinfections were significantly more common than single-pathogen infections, according to an analysis of infection patterns (Figure 2). With 27 coinfections compared to just 4 single infections, the human rhinovirus/enterovirus notably displayed the highest coinfection tendency. Similarly, RSV showed frequent coinfection patterns, with 21 coinfection cases versus 6 single infections. Adenovirus showed 17 coinfection cases compared to 3 single infections, and human metapneumovirus showed 8 coinfections compared to 3 single infections.

Mixed infection prevalence

A total of 33 different types of mixed organisms were identified, with 21 different combinations. Out of the 60 specimens, 55% (33/60) tested positive for more than one analyte. The most common combination was the presence of both rhinovirus/enterovirus and RSV, which was found in 21.2% (7/33) of the cases. This was followed by a combination of adenovirus and RSV, which accounted for 12.1% (4/33) of the cases. (Table 3). Among the mixed organism-positive patients, the majority were observed to have human rhino/entero (46%, 15/33), adenovirus (33%, 11/33), RSV (21%, 7/33), and HMPV (15%, 5/33) (Figure 3).

Age-stratified pathogen distribution

The most common infection found was human rhinovirus/enterovirus, which was found in 68.1% (15/22) of infants under 1 year and 44.7% (17/38) of children ages 1 to 5. The second most prevalent infection was RSV, which was found in 50% (11/22) of infants and 39.5% (15/38) of older children. The frequency of adenovirus was constant across age groups, affecting 31.6% (12/38) of children aged 1-5 years and 36.6% (8/22) of infants. HMPV showed an intriguing age-related trend, with babies (13.6%, 3/22) having a lower prevalence than the 1-5-year group (21.5%, 8/38). Human parainfluenza virus 4 (HPIV 4) was detected exclusively in the older age group (10.5%, 4/38), while other parainfluenza viruses (HPIV 2 and 3) showed low prevalence across both groups. We found bacterial infections less often. *Chlamydia pneumoniae* was found in 4.5% (1/22) of infants and 7.9% (3/38) of older children. *Mycoplasma pneumoniae* was found in only one case (2.6%) in the 1-5 years' group.

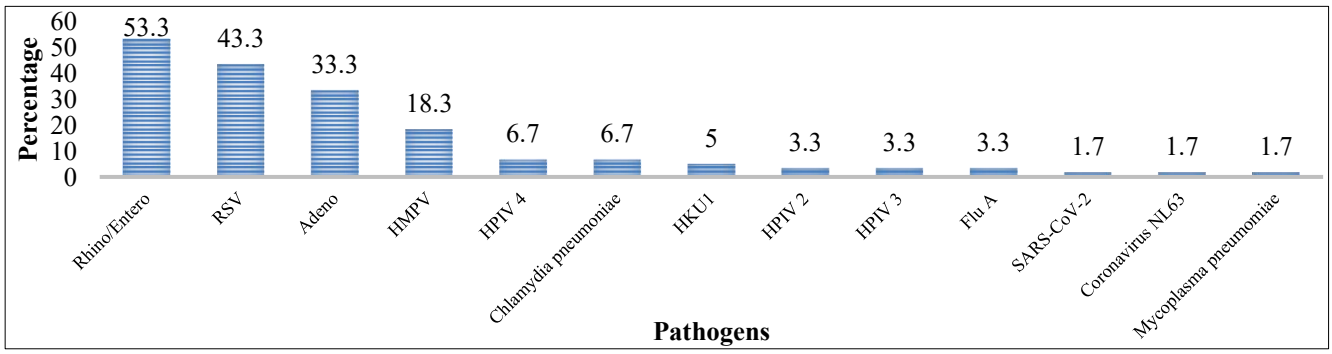

Figure 1: Positive spectrum of respiratory pathogens detected by FARP 2.1 plus.

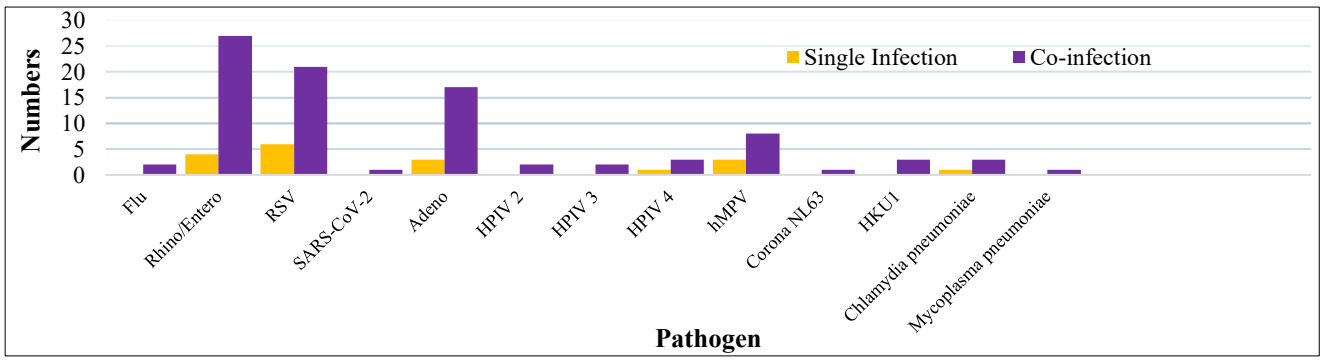

Figure 2: Comparison of viral and bacterial frequency in single infection and coinfection.

Table 3: Distribution of mixed-pathogen combinations in NPS specimens.

Analyte 1	Analyte 2	Analyte 3	Analyte 4	Number
Rhinovirus/enterovirus	Respiratory syncytial virus			7
Adenovirus	Respiratory syncytial virus			4
Adenovirus	Rhinovirus/enterovirus			3
Rhinovirus/enterovirus	Human metapneumovirus			3
Rhinovirus/enterovirus	Adenovirus	Respiratory syncytial virus	Rhinovirus/ enterovirus	1
Respiratory syncytial virus	Human metapneumovirus			1
Adenovirus	Coronavirus NL63			1
Rhinovirus/enterovirus	Parainfluenza virus 2			1
Chlamydia pneumoniae	Parainfluenza virus 3			1
Rhinovirus/enterovirus	Coronavirus HKU1			1
Respiratory syncytial virus	Influenza A H1-2009			1
Rhinovirus/enterovirus	Respiratory syncytial virus	SARS-COV-2		1
Adenovirus	Human metapneumovirus	*Chlamydia pneumoniae*		1
Adenovirus	Human metapneumovirus	Rhinovirus/enterovirus		1
Adenovirus	Rhinovirus/enterovirus	Influenza A H1-2009		1
Rhinovirus/enterovirus	Parainfluenza virus 2	Coronavirus HKU1		1
Adenovirus	Parainfluenza virus 4	Coronavirus HKU1		1
Adenovirus	Respiratory syncytial virus	Rhinovirus/enterovirus	Human metapneumovirus	1
Adenovirus	Respiratory syncytial virus	Rhinovirus/enterovirus	Parainfluenza virus 2	1

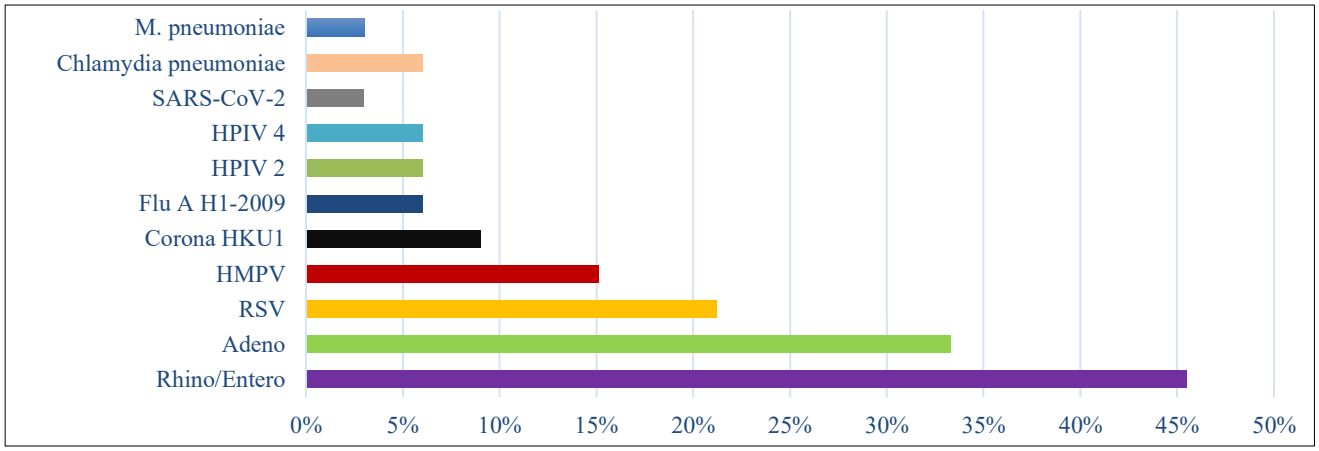

Figure 3: The percentage of mixed-pathogen positive specimens positive for the specific pathogen.

Table 4: Prevalence of respiratory pathogens tested in different age groups by Biofire FARP 2.1 plus.

Pathogen	<1 year		1-5 years		P value
Virus	**Pos**	**Prevalence (n=22) (%)**	**Pos**	**Prevalence (n=38) (%)**	
Human rhino/entero	15	68.1	17	44.7	0.109
RSV	11	50	15	39.5	0.589
Adeno	8	36.6	12	31.6	0.780
HMPV	3	13.6	8	21.5	0.731
HPIV 4	0	0	4	10.5	0.286
Coronavirus HKU1	2	9.09	1	2.6	0.548
Flu A	1	4.5	1	2.6	1.000
HPIV 2	1	4.5	1	2.6	1.000
HPIV 3	0	0	2	5.3	0.528
SARS-CoV-2	1	4.5	0	0	0.367

Continued.

Pathogen	<1 year		1-5 years		P value
Virus	**Pos**	**Prevalence (n=22) (%)**	**Pos**	**Prevalence (n=38) (%)**	
Coronavirus NL63	0	0	1	2.6	1.000
Bacteria					
Chlamydia pneumoniae	1	4.5	3	7.9	1.000
Mycoplsma pneumoniae	0	0	1	2.6	1.000
Total	21	95.5	36	94.7	1.000

DISCUSSION

ARTI is a significant health issue that has a profound impact on patients and society. It is particularly prevalent in children and certain individuals with compromised immune systems. The use of molecular diagnosis, specifically the multiplex PCR assay, has proven to be a more effective tool compared to routine diagnostic tests. The objective of this study was to rapidly detect the causative organism of acute upper respiratory tract infection by FARP in outpatients visiting BITID with suspected respiratory tract infections. A total of 60 patients were included in the study, with 35 being male and 25 females.

The high positivity rate (95%) achieved by the FARP 2.1 plus underscores the utility of comprehensive molecular diagnostic platforms in pediatric respiratory infections. Compared to conventional diagnostic techniques, which usually provide positivity rates of 60-80%, this detection rate is significantly greater, indicating that in ARTI instances, multiplex PCR panels can greatly increase diagnostic yield.[19,20] Other studies that have used multiplex molecular tests have indicated similar high detection rates, with rates ranging from 85-95%.[21,22]

Viral pathogens, including rhinovirus/enterovirus and RSV (53.3% and 43.3%), are prevalent, which is consistent with known epidemiological trends in pediatric respiratory illnesses.[23,24] Because of their underdeveloped immune systems and limited past exposure, younger infants are more susceptible to these common infections, which could account for the higher prevalence of rhinovirus and enterovirus in this age group.[25,26]

With the vast majority of pathogens found in coinfection scenarios rather than single-infection settings, the study's wide coinfection patterns underscore the intricate etiology of pediatric ARTI. The finding that 84.4% of rhinovirus/enterovirus detections (27/32) occurred as coinfections, along with 80.8% of RSV cases (21/26) and 85% of adenovirus cases (17/20), challenges the traditional single-pathogen disease model and suggests that respiratory viral interactions may be the norm rather than the exception in pediatric ARTI.

This high proportion of coinfection is in line with recent molecular epidemiology studies, which have reported coinfection rates ranging from 40-80% in pediatric respiratory infections when sensitive molecular methods are employed.[27,28,29]

Compared to the 10-30% usually reported in pediatric populations, our study found a mixed respiratory virus infection rate of 55% (33/60).[30] This elevated rate may reflect enhanced diagnostic sensitivity with multiplex PCR testing or characteristics specific to our study population. The most common specific combination of rhinovirus/enterovirus with RSV (21.2% of mixed cases) is consistent with literature showing frequent RSV coinfections that may increase disease severity. The predominance of rhinovirus/enterovirus in mixed infections (46% of cases) aligns with recent observations during the COVID-19 pandemic, where rhinovirus emerged as the most prevalent co-circulating respiratory pathogen.[31,32]

Human rhinovirus/enterovirus emerged as the most frequently detected pathogen in our study, affecting 68.1% of infants under 1 year and 44.7% of children aged 1-5 years. This pattern aligns closely with findings from other studies, which reported rhinoviruses as the most common viral cause of respiratory infections in children, particularly in younger age groups.[33,34] RSV was identified as the second most common pathogen, detected in 50% of infants and 39.5% of older children. This age distribution pattern is consistent with established RSV epidemiology.[35] Adenovirus showed remarkably consistent prevalence across both age groups (36.6% in infants versus 31.6% in children aged 1-5 years), which differs from some previous studies that have shown age-related variations. One study reported similar findings in their comprehensive analysis of respiratory viruses, noting that adenovirus maintains relatively stable detection rates across pediatric age groups, unlike other respiratory viruses that show more pronounced age-related patterns.[36] The higher prevalence of HMPV in children aged 1-5 years compared to infants (21.5% versus 13.6%) represents an interesting finding. This age distribution may reflect the fact that HMPV typically causes more clinically apparent disease in slightly older children.[37] The low prevalence of bacterial pathogens in our study (*Chlamydia pneumoniae*: 4.5-7.9%, *Mycoplasma pneumoniae*: 0-2.6%) aligns with contemporary understanding of pediatric respiratory infections. This finding is consistent with this study.[38]

The results of this study should be interpreted in light of its limitations, which include its single-center design and partially small sample size. The generalizability of these results would be strengthened by future multicenter investigations with larger populations. The clinical importance of identified pathogens and coinfections would also be better understood if pathogen detection were

correlated with clinical severity levels and treatment outcomes. The high sensitivity of molecular techniques may identify viral shedding that is clinically irrelevant, so data must be carefully interpreted in clinical settings.

CONCLUSION

In pediatric ARTI, the FilmArray diagnostic technique showed complicated patterns of single and mixed-pathogen infections with a high diagnostic yield. Future therapeutic and preventative measures in pediatric respiratory medicine may be influenced by the age-specific distribution patterns and high prevalence of coinfections, which highlight the need for complex clinical interpretation of molecular diagnostic results.

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