Hindawi
Advances in Virology
Volume 2024, Article ID 7613948, 9 pages
https://doi.org/10.1155/2024/7613948

Research Article

Prevalence of Influenza Viruses A and B, Adenovirus, Respiratory Syncytial Virus, and Human Metapneumonia Viruses among Children with Acute Respiratory Tract Infection

Rana Farzi,[1] Neda Pirbonyeh,[1,2] Mohammad Rahim Kadivar,[3,4] and Afagh Moattari [1]

[1]*Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[2]*Burn and Wound Healing Research Center, Microbiology Department, Shiraz University of Medical Sciences, Shiraz, Iran*
[3]*Department of Pediatrics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[4]*Professor Alborzi Clinical Microbiology Research Center, Namazi Hospital, Shiraz Medical University, Shiraz, Iran*

Correspondence should be addressed to Afagh Moattari; moattaria@sums.ac.ir

Received 30 July 2022; Revised 4 October 2023; Accepted 23 December 2023; Published 22 January 2024

Academic Editor: Ahmed Majeed Al-Shammari

Background. Acute respiratory tract infection (ARTI) is a significant cause of morbidity and mortality among children worldwide. The majority of acute respiratory infections in children are caused by viruses, with respiratory syncytial virus (RSV) being the most frequently encountered. Other important viral pathogens include human metapneumovirus, human coronaviruses, adenovirus, and influenza. These infections can lead to complications such as bronchitis and pneumonia. So, this study aimed to evaluate the prevalence of influenza viruses A and B, adenovirus, respiratory syncytial virus (RSV), and human metapneumovirus (HMPV) in children with ARTI. *Methods*. The molecular diagnostic of polymerase chain reaction approach was used to detect influenza (A and B), metapneumovirus, respiratory syncytial virus (RSV), and adenovirus in respiratory samples of children with acute respiratory infection hospitalization in a teaching hospital of the Shiraz University of Medical Sciences in January 2016–March 2017. *Results*. Of the 340 patients examined, 208 (61.20%) were male and the median age was 3.13 ± 2.38 years. Respiratory viruses were found in 179 (52.64%) patients. The male-to-female ratio was 1.63 : 1 in patients who were viral positive. Detection rates for influenza A, adenovirus, influenza B, RSV, and HMPV were 28.23%, 24.70%, 8.52%, 3.23%, and 2.64%, respectively, and coinfections were detected in 24.02%. The most common combination of two-virus coinfections was IFVA/AdV, followed by IFVB/AdV, AdV, IFVB/IFVA, RSV/IFVA, HMPV/AdV, RSV/AdV, and HMPV/IFVA. *Conclusion*. The high prevalence of respiratory viruses in children hospitalized with ARTI suggests that viral infection may play a role in disease pathogenesis. This should be confirmed through the conduct of case-control studies and may inform the role of vaccination to prevent respiratory viral infections.

1. Introduction

Community-acquired acute respiratory tract infection (ARTI) is a major cause of illness and fatality throughout the world [1, 2], Also, every year 4-5 million children in developing countries are hospitalized due to this infection [2, 3]. Older people, especially the elderly, young children, and immunocompromised people are more at risk of death [1]. ARTI is the most prevalent reason for referral to medical clinics and accounts for 70% of the respiratory diseases in young children and newborns under one year of age [4, 5]. These infections affect the upper and lower respiratory tracts from the pharynx to the alveoli, with a spectrum of disease ranging from mild cold to severe pneumonia illness [6]. ARTI includes rhinopharyngitis, pharyngitis, sinusitis, acute otitis media, epiglottitis, laryngitis, laryngotracheobronchitis, bronchitis, bronchiolitis, and exacerbation of asthma and pneumonia [7]. The circulation of these infections in the community is common and transmission occurs through the inhalation of aerosols and touching contaminated surfaces with self-inoculation onto mucosal surfaces [4]. These infections are easily transmitted and spread in the society rapidly and pose a huge economic burden on the society [6]. Among causative agents of the respiratory tract pathogens, viruses play a major role in the development of ARTI [4] and are responsible for 30%–40% of ARTIs [8]. Viruses commonly associated with ARTIs include respiratory syncytial virus (RSV), influenza virus A (IFVA), influenza virus B (IFVB), adenovirus (AdV), and human metapneumovirus (HMPV) [3, 9]. These viruses use specific receptor and generate infection in respiratory tract infection (Figure 1). Other viruses, including human parainfluenza viruses (HPIVs), rhinovirus (RV), and human coronavirus (HCov), are also associated with ARTI [10, 11]. Respiratory viral pathogens may cause ARTI as a single-pathogen infection or as coinfections with other viruses or bacteria. The most important bacterial agents involved in the respiratory infection are *Legionella pneumophila* (LP), *Chlamydophila pneumoniae* (CP), *Mycoplasma pneumoniae* (MP), and *Bordetella pertussis* (BP) and also Pneumococcus and Hemophilus influenzae have partial role in the respiratory infection [1, 12]. Several studies have suggested that coinfection may be associated with increased severity of ARTI [13–16]. Identification of the commonly occurring viruses informs prevention strategies, including vaccine production [4]. The aim of this study was to investigate the prevalence of IFVA, IFVB, AdV, RSV, and HMPV among children hospitalized with ARTI.

2. Methods

2.1. Study Population. This was a cross-sectional study of children under 14 years of age admitted with an LT-ARI diagnosis at hospitals affiliated with the Shiraz University of Medical Sciences in the period between January 2016 and March 2017. LT-ARI was defined as any acute lower respiratory tract infection of sufficient severity to warrant admission to the hospital. All types of LT-ARI were included, from bronchiolitis to pneumonia, with or without wheezing, fever, rhinorrhea, or respiratory distress.

2.2. Collection and Preparation of the Samples. Throat and nasopharyngeal swab samples were collected from the study participants. Samples were refrigerated at 2–8°C and transported on ice to the Virology Department of Shiraz University of Medical Science and stored at −80°C until analyzed.

2.3. Nucleic Acid Extraction and Virus Detection. Nucleic acid (DNA or RNA) extraction was performed by using the High Pure Viral Nucleic Acid kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. For AdV DNA detection in the throat and nasopharyngeal samples, PCR was performed using Master Mix RED (Amplicon, A180306).

Detection of HMPV and RSV was performed using the one step RT-PCR kit (QIAGEN GmbH, Germany). IFVA and IFVB were detected using a one-step real-time (RT) PCR kit (QIAGEN GmbH, Germany), according to the manufacturer's instructions.

The primers and probe sequences used in this study are summarized in Table 1.

2.4. Statistical Analysis. Statistical analysis was performed using SPSS software, version 25. Chi-square or Fisher's exact tests were used for comparison of the proportions. The significance level was determined at $p < 0.05$.

2.5. Ethical Considerations. Ethical approval for this study was obtained from SUMS Medical Ethics (IR.SUMC.REC.1396.S564). Written informed consent was obtained from all the study patients.

3. Result

Out of the 340 respiratory patients, 132 (38.80%) were female and 208 (61.20%) were male and the patients' median age was 3.13 ± 2.38 years and infection rates were higher in under three years and 3–5 age groups.

Investigation revealed that 179 (52.64%) patients with respiratory infection had at least one respiratory virus. Considering that the samples were taken from hospitalized patients with respiratory symptom in hospital wards during the peak of respiratory infections in autumn and winter, the high rates are not biased. Demographic data of all the children admitted with acute respiratory infections are shown in Table 2. The rate of viral respiratory infections was estimated at 68 (37.98%) for female and 111 (62.01) for male. The male-to-female ratio was 1.63 : 1 (111 : 68) in patients who were viral positive. The viral infection rates were more in <5 age. The respiratory virus-infected patients' median age was $3.30 + 2.46$ years. In terms of disease symptoms, the most common symptoms in these patients include fever, cough, sore throat, muscle pain, wheezing, anorexia, runny nose, and headache.

Diarrhea and vomiting were also observed in some patients at a young age.

The frequency of each viral infection was IFVA: 96 (28.23%), AdV: 84 (24.70%), IFVB: 29 (8.52%), HMPV: 9 (2.64%), and RSV: 11 (3.23%) of the samples.

The most age group infected with the IFVA, IFVB, and AdV was the age group under 10 years old, and the most infected age group with RSV and HMPV was under 5 and under three years age group.

The rate of monovirus infection in patients with viral respiratory infections was 136/170 (80.00%). This was despite the fact that some children were infected with more than one virus (coinfection), and its frequency was 43/170 (25.29%) (Table 3). Agarose gel electrophoresis image of PCR of RSV (A), HMPV(B), AdV(c) is shown in Figures 2(a)–2(c).

Multiple infections were detected in 43/179 (24.02%) episodes, of which 37/179 (20.67%) were with two viruses, 5/179 (2.79%) were with three viruses, and 1/179 (0.55%) was with four viruses. The most common combination of two-virus coinfections was IFVA/AdV, followed by IFVB/Adv, IFVB/IFVA, RSV/IFVA, HMPV/AdV, RSV/AdV, and HMPV/IFVA. The result is shown in Table 3.

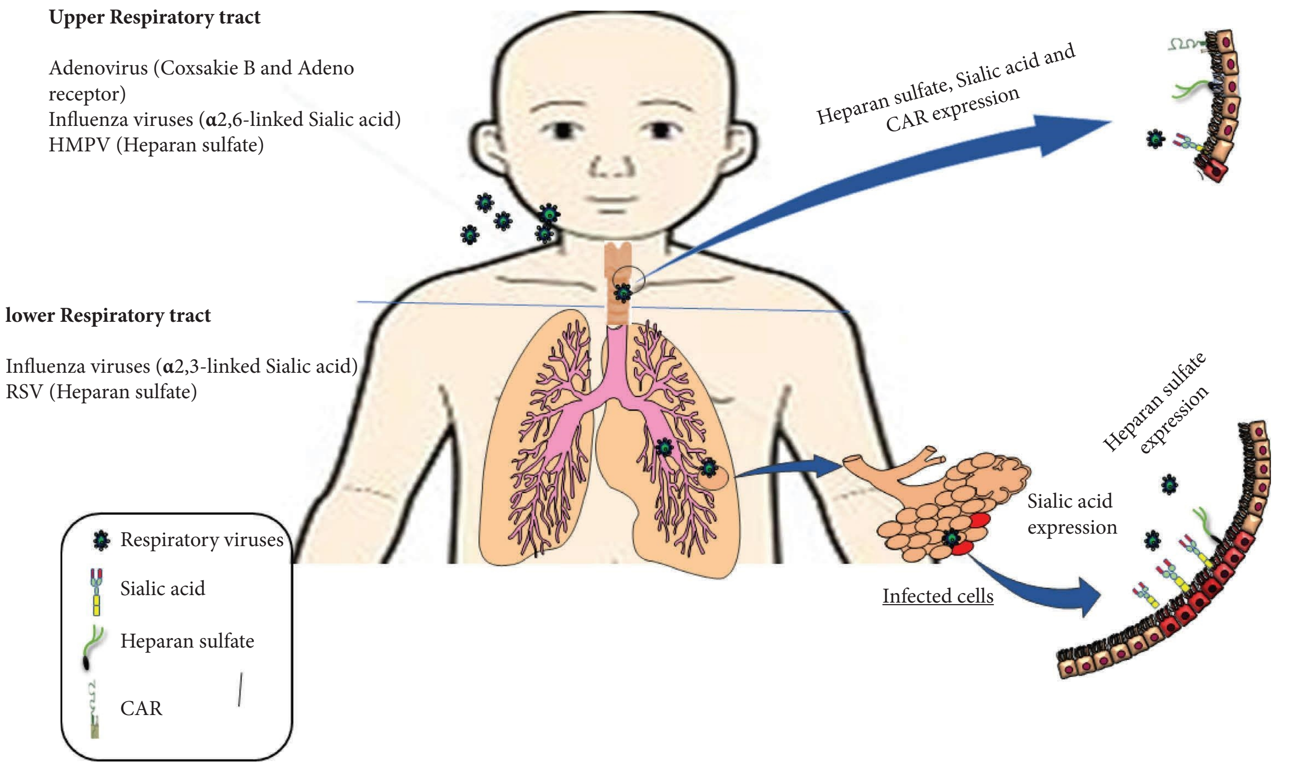


Figure 1: Occurrence of viral ART infection in children.

Table 1: Primer sequences, product size, and annealing temperature.

Virus	Primer sequence	Product size (bp)	Method	Tm	Reference
AdV	TTCCCCATGGCICAYAACAC CCCTGGTAKCCRATRTTGTA	482	Universal PCR	60°C	[17]
RSV	AACAGTTTAACATTACCAAGTGA TGATTACTTGAGATATTGATGC	379	Universal PCR	58°C	[18]
HMPV	GAGCAAATTGAAAATCCCAGACA GAAAACTGCCGCACAACATTTAG	347	Universal PCR	58°C	[19]
IFAV	GACCRATCCTGTCACCTCTGAC AGGGCATTYTGGACAAAKCGTCTA FAM-TGCAGTCCTCGCTCACTGGGCAGC- BHQ-1	106	One step RT-PCR	58°C	[20]
IFBV	TTCTTTCCCACCGAACCAAC GAGACACAATTGCCTACCTGCTT FAM-AGAAGATGGAGAAGGCAAAGCAGAACTAGC-BHQ-1	95	One step RT-PCR	58°C	[21]

The combination of IFA and AdV were predominant among cases with three and four coinfecting viruses. Coinfection was more common in males 28/43 (65.11%) than females 15/43 (34.88%). Clinical data were available for 178 children that were infected with at least one virus, and the most common symptoms were fever and cough. There was no significant correlation between the clinical symptoms and the number of detected viruses.

4. Discussion

ARTI is one of the most important public health problems due to its high incidence and ease of spread in the society [8]. Respiratory viruses are the most common pathogens in the development of ARTI [4, 8]. The study of respiratory virus prevalence is important in the control and treatment of these infections [4]. RSV, IFVA/B, PIV1, 2, and 3, HMPV, and AdV are considered to be the most common causative viruses for ARTI [3]. We, therefore, evaluated the prevalence of IFVA, IFVB, RSV, HMPV, and AdV in children hospitalized with ARTI.

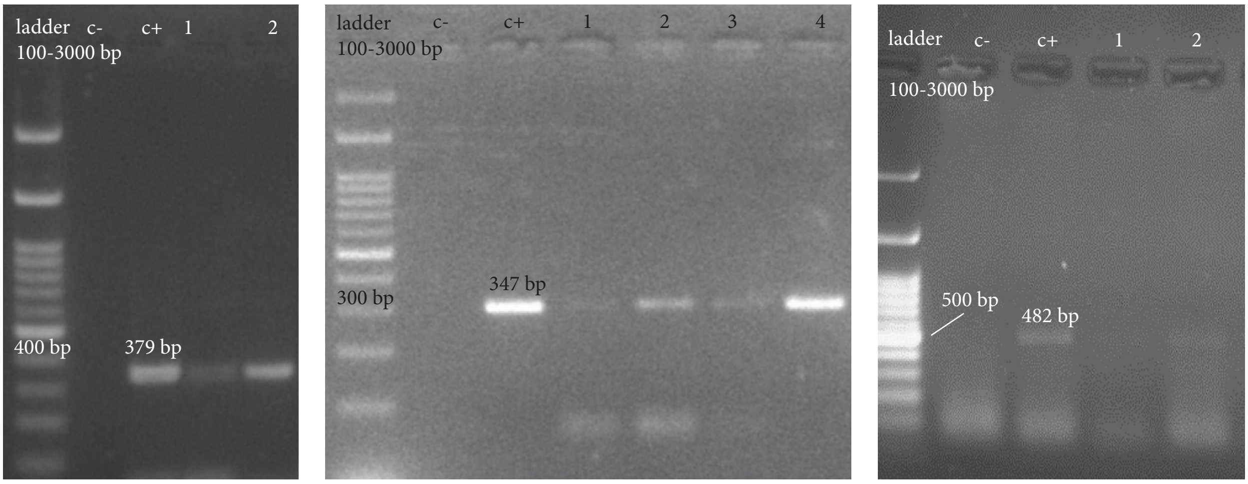
In our study, samples were obtained from symptomatic patients, and at least one respiratory virus was detected in 179/340 (52.64%) children. Perhaps, one of the reasons for the high prevalence of viral infections in this study is related to the study population because these patients had acute respiratory symptoms and were admitted to the hospital after initial treatment with the opinion of a specialist. In the

Table 2: Demographic data of all the children admitted with acute respiratory infections.

	Respiratory infection ($n = 340$)	Viral respiratory infection ($n = 179$)	HMPV ($n = 9$)	RSV ($n = 11$)	INFB ($n = 29$)	INFA ($n = 96$)	Ade ($n = 84$)
Sex							
Male	208 (61.20%)	111 (62.01%)	7 (77.77%)	6 (54.54%)	19 (65.51%)	59 (61.45%)	53 (63.09%)
Female	132 (38.80%)	68 (37.98%)	2 (22.22%)	5 (45.45%)	10 (34.48%)	37 (38.54%)	31 (36.90%)
Age							
Min	1	1	1	1	1	1	1
Maxim	15	15	12	5	9	15	10
Mean ± sd	3.13 ± 2.38	3.30 ± 2.46	3.88 ± 4.04	2.36 ± 1.50	2.82 ± 1.89	3.40 ± 2.71	3.19 ± 2.23
Age groups							
<3	151 (44.41%)	75 (41.89%)	5 (55.55%)	6 (54.54%)	13 (44.82%)	42 (43.75%)	38 (45.23%)
3–5	108 (31.76%)	60 (33.51%)	1 (11.11%)	4 (36.36%)	10 (34.48%)	29 (30.20%)	26 (30.95%)
5–10	75 (22.06%)	40 (22.34%)	2 (22.22%)	1 (9.09%)	6 (20.68%)	22 (22.91%)	20 (23.80%)
10–19	6 (1.76%)	4 (2.23%)	1 (11.11%)	0	0	3 (3.12%)	0
History							
Underlying disease	16 (4.70%)	11 (6.14%)	0	0	2 (6.89%)	5 (5.20%)	6 (7.14%)
Travel	37 (10.88%)	16 (8.93%)	0	2 (18.18%)	5 (17.24%)	9 (9.37%)	8 (9.52%)
Influenza vaccination	16 (4.70%)	7 (3.91%)	1 (11.11%)	1 (9.09%)	0	5 (5.20%)	2 (2.38%)
Pneumonia	17 (5.00%)	9 (5.02%)	1 (11.11%)	1 (9.09%)	1 (3.44%)	3 (3.12%)	4 (4.76%)
Symptoms							
Fever	307 (90.29%)	164 (91.62%)	9 (100%)	11 (100%)	26 (89.65%)	89 (92.70%)	76 (90.47%)
Anorexia	145 (42.64%)	78 (43.57%)	1 (11.11%)	5 (45.45%)	19 (65.51%)	44 (45.83%)	30 (35.71%)
Runny nose	151 (44.41%)	73 (40.78%)	2 (22.22%)	5 (45.45%)	18 (62.06%)	39 (40.62%)	32 (38.09%)
Muscle pain	183 (53.82%)	94 (52.51%)	4 (44.44%)	7 (63.63%)	13 (44.82%)	47 (48.95%)	50 (59.52%)
Sore throat	175 (51.47%)	106 (59.21%)	5 (55.55%)	7 (63.63%)	15 (51.72%)	59 (61.45%)	46 (54.76%)
Headache	78 (22.94%)	41 (22.90%)	1 (11.11%)	1 (9.09%)	8 (27.58%)	25 (26.04%)	15 (17.85%)
Diarrhea	18 (5.29%)	11 (6.14%)	0	1 (9.09%)	2 (6.89%)	7 (7.29%)	4 (4.76%)
Cough	222 (65.29%)	116 (64.80%)	3 (33.33%)	9 (81.81%)	18 (62.06%)	62 (64.58%)	56 (66.66%)
Wheezing	176 (51.76%)	93 (51.95%)	5 (55.55%)	5 (45.45%)	14 (48.27%)	51 (53.12%)	46 (54.76%)
Bronchitis	26 (7.64%)	15 (8.37%)	1 (11.11%)	0	2 (6.89%)	7 (7.29%)	7 (8.33%)
Vomit	15 (4.41%)	8 (4.46%)	0	1 (9.09%)	2 (6.89%)	4 (4.16%)	3 (3.57%)

Table 3: Frequency of mono- and coinfection with respiratory viruses with a pattern.

			Frequency ($n = 179$)	Percent in each groups (%)
Monoinfection ($n = 136$) (53 female and 83 male)		HMPV	4 (2.2%)	2.94
		RSV	4 (2.2%)	2.94
		INFB	14 (7.8%)	10.29
		INFA	63 (35.2%)	46.32
		AdV	51 (28.5%)	37.5
Coinfection ($n = 43$) (15 female and 28 male)	Two viruses ($n = 37$)	HMPV, INFA	2 (1.1%)	5.40
		HMPV, AdV	2 (1.1%)	5.40
		RSV, INFA	3 (1.7%)	8.10
		RSV, AdV	2 (1.1%)	5.40
		INFB, INFA	5 (2.8%)	13.51
		INFB, AdV	6 (3.4%)	16.21
		INFA, AdV	17 (9.5%)	45.94
	Three viruses ($n = 5$)	RSV, INFA, AdV	2 (1.1%)	40
		INFB, INFA, AdV	3 (1.7%)	60
	Four viruses ($n = 1$)	HMPV, INFB, INFA, AdV	1 (0.6%)	100
		Total	179 (100%)	



(a) (b) (c)

Figure 2: Detection of respiratory viruses. c−, negative control; c+, positive control. (a) Respiratory syncytial virus, (b) human metapneumonia viruses, and (c) adenovirus.

study of Lei et al. [22], they found that the detection rate of viral respiratory infection among children hospitalized for ARI was 77.2%. Previous studies showed that about 31.2–86% of respiratory infection was caused by virus [23–25]. Bacteria or other respiratory viruses such as enteroviruses, coronaviruses, bocaviruses, or unidentified respiratory pathogens may be a cause of these undiagnosed infections [23]. Previous studies from Tabriz and Tehran reported that the prevalence of viral agents in samples from children with ARTI were (36%) [26] and (35.4%), respectively [27]. Studies in other countries have also shown that the prevalence of viral infections varies from 27% to 91.6% [28–38]. The large differences in viral detection rates may be due to heterogeneity within the study population, variability of genetic factors, the number and type of viral pathogens included for testing, methods used for testing, and geographic variation [34, 35, 39, 40].

In this study, the prevalence of viral respiratory infections was higher in boys than in girls. In general, men at younger and older ages are more susceptible to severe consequences of respiratory viral infections. This is despite the fact that during the reproductive years, women are often at greater risk than men with more severe consequences of viral respiratory infections. Maturity and gender influence the pathogenesis of respiratory viral infections [41].

In our study, the prevalence of viral respiratory infections in children under 5 years of age is higher than other ages. Most often, viral infections of the respiratory tract spread when children's hands come into contact with the nasal secretions of an infected person [42]. Children under 5 years of age are usually more exposed to viral respiratory infections due to the lack of knowledge about social healthcare.

In the present study, the prevalence of IFVA, AdV, IFVB, RSV, and HMPV infections was 28.23%, 24.70%, 8.52%, 3.23%, and 2.64%, respectively. Previous studies reported the detection rates for IFVA, IFVB, RSV, and AdV as 4.7%–46%, 2.5%–40%, 9.7%–28.0%, and 4%–16.9%, respectively [26].

The prevalence of respiratory viruses tends to increase in autumn and winter in temperate climates. High rates of transmission/infection in children and infants may be related to immature immune systems, lack of prior exposure to these pathogens, living conditions which promote overcrowding or environmental air pollution, and greater pathogen exposure. Lack of hygiene may also lead to a higher rate of infections in infants and young children [35, 43–45].

Although in this study, we showed significant differences in clinical data and symptoms between the different respiratory viruses studied (Table 2), a specific clinically recognizable pattern for each virus group cannot be defined because all respiratory viruses in terms of clinical symptoms overlap.

Clinicians frequently do not consider more than one aetiologic agent responsible for the respiratory infection and often order diagnostic tests for single pathogen, especially influenza viruses. However, in some patients with respiratory illness, coinfection with different viruses is common [46] Hence, we evaluated viral respiratory coinfection in pediatric patients. At the host level, the result of dual infection is usually viral interference, and in this case, the replication of one virus causes competitive inhibition of the other virus, but in some cases, it can increase viral replication. In coinfection, the time interval between virus exposure and the route of infection seems to affect the pathogenicity of coinfection [47]. Coinfections can also change the epidemiology of viral infections. For example, a fast-replicating virus can interfere with the reproduction of other viruses and inhibit the presence of other low-replicating viruses. The pathophysiology behind dual viral infections can explain some of the epidemiology of viral-viral infections seen at the population level [47].

In our study, multiple viral infections occurred in 24.40% (43/179) patients. In the study of Chun-Yu Yen et al., 27% of the children had multiple viral infections [48]. The result is similar to the previous studies that the detection of multiple viruses simultaneously in pediatric patients ranges from 10 to 30% [49].

In the present study, IFVA was the pathogen most commonly associated with viral-viral coinfections in ARTI patients. Although no statistically significant association was found between coinfecting viruses, Greer et al. [50] reported a negative associations between infection with human rhinovirus and AdV, HMPV, and RSV. Similarly, Brunstein et al. [51] reported a low prevalence of IFVA in the face of RSV, parainfluenza, HMPV, and human rhinovirus, albeit not statistically significant. Also, in a study by Tanner et al. [23], prominent associations between coinfecting respiratory viruses were reported with a lower frequency of AdV, RSV, parainfluenza, HMPV, and human rhinovirus in cases that were positive for IFVA and vice versa.

5. Conclusion

ARTI is a complex and diverse group of diseases that commonly affect infants and children and range in severity from mild to severe and is life threatening. Reliance on clinical symptoms to identify the causative pathogen is not possible, given the broad range of potential organisms. We describe a high prevalence of respiratory viral infections among Iranian children hospitalized with ARTI and a relatively high rate of viral-viral coinfections. IFVA was the most commonly detected virus, either monoinfection or coinfection.

There is a growing appreciation that viruses are involved in the pathogenesis of ARTI. The use of vaccines, including seasonal influenza vaccine, may be helpful in preventing ARTI in children and adolescents.

5.1. Limitation. In this study, the relationship between the viral load and clinical features was not defined. More research is needed to understand the prevalence of these viruses and their impact on disease severity in children with acute respiratory infections.

Data Availability

The data used in this study will be shared on request to the corresponding author with the permission of Mohammad Rahim Kadivar.

Ethical Approval

Ethical approval for this study was obtained from SUMS Medical Ethics (IR.SUMC.REC. 1396.S564).

Consent

Written informed consent was obtained from all the study patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] S. Goktas and M. C. Sirin, "Prevalence and seasonal distribution of respiratory viruses during the 2014-2015 season in Istanbul," *Jundishapur Journal of Microbiology*, vol. 9, no. 9, 2016.

[2] S. S. Malekshahi, N. Z. Shafiei-Jandaghi, J. Yavarian, A. Shadab, M. Naseri, and T. M. Azad, "Detection of respiratory Co-infections in children less than five years with adenovirus infection," *Archives of Pediatric Infectious Diseases*, vol. 5, no. 1, 2017.

[3] W. K. Liu, Q. Liu, D. H. Chen et al., "Epidemiology of acute respiratory infections in children in Guangzhou: a three-year study," *PLoS One*, vol. 9, no. 5, 2014.

[4] L. Fernandes-Matano, I. E. Monroy-Muñoz, J. Angeles-Martínez et al., "Prevalence of non-influenza respiratory viruses in acute respiratory infection cases in Mexico," *PLoS One*, vol. 12, no. 5, 2017.

[5] A. Moattari, A. Emami, N. Pirbonyeh, and R. Yaghoobi, "Detection of Adenovirus infection among children with acute respiratory disease during 2010-2012 in Shiraz, Iran," *Archives of Pediatric Infectious Diseases*, vol. 3, no. 2, 2014.

[6] J. M. Kim, H. D. Jung, H. M. Cheong et al., "Nation-wide surveillance of human acute respiratory virus infections between 2013 and 2015 in Korea," *Journal of Medical Virology*, vol. 90, no. 7, pp. 1177–1183, 2018.

[7] S. Yahia, A. Y. Kandeel, E. Hammad, and A.-H. El-Gilany, "Human Metapneumovirus (hMPV) in acute respiratory infection: a clinic-based study in Egypt," *Indian Journal of Pediatrics*, vol. 79, no. 10, pp. 1323–1327, 2012.

[8] H. T. Othman, W. A. S. A. Elhamed, D. M. Hassan, M. S. Soliman, and R. W. Abdel Baset, "Respiratory syncytial virus and human metapneumovirus in severe lower respiratory tract infections in children under two," *The Journal of Infection in Developing Countries*, vol. 10, no. 03, pp. 283–289, 2016.

[9] A. Pormohammad, S. Ghorbani, A. Khatami et al., "Comparison of influenza type A and B with COVID-19: a global systematic review and meta-analysis on clinical, laboratory and radiographic findings," *Reviews in Medical Virology*, vol. 31, no. 3, 2021.

[10] A. Pormohammad, S. Ghorbani, A. Khatami et al., "Comparison of confirmed COVID-19 with SARS and MERS cases-Clinical characteristics, laboratory findings, radiographic signs and outcomes: a systematic review and meta-analysis," *Reviews in Medical Virology*, vol. 30, no. 4, 2020.

[11] A. Pormohammad, S. Ghorbani, B. Baradaran et al., "Clinical Characteristics, laboratory findings, radiographic signs and outcomes of 52,251 patients with confirmed covid-19 infection: a systematic review and meta-analysis," 2020, https://www.preprints.org/manuscript/202003.0252/v1.

[12] J. O. Wishaupt, T. van der Ploeg, R. de Groot, F. G. A. Versteegh, and N. G. Hartwig, "Single-and multiple viral respiratory infections in children: disease and management cannot be related to a specific pathogen," *BMC Infectious Diseases*, vol. 17, no. 1, p. 62, 2017.

[13] H. K. Brand, R. de Groot, J. M. D. Galama et al., "Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis," *Pediatric Pulmonology*, vol. 47, no. 4, pp. 393–400, 2012.

[14] P. Martinez, J. Cordero, C. Valverde et al., "Co-infección viral respiratoria en niños hospitalizados por infección respiratoria aguda y su impacto en la gravedad clínica," *Chilean journal of infectious diseases*, vol. 29, no. 2, pp. 169–174, 2012.

[15] S. Ricart, M. A. Marcos, M. Sarda et al., "Clinical risk factors are more relevant than respiratory viruses in predicting bronchiolitis severity," *Pediatric Pulmonology*, vol. 48, no. 5, pp. 456–463, 2013.

[16] F. G. A. Versteegh, G. J. Weverling, M. F. Peeters et al., "Community-acquired pathogens associated with prolonged coughing in children: a prospective cohort study," *Clinical Microbiology and Infection*, vol. 11, no. 10, pp. 801–807, 2005.

[17] H. Wang, Y. Zheng, J. Deng, X. Chen, P. Liu, and X. Li, "Molecular epidemiology of respiratory adenovirus detection in hospitalized children in Shenzhen, China," *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 9, pp. 15011–15017, 2015.

[18] T. Mazzulli, T. C. Peret, A. McGeer et al., "Molecular characterization of a nosocomial outbreak of human respiratory syncytial virus on an adult leukemia/lymphoma ward," *The Journal of Infectious Diseases*, vol. 180, no. 5, pp. 1686–1689, 1999.

[19] A. R. Falsey, D. Erdman, L. J. Anderson, and E. E. Walsh, "Human metapneumovirus infections in young and elderly adults," *The Journal of Infectious Diseases*, vol. 187, no. 5, pp. 785–790, 2003.

[20] World Health Organization, *CDC Protocol of Real Time RT-PCR for Swine Influenza A (H1N1)*, WHO, Geneva, Switzerland, 2009.

[21] P. Mehrbod, S. Eybpoosh, F. Fotouhi, H. Shokouhi Targhi, V. Mazaheri, and B. Farahmand, "Association of IFITM3 rs12252 polymorphisms, BMI, diabetes, and hypercholesterolemia with mild flu in an Iranian population," *Virology Journal*, vol. 14, no. 1, p. 218, 2017.

[22] C. Lei, C. T. Lou, K. Io et al., "Viral etiology among children hospitalized for acute respiratory tract infections and its association with meteorological factors and air pollutants: a time-series study (2014-2017) in Macao," *BMC Infectious Diseases*, vol. 22, no. 1, p. 588, 2022.

[23] H. Tanner, E. Boxall, and H. Osman, "Respiratory viral infections during the 2009–2010 winter season in Central England, UK: incidence and patterns of multiple virus co-infections," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 31, no. 11, pp. 3001–3006, 2012.

[24] E. Choi, K.-S. Ha, D. J. Song, J. H. Lee, and K. C. Lee, "Clinical and laboratory profiles of hospitalized children with acute respiratory virus infection," *Korean Journal of Pediatrics*, vol. 61, no. 6, p. 180, 2018.

[25] O. Kurskaya, T. Ryabichenko, N. Leonova et al., "Viral etiology of acute respiratory infections in hospitalized children in Novosibirsk City, Russia (2013–2017)," *PLoS One*, vol. 13, no. 9, 2018.

[26] N. A. Faezi, A. Z. Bialvaei, H. E. Leylabadlo, H. Soleimani, M. Yousefi, and H. S. Kafil, "Viral infections in patients with acute respiratory infection in Northwest of Iran," *Molecular Genetics, Microbiology and Virology*, vol. 31, no. 3, pp. 163–167, 2016.

[27] J. Yavarian, N. Zahra Shafiei-Jandaghi, A. Rahimi-Foroushani, A. Shadab, M. Ghamarchehreh, and T. Mokhtari-Azad, "Human metapneumovirus and influenza viruses in children with severe acute respiratory infections in Iran," *Iranian Journal of Pediatrics*, vol. 28, no. 1, 2018.

[28] M. S. Al-Ayed, A. M. Asaad, M. A. Qureshi, and M. S. Ameen, "Viral etiology of respiratory infections in children in southwestern Saudi Arabia using multiplex reverse-transcriptase polymerase chain reaction," *Saudi Medical Journal*, vol. 35, no. 11, p. 1348, 2014.

[29] X. Huo, Y. Qin, X. Qi et al., "Surveillance of 16 respiratory viruses in patients with influenza-like illness in Nanjing, China," *Journal of Medical Virology*, vol. 84, no. 12, pp. 1980–1984, 2012.

[30] E. P. Schlaudecker, J. P. Heck, E. T. MacIntyre et al., "Etiology and seasonality of viral respiratory infections in rural Honduran children," *The Pediatric Infectious Disease Journal*, vol. 31, no. 11, pp. 1113–1118, 2012.

[31] S. Essa, A. Owayed, H. Altawalah, M. Khadadah, N. Behbehani, and W. Al-Nakib, "Mixed viral infections circulating in hospitalized patients with respiratory tract infections in Kuwait," *Advances in virology*, vol. 2015, Article ID 714062, 8 pages, 2015.

[32] S. Kouni, P. Karakitsos, A. Chranioti, M. Theodoridou, G. Chrousos, and A. Michos, "Evaluation of viral co-infections in hospitalized and non-hospitalized children with respiratory infections using microarrays," *Clinical Microbiology and Infection*, vol. 19, no. 8, pp. 772–777, 2013.

[33] K. Ramaekers, E. Keyaerts, A. Rector et al., "Prevalence and seasonality of six respiratory viruses during five consecutive epidemic seasons in Belgium," *Journal of Clinical Virology*, vol. 94, pp. 72–78, 2017.

[34] S. A. Eifan, A. Hanif, S. M. AlJohani, and M. Atif, "Respiratory tract viral infections and coinfections identified by Anyplex™ II RV16 detection kit in pediatric patients at a riyadh tertiary care hospital," *BioMed Research International*, vol. 2017, Article ID 1928795, 10 pages, 2017.

[35] M. M. van der Zalm, B. E. van Ewijk, B. Wilbrink, C. S. Uiterwaal, T. F. Wolfs, and C. K. van der Ent, "Respiratory pathogens in children with and without respiratory symptoms," *The Journal of Pediatrics*, vol. 154, no. 3, pp. 396–400.e1, 2009.

[36] S. Bicer, T. Giray, D. Çöl et al., "Virological and clinical characterizations of respiratory infections in hospitalized children," *The Italian journal of pediatrics*, vol. 39, 22 pages, 2013.

[37] A. Martínez-Roig, M. Salvadó, M. Caballero-Rabasco, A. Sánchez-Buenavida, N. López-Segura, and M. Bonet-Alcaina, "Viral coinfection in childhood respiratory tract infections," *Bronconeumology Archives*, vol. 51, no. 1, pp. 5–9, 2015.

[38] G. Zhang, Y. Hu, H. Wang, L. Zhang, Y. Bao, and X. Zhou, "High incidence of multiple viral infections identified in upper respiratory tract infected children under three years of age in Shanghai, China," *PLoS One*, vol. 7, 2012.

[39] J. del Valle Mendoza, A. Cornejo-Tapia, P. Weilg et al., "Incidence of respiratory viruses in peruvian children with acute respiratory infections," *Journal of Medical Virology*, vol. 87, no. 6, pp. 917–924, 2015.

[40] M. Suryadevara, E. Cummings, C. A. Bonville et al., "Viral etiology of acute febrile respiratory illnesses in hospitalized children younger than 24 months," *Clinical Pediatrics*, vol. 50, no. 6, pp. 513–517, 2011.

[41] R. L. Ursin and S. L. Klein, "Sex differences in respiratory viral pathogenesis and treatments," *Annual Review of Virology*, vol. 8, no. 1, pp. 393–414, 2021.

[42] C. X. Gao, Y. Li, J. Wei et al., "Multi-route respiratory infection: when a transmission route may dominate," *Science of the Total Environment*, vol. 752, Article ID 141856, 2021.

[43] F. Gülen, B. Yıldız, C. Çiçek, E. Demir, and R. Tanaç, "Ten year retrospective evaluation of the seasonal distribution of agent viruses in childhood respiratory tract infections," *Turkish Pediatrics Archive*, vol. 49, no. 1, pp. 42–46, 2017.

[44] E. G. Huijskens, R. C. Biesmans, A. G. Buiting, C. C. Obihara, and J. W. Rossen, “Diagnostic value of respiratory virus detection in symptomatic children using real-time PCR,” *Virology Journal*, vol. 9, no. 1, p. 276, 2012.

[45] G. Huang, D. Yu, N. Mao et al., “Viral etiology of acute respiratory infection in Gansu Province, China, 2011,” *PLoS One*, vol. 8, no. 5, 2013.

[46] I. Stefanska, M. Romanowska, S. Donevski, D. Gawryluk, and L. B. Brydak, “Co-infections with influenza and other respiratory viruses,” *Advances in Experimental Medicine and Biology*, vol. 756, pp. 291–301, 2013.

[47] S. D. Meskill and S. C. O’Bryant, “Respiratory virus Co-infection in acute respiratory infections in children,” *Current Infectious Disease Reports*, vol. 22, no. 1, p. 3, 2020.

[48] C. Y. Yen, W. T. Wu, C. Y. Chang et al., “Viral etiologies of acute respiratory tract infections among hospitalized children-A comparison between single and multiple viral infections,” *Journal of Microbiology, Immunology, and Infection*, vol. 52, no. 6, pp. 902–910, 2019.

[49] A. Franz, O. Adams, R. Willems et al., “Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection,” *Journal of Clinical Virology*, vol. 48, no. 4, pp. 239–245, 2010.

[50] R. M. Greer, P. McErlean, K. E. Arden et al., “Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections?” *Journal of Clinical Virology*, vol. 45, no. 1, pp. 10–15, 2009.

[51] J. D. Brunstein, C. L. Cline, S. McKinney, and E. Thomas, “Evidence from multiplex molecular assays for complex multipathogen interactions in acute respiratory infections,” *Journal of Clinical Microbiology*, vol. 46, no. 1, pp. 97–102, 2008.