Research article

Viral etiology of severe lower respiratory tract infections in SARS-CoV-2 negative hospitalized patients during the COVID-19 pandemic in Kuwait

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ABSTRACT

Background: The prevalence of respiratory infections is largely underexplored in Kuwait. The aim of our study is to determine the etiology of infections from patients who are SARS-CoV-2 negative hospitalized with severe lower respiratory tract infections (LRTIs) in Kuwait during the coronavirus disease 2019 (COVID-19) pandemic.
Methods: We conducted an observational cross-sectional study among severe LRTI patients between September 2021 and March 2022. Respiratory samples from 545 non-COVID-19 severe LRTIs patients were prospectively evaluated with FTD Respiratory 21 Plus® real-time PCR, targeting 20 different viruses and 1 atypical bacterial pathogen.
Results: Among all 545 hospitalized cases, 411 (75.4 %) tested positive for at least one respiratory pathogen. The most common were rhinovirus (HRV) (32.7 %), respiratory syncytial virus (RSV) (20.9 %), metapneumovirus (HMPV) (14.1 %), bocavirus (13.2 %), and influenza A (12.7 %). The proportion of pathogens detected was highest in the under-5 age group, while HKU1 (44.4 %) predominated in the elderly (>50 years).
Conclusion: Our study reveals a high prevalence of respiratory viruses in severe acute lower respiratory tract infections among non-COVID-19 hospitalized patients in Kuwait. HRV remains the main etiology affecting the country, particularly in infants. These results underscore the necessity of employing multiplex PCR for accurate diagnosis and describing the epidemiology of infections among severe lower respiratory tract infections. This will facilitate the use of specific antiviral therapy and help avoid excessive or inappropriate antibiotic therapy.

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1. Introduction

Severe lower respiratory tract infections (LRTIs) represent common causes of acute local and systemic illness and are frequently encountered among patients admitted to intensive care units (ICUs) and pediatric intensive care units (PICUs). These infections pose significant morbidity and mortality risks across all age groups, including children, adults, and immunocompromised individuals [1,2]. LRTIs are responsible for around 1.4 million deaths a year in children under the age of five worldwide. Respiratory infections are caused by a range of different pathogens, including viruses, bacteria, and fungi [3,4], but most respiratory infections are viral in origin [5–7]. Indeed, respiratory syncytial virus (RSV), influenza (Flu), adenoviruses (AdV), human metapneumovirus (HMPV), para-influenza viruses, and human rhinoviruses (RV) are recognized as significant pathogens in the etiology of acute respiratory infections [5,8,9]. Because respiratory viral infections are characterized by similar respiratory symptoms, it is difficult to make an etiological diagnosis based on symptoms alone [5,10].

Over the last decade, improvements in detection techniques have made a major contribution to the diagnosis of respiratory viral infections and to providing a more accurate diagnosis of the pathogens responsible [11,12]. In addition, previous studies have shown that non-pharmaceutical interventions (NPIs), namely the wearing of masks, social distancing, confinement, and hand disinfection during the COVID-19 pandemic, had a significant impact on the prevalence and seasonality of various respiratory viral infections [13–17]. Rapid laboratory diagnosis of LRTIs is needed to support and guide clinical decisions for appropriate patient management and to avoid antibiotic misuse [18]. In this context, multiplex polymerase chain reaction (PCR) has been used to identify many different pathogens in hospitalized patients suffering from acute respiratory illnesses [18–21]. The etiology of acute respiratory infections in patients hospitalized in Kuwait is still largely underexplored. Furthermore, accurate data on the incidence and epidemiological distribution of respiratory viruses are important for public health in general and for disease prevention and control [22,23].

The aim of our study is to determine the etiology of infections from patients who are SARS-CoV-2 negative hospitalized with severe lower respiratory tract infections (LRTIs) in Kuwait during the coronavirus disease 2019 (COVID-19) pandemic. This investigation seeks to identify and monitor the viruses circulating within the country during this critical period. Additionally, the study endeavors to provide a comprehensive description of the pathogens associated with severe LRTIs among hospitalized cases, describing distribution according to age and the month of occurrence. This multifaceted approach will contribute valuable insights into the epidemiology of severe LRTIs during the context of the ongoing pandemic in Kuwait.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Kuwait Ministry of Health's Standing Committee for the Coordination of Medical and Health Research (No. 2021). Participants received an information note explaining the purpose of the study and an informed consent form. The study was carried out in accordance with the ethical guidelines of the Declaration of Helsinki.

2.2. Study design and clinical specimens

A cross-sectional study was conducted on hospitalized non-COVID-19 patients with severe lower respiratory tract infections. Physicians provided clinical records. We defined a severe LRTI patient as someone experiencing acute respiratory illness within 15 days of onset, with a history of acute febrile illness ($\geq 38.0\,^\circ$C), a diagnosis of lower respiratory infection including rales or rhonchi on pulmonary auscultation, tachypnea, chest retractions, sputum production, cyanosis, cough, and radiologic findings, and requiring hospitalization. The exclusion criteria included cases hospitalized for illnesses other than respiratory infections.

A total of 545 patients were enrolled from Sabah Medical Hospital over seven consecutive months from September 2021 to March 2022. Specimens were sent to the virology unit at the Yacoub Behbehani centre for the diagnosis of COVID-19. Fluid samples were taken by a specialist team and collected in viral transport medium (VTM). All samples were stored at $-80\,^\circ$C until used with FTD Respiratory 21 Plus® real-time multiplex Plus®.3.2.

2.3. Respiratory infection detection

Total nucleic acids were extracted using the Purifier TM Modesty automated extraction instrument (Genfine Biotech, Beijing-China, LTD), according to the manufacturer's instructions (GenFine Biotech). Real-time RT-PCR was performed using the cobas SARS-CoV-2 kit on the cobas 6800 instrument (Roche Molecular Systems, Rotkreuz, Switzerland).

FTD Respiratory 21 Plus®.3.2 real-time multiplex PCR (FTD, Fast-Track Diagnostics, Junglinster, Luxembourg) was performed according to the manufacturer's protocol. The test targets 20 different viruses and 1 atypical pathogenic bacterium. The viruses and bacteria detected are as follows: enterovirus (EV), human adenovirus (AdV), human bocavirus (HBoV), human coronavirus (HCoV) NL63, 229E, OC43, and HKU1, human metapneumovirus (HMPV) A and B, human parainfluenza virus (HPIV) 1, 2, 3, and 4, human rhinovirus (HRV), human respiratory syncytial virus (RSV) A and B, human parechovirus (HPeV); influenza A virus of unknown subtype; influenza A H1N1 virus; influenza B virus; and *Mycoplasma pneumoniae.*

The real-time multiplex reverse transcription chain reaction (RT-PCR) was performed on the LightCycler 480 (F. Hoffmann-La Roche AG, Basel, Switzerland). The FTD21 assay is an in vitro diagnostic (IVD) test and carries the CE mark. It has an overall diagnostic sensitivity of 93.94 % and an overall diagnostic specificity of 98.93 % (https://www.siemens-healthineers.com/molecular-

diagnostics).

2.4. Statistical analysis

Categorical variables were expressed as percentages and compared using the chi-square test. Quantitative variables are presented as median and range. The chi-square test for trends was used to compare groups. A p-value <0.05 was considered statistically significant. All p-values were two-tailed. All statistical analyses were performed using GraphPad PRISM version 6.0e (GraphPad Software, San Diego, CA, USA) and R package (https://www.r-project.org).

3. Results

3.1. Patient characteristics

A total of 545 patients were collected from Sabah Medical Hospital over seven consecutive months from September 2021 to March 2022. The baseline characteristics and outcomes of the study patients are summarized in Table 1. The median age of patients enrolled in this study was 3 years (1 week- 97 years), with the majority of patients aged less than younger than 5 years (51.6 %) (Table 1). Females accounted for 52.1 % of the study population, while males represented 47.9 %. The majority of deaths from respiratory infections were observed among babies aged 1 week to less than one year, comprising 35 cases (6.6 %).

3.2. Viral and atypical bacterial etiologies

All 545 patients tested negative for SARS-CoV-2 infection. Of the 545 respiratory samples collected between September 2021 and March 2022, 411 (75.4 %) were positive for at least one respiratory pathogen, whereas infection with multiple pathogens was less frequently observed (128/545 cases, 23.8 %) (Table 2).

HRV was the most frequently detected virus, present in 178 (32.7 %) of all patients, followed by RSV, HMPV, HBoV, and influenza A (20.9 %, 14.1 %, 13.2 %, and 12.7 %, respectively) (Fig. 1). Other respiratory viruses were reported at lower frequencies, including AdV (8.0 %), influenza B (7.3 %), HPIV4 (5.1 %), coronavirus OC43 (4.2 %), HPIV4 (3.9 %), coronavirus 229E (2.4 %), coronavirus HKU1 (1.7 %), HPeV (1.3 %), EV (1.0 %), and HPIV1 (1.0 %). Infection with *M. pneumoniae* was found in one case (0.2 %) (Fig. 1). The absence of detection of coronavirus NL63 was noted in our study population.

In multiple infections, the most frequent co-infection was that of HRV/AdV observed in 10.9 % (14/128), followed by RSV/HBoV (7.8 %, 10/128), RV/RSV in 7.0 % (9/128), and HBoV/influenza A in 6.3 % (8/128).

The large majority of deaths probably due to respiratory infections occurred in babies aged 1 week to less than one year (35 cases; 6.5 %). The distribution of deaths by pathogen is shown in Fig. 1. Among the newborn deaths, the most associated pathogens were RSV (40 %, 14/35), HRV (22.9 %, 8/35), HMPV (14.3 %, 5/35) and HPIV4 (8.6 %, 3/35) (Fig. 1).

Table 1
Baseline characteristics of study group.

	N = 545 No. (%)
Age, years	
< 1	34 (6.2)
1–4	281 (51.6)
5–10	52 (9.5)
11–30	56 (10.3)
31–50	59 (10.8)
>50	63 (11.6)
Gender	
Female	284 (52.1)
Male	261 (47.9)
Nationality[a]	
Kuwaiti	420 (77.9)
Non-Kuwaiti	119 (22.1)
Outcome[a]	
Alive	508 (93.6)
Dead	35 (6.4)
Sample type	
Nasopharyngeal/Throat swabs	523 (96.0)
Sputum	12 (2.2)
Bronchoalveolar lavage/Tracheal aspirate	7 (1.3)
Endotracheal secretion	3 (0.6)

[a] Data are missing.

Table 2

Distribution of respiratory pathogens by age group.

| Pathogen	Total	Positive cases per age group, years						
		<1 No. (%)	1–4 No. (%)	5–10 years No. (%)	11–30 years No. (%)	31–50 years No. (%)	>50 years No. (%)	p value
Adenovirus	47	2 (4.3)	**33 (70.2)**	3 (6.4)	4 (8.5)	2 (4.3)	3 (6.4)	<0.0001
Bocavirus	76	4 (5.3)	**55 (72.4)**	7 (9.1)	3 (4.0)	2 (2.6)	5 (6.6)	<0.0001
Coronavirus 229E	13	0 (0.0)	**5 (38.5)**	3 (23.1)	1 (7.7)	2 (15.4)	2 (15.4)	0.145
Coronavirus HKU1	9	0 (0.0)	3 (33.3)	0 (0.0)	1 (11.1)	1 (11.1)	**4 (44.4)**	0.056
Coronavirus NL63	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	–
Coronavirus OC43	23	0 (0.0)	**14 (60.9)**	1 (4.4)	3 (13.0)	1 (4.4)	4 (17.4)	<0.0001
Enteroviruses	5	1 (20.0)	**3 (60.0)**	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	0.080
Human metapneumovirus A/B	78	5 (6.4)	**48 (61.5)**	6 (8.0)	10 (12.8)	3 (3.9)	6 (7.7)	<0.0001
Parechovirus	7	0 (0.0)	**7 (100.0)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.0001
Influenza A	68	0 (0.0)	14 (20.6)	8 (11.8)	14 (20.6)	13 (19.1)	**19 (27.9)**	<0.001
Influenza B	39	2 (5.1)	**15 (38.5)**	6 (15.4)	5 (12.8)	10 (25.6)	1 (2.6)	<0.001
Mycoplasma pneumoniae	1	0 (0.0)	0 (0.0)	0 (0.0)	**1 (100.0)**	0 (0.0)	0 (0.0)	0.306
Parainfluenza virus	54	3 (5.6)	**30 (55.6)**	5 (9.3)	5 (9.3)	5 (9.3)	6 (11.1)	<0.0001
Respiratory syncytial virus A/B	116	15 (12.9)	**73 (62.9)**	6 (5.2)	5 (4.3)	10 (8.6)	7 (6.0)	<0.0001
Rhinovirus	179	6 (3.4)	**106 (59.2)**	22 (12.3)	17 (9.5)	14 (7.8)	14 (7.8)	<0.0001
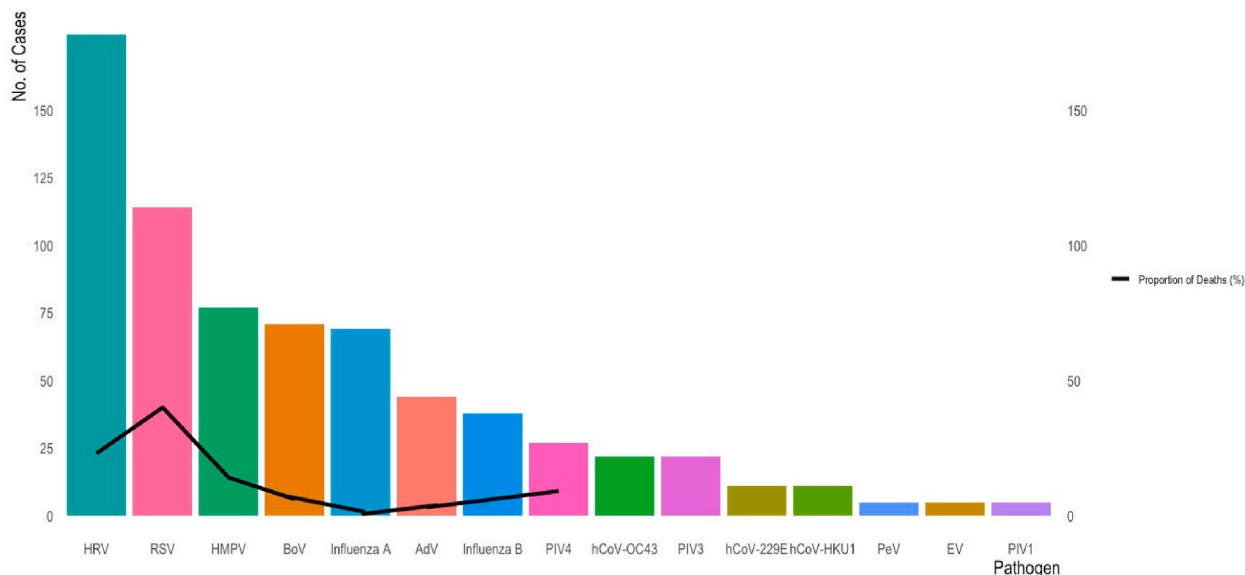
Fig. 1. Viral pathogens causing severe lower respiratory tract infections (LRTIs) morbidity, and deaths in hospitalized patients at Sabah Medical Hospital from September 2021 to March 2022. HRV, human rhinovirus; AdV, adenovirus; RSV, respiratory syncytial virus; PIV4, parainfluenza virus 4; HMPV, human metapneumovirus; PIV, parainfluenza virus; HBoV, human bocavirus; EV, enterovirus; hCoV, human coronavirus.

3.3. Distribution of respiratory pathogens by month

Fig. 2 illustrates the trend in the number of hospitalized non-COVID-19 cases over the study period, with a clear peak observed in hospitalizations for non-COVID-19 cases during November and December. Rates of single respiratory pathogen infections remained consistent throughout the seven-month study period (September 2021 to March 2022) (Table 2). The number of viruses detected peaked during November to January. Interestingly, the prevalence of several respiratory viruses varied by month over the study period. AdV, coronavirus HKU1, HMPV, influenza A, influenza B, RSV, and HRV exhibited significant variations depending on the month of the year (Fig. 2).

Peaks in HKU1 and AdV were observed in September, while HMPV was most active in January and February. Influenza A and influenza B had the highest detection rates from November to January. RSV accounted for a substantial number of detections from October to November. The highest rates of HRV infections were reported in February and March. On the other hand, coronaviruses
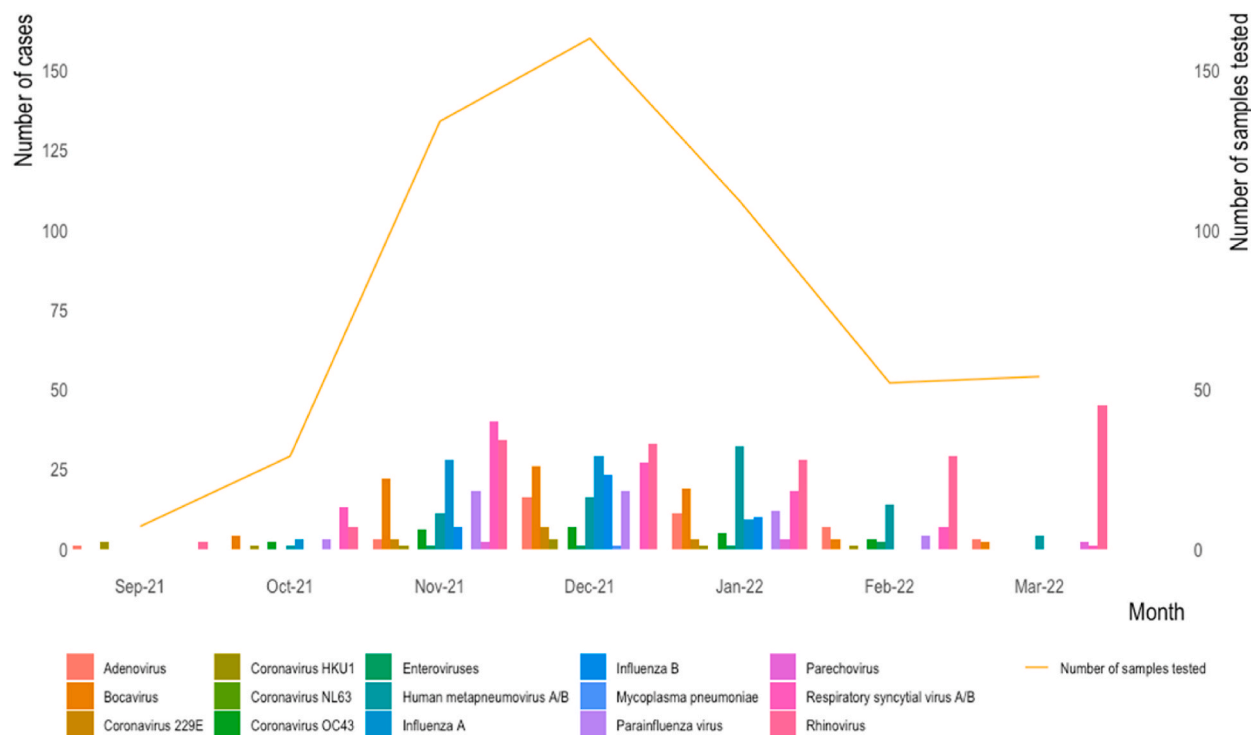
Fig. 2. Distribution of different respiratory pathogens among hospitalized non-COVID-19 cases over the study period.

229E, OC43, HBoV, EV, and HPeV did not exhibit month-specific peaks, and their detection rates remained relatively constant over the study period (Fig. 2).

3.4. Age-related distribution of respiratory pathogens

The frequency of each pathogen in each respective age group is presented in Table 2. A statistically significant difference was observed between all age groups regarding the most detected pathogens ($p < 0.05$). Notably, the under-5 age group was the most affected (Table 2). It is noteworthy that all detected cases of HPeV were exclusively in children under 5 years of age (100 %). HBoV was the most frequently identified pathogen, with a detection rate of 72.4 %, followed by AdV (70.2 %), RSV (62.9 %), HMPV (61.5 %), EV (60 %), and RV (59.2 %). In contrast, HKU1 (44.4 %) predominated in the over-50 age group, followed by Influenza A (28.0 %) (Table 2).

4. Discussion

This is a cross-sectional study to determine the etiology severe respiratory infections and the prevalence of these respiratory pathogens in infants, children, and adults hospitalized in PICUs and ICUs in Kuwait for seven consecutive months (September 2021–March 2022) during the COVID-19 epidemic. Most surveys worldwide focus on infants and children. Furthermore, NPIs such as social distancing, wearing masks, and hygiene promotion were strongly promoted as the pandemic progressed, reducing not only the rate of COVID-19 but also that of other circulating viruses [24]. To our knowledge, this is the first study to attempt to describe the etiology of severe lower respiratory tract infections in patients hospitalized during the COVID-19 pandemic in Kuwait. The etiology of acute respiratory infections is diverse and complicated, and may vary according to sex, age and season [25,26]. This survey showed that 51.6 % of cases were under-5 years of age. This result appears to be consistent with previous reports from Kuwait using molecular methods for virus detection [27,28]. In the present study, respiratory viruses were detected in 75.4 % of patients between September 2021 and March 2022. This rate is significantly higher compared to pre-COVID-19 data in Kuwait, 28.4 %–47.8 % of hospitalized patients with respiratory tract infections from 2010 to 2015 [20,21]. However, this result seems comparable to that of previous surveillance studies using multiplex detection of respiratory viruses. Indeed, higher positivity rates have been reported in the USA (93.0 %) [29], India (82.0 %) [30], Qatar (78.0 %) [31], Bangladesh (73.0 %) [32], Madagascar (75.1–83.1 %) [33,34], Turkey (71.4 %) [35], Brazil (61.8 %) [22] and Cameron (61.0%–65.4 %) [36,37]. In contrast, lower rates have been reported in other surveys. For example, a lower rate of positivity for viral respiratory infections (30.7 %) in hospitalized patients with severe acute respiratory infections was reported in sentinel surveillance conducted in the Eastern Mediterranean region (Egypt, Jordan, Oman, Qatar, and Yemen) between December 2007 and February 2014 [38]. In addition, a retrospective study conducted in Qatar between 2012 and

2017 on cases of influenza illness showed that 46.5 % of patients tested were positive for at least one respiratory pathogen [23]. Similarly, lower positivity rates have been reported in Saudi Arabia (48.0 %) [39], the Philippines (43.0 %) [40], Peru (42.6 %) [41], Italy (41.7 %) [42], Colombia (36.0 %) [43] and China (34.6 %) [44]. These differences may be due to a variety of factors, including the study population, study design, and diagnosis tools.

The co-infection rate in this study (23.8 %) was comparable to previous investigations [29,45], but relatively high compared to the previous co-infection rate (15.4 %) reported in a previous study in Kuwait [27].

In our study, HRV and RSV were the most frequently detected pathogens, followed by HMPV (14.1 %), HBoV, and influenza A during a period of NPIs strengthening. The profile of viruses detected in patients with lower respiratory tract infections was similar to previous data in Kuwait using molecular methods for virus detection [27,28,46]. Furthermore, the predominance of HRV in our series of patients appears consistent with the results of other reports from developed and developing countries using multiplex PCR. These reports show a high incidence of HRV infection during the COVID-19 pandemic [25,33,47–52]. Notably, HRV, RSV, and influenza A are indeed among the most prevalent viruses responsible for respiratory infections, causing significant morbidity and mortality worldwide [53–55].

The rates of single infection with respiratory pathogens remained similar throughout the months of follow-up. However, we observed peaks for certain respiratory viruses. AdV, HKU1, and RSV exhibited a high incidence in November, while influenza A, influenza B, and HMPV were more prevalent in December and February. HRV isolates were detected in every month of the follow-up, with the highest positive rates recorded in February–March. This is consistent with previous surveillance studies conducted in Kuwait between 2010 and 2015 [27,28,46], and they are similar to reports from countries in the Arabian Peninsula [23,53,54].

We observed that the under-5 age group had higher proportions of pathogens detected. In this age group, HBV, AdV, RSV, EV, and RV are the most frequently identified pathogens, considered to be the most common viruses causing LRTIs in children aged younger than 5 years [55–57]. Previous reports suggest that HBoV may be the etiological agent responsible for respiratory tract diseases in young children [58]. There was also a direct correlation between high HBoV and severity in children under five years of age in patients co-infected with at least one other respiratory virus [59]. Serological surveys have shown that the rate of HBoV seropositivity increases with age, reaching 100 % [60,61], suggesting that most children are exposed to HBoV during the first 6 years of life [61–63].

RSV is widely acknowledged as a primary cause of severe LRTIs in infants and young children, particularly among those less than one year of age, often leading to hospitalization and significant morbidity and mortality. However, our study showed a low percentage of RSV cases, indicating a different epidemiological pattern in this population compared to previous research [56–58]. The disparity between our findings and the existing literature may be attributed to the stringent infection control practices enforced during the COVID-19 pandemic.

This discrepancy underscores the importance of context-specific epidemiological surveillance and clinical research to guide local public health strategies and clinical management practices. The presence of RSV in neonates and young children suggests that a maternal RSV vaccine, administered to pregnant women, may offer protection to infants up to six months of age [59].

All HPeV detected were only found in children under 10 years of age (100 %), indicating that most HPeV infections are often acquired in early childhood and is in agreement with previous studies [57,58]. HKU1 (44.4 %) predominates in the over-50 years. This result appears to be consistent with a previous study [64].

Most cases of hospitalization and death occurred in young children. In addition, all deaths occurred in newborns hospitalized for acute lower respiratory tract infections. This study confirms that young children are the group most vulnerable to severe acute respiratory infections requiring hospitalization, which is comparable to previous reports [31, 34, 65].

Understanding the seasonality of acute respiratory infections (ARI) is crucial for effective public health planning and resource allocation, particularly in regions like Kuwait where environmental and socio-demographic factors may influence transmission dynamics. Our study primarily focused on the virological aspects of ARI cases during the pandemic period. Still, it is important to acknowledge the broader context of ARI seasonality in Kuwait. While specific data on ARI hospitalizations in Kuwait are scarce, previous studies have suggested that respiratory virus activity in the region may follow seasonal trends similar to those observed in temperate climates [60–62].

The strength of our study indeed lies in the utilization of a multiplex PCR testing approach to explore the etiology of infections in patients hospitalized with severe lower respiratory tract infections. This method allows for the simultaneous detection of multiple viral pathogens, providing valuable insights into the viral causes of respiratory infections. However, it is important to note that our approach does not include respiratory bacterial cultures. As a result, bacterial pathogens may not have been adequately assessed in our study, potentially limiting our understanding of the complete etiology of severe lower respiratory tract infections. Another limitation of our study is the numbers of cases was limited and were taken from only one center, which is not representative of the whole population, preventing us from making a true estimate of prevalence at the national level. Secondly, the clinical course during hospital stays and the radiological findings of cases were not collected during follow-up. Moreover, the use of different sample types (nasopharyngeal/throat swab, sputum, bronchoalveolar lavage/tracheal aspirate, and endotracheal secretion) could affect the sensitivity and specificity of the test, which is a limitation of our study. Another limitation of our study is the lack of data for a full calendar year. Without complete data spanning all seasons, it is challenging to accurately assess the seasonality of virus transmission patterns. Future research endeavors should prioritize collecting data over multiple years to comprehensively capture seasonal variations and enhance our understanding of virus transmission dynamics over time in Kuwait.

5. Conclusions

Our data showed the dynamics of respiratory pathogens, other than SARS-CoV-2, in Kuwait for the first time during the COVID-19

pandemic. We observed a high rate of respiratory viruses in the etiology of hospital admissions associated with severe lower respiratory tract infections in Kuwait, highlighting the importance of maintaining national surveillance of lower respiratory tract infections. Our study clearly demonstrated that HRV and RSV were the most frequently identified viruses in almost all age groups. Increased laboratory-based surveillance of hospital admissions due to respiratory diseases could help identify pathogens and at-risk groups with a view to targeted use of vaccines and other preventive measures.

In this context, multiplex PCR could be used as a good strategy for the rapid detection and precise identification for a large number of respiratory viruses. We, therefore, stress the need for such surveillance at the national level to determine the dynamics and etiology of severe lower respiratory tract infections in the population for evidence-based public and individual health programs.

Patient consent for publication

No consent to publish was needed for this study, as we did not use any identity related to individual participants.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the permanent Committee for Coordination of Medical and Health Research, Ministry of health, Kuwait. Informed consent was obtained from all subjects involved in the study (N°2021).

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Data availability statement

The corresponding authors have full access to all the data in the study. Data will be made available on request.

CRediT authorship contribution statement

Haya Altawalah: Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wadha Alfouzan:** Writing – review & editing, Conceptualization. **Talal Al-Fadalah:** Methodology. **Mariam Ali Zalzala:** Methodology, Data curation. **Sayeh Ezzikouri:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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