Circulation Pattern and Genetic Variation of Rhinovirus Infection among Hospitalized Children on Hainan Island, before and after the Dynamic Zero-COVID Policy, from 2021 to 2023

Meifang Xiao[1], Afreen Banu[1], Yibo Jia[2], Gaoyu Wang[2], Meng Chang[2], Jing An[3], Yi Huang[2], Xiaoyuan Hu[2], Chuanning Tang[2], Zihan Li[2], Yi Niu[2], Xiuying Tian[2], Wanxin Deng[2], Cheng Tang[2], Jiang Du[2], Xiuji Cui[2], Jasper Chan[4], Ruoyan Peng[2], and Feifei Yin[2]

[1]Lincoln University College Faculty of Medicine
[2]Hainan Medical University
[3]Hainan Women and Children's Medical Center
[4]The University of Hong Kong State Key Laboratory of Emerging Infectious Diseases

May 20, 2024

Abstract

Objective Throughout the COVID-19 pandemic, Rhinovirus (RV) remained notable persistence, maintaining its presence while other seasonal respiratory viruses were largely suppressed by pandemic restrictions during national lockdowns. This research explores the epidemiological dynamics of RV infections among pediatric populations on Hainan Island, China, specifically focusing on the impact before and after the zero-COVID policy was lifted. **Methods** From January 2021 to December 2023, 19,680 samples were collected from pediatric patients hospitalized with acute lower respiratory tract infections (ARTIs) at the Hainan Maternal and Child Health Hospital. The infection of RV was detected by tNGS. RV species and subtypes were identified in 32 RV-positive samples representing diverse time points by analyzing the VP4/VP2 partial regions. **Results** Among the 19,680 pediatric inpatients with ARTIs analyzed, 21.55% were found to be positive for RV infection, with notable peaks observed in April 2021 and November 2022. A gradual annual decline in RV infections was observed, alongside a seasonal pattern of higher prevalence during the colder months. The highest proportion of RV infections was observed in the 0-1 year age group. Phylogenetic analysis revealed 23 distinct RV subtypes, with a shift in dominance from RV-A to RV-C in 2022, suggesting evolving RV dynamics. **Conclusions** The research emphasizes the necessity for ongoing surveillance and targeted management, particularly for populations highly susceptible to severe illnesses caused by RV infections.

Introduction

During the COVID-19 pandemic, a virological anomaly that garnered attention was the persistent global prevalence of rhinoviruses (RV). The non-pharmaceutical interventions implemented to mitigate SARS-CoV-2 transmission—such as mask-wearing, social distancing, and lockdowns—resulted in a substantial reduction in the incidence of respiratory viruses, including influenza, parainfluenza, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV)[1, 2]. However, in notable contradistinction, RV demonstrated adaptability and appeared to coexist with SARS-CoV-2 at a rate consistent with its typical seasonal patterns, despite the decrease in interpersonal contacts due to these public health measures[3]. RV is renowned for its efficient transmission through both airborne particles and fomites. As non-enveloped viruses, rhinoviruses exhibit prolonged survivability on surfaces and resistance to disinfectants containing alcohol, facilitating effective fomite transmission[4-6]. This characteristic likely played a pivotal role in the sustained propagation of RV within populations, even in the face of various pandemic measures such as masking, social distancing, and lockdowns.

RV, commonly known as the "common cold virus," has historically been overlooked as a causative agent of severe illness[7]. Nevertheless, in recent years, the clinical significance of RV has garnered considerable attention. Beyond causing common colds, RV infections manifest in a spectrum of clinical outcomes, ranging from asymptomatic infections to severe lower respiratory conditions, including bronchiolitis, pneumonia, and asthma exacerbations[8-10]. The diverse clinical presentations of RV infections indicate a potential association between illness severity and various host and environmental factors[11]. Additionally, factors intrinsic to the virus may influence the transition from asymptomatic infection to severe illness. However, the complexity of this research is compounded by RV's status as the most diverse virus family within the enterovirus genera. The current RV classification encompasses three species (RV-A, RV-B, and RV-C), further divided into 169 subtypes[12]. Identifying the most clinically relevant subtypes within this diversity is crucial for developing targeted therapeutic strategies against this virus. Despite concerted efforts, establishing meaningful connections between RV species as well as subtypes and symptoms, seasonal patterns, severity, and interactions with other viruses has proven challenging.

Following the extensive COVID-19 epidemic in February 2020, China transitioned into a phase of normalized prevention and control measures starting in May 2020[13]. Responding to the heightened transmissibility of the Delta variant, China implemented a novel strategy termed "Dynamic COVID-zero" in August 2021. This strategic approach encapsulates China's accumulated knowledge in effectively managing the Delta variant spread, aiming to control the epidemic with heightened efficiency, reduced cost, and in a shorter timeframe[14]. The core of this strategy lies in the precise implementation of comprehensive measures to address localized COVID-19 cases, swiftly sever the transmission chain, and promptly terminate the epidemic[15].

However, in response to the emergence of the SARS-CoV-2 Omicron subvariants, characterized by attenuated pathogenicity, coupled with the broadening vaccination coverage, these stringent policies were lifted on December 7, 2022. The abrupt shift in Non-Pharmaceutical Interventions (NPIs), combined with the accrued 'immunity debt' and viral evolution, potentially contributed to the resurgence of other respiratory viruses[16]. Notably, an outbreak of influenza A virus among children was documented following the conclusion of the zero-COVID policy in Shanghai, China[17]. Previous research has indicated that viral interference among influenza virus, rhinovirus, and other respiratory viruses can influence viral infections at both the individual and population levels[18]. Hence, it becomes essential to closely monitor the alterations in the pattern of RV infections post the ending of the zero-COVID policy.

This study investigates epidemiological shifts in RV infections among children on Hainan Islands, with a focus on the periods before and after the termination of the zero-COVID policy. Hainan, a tropical island located at China's southernmost tip, is known for its warm temperatures, high humidity, and status as a major free trade port and tourist destination. These unique climatic and geographical attributes potentially present distinct health challenges. Specifically, the research provides insights into how policy changes, notably the ending of the zero-COVID policy, influenced the dynamics of respiratory viruses from 2021 to 2023. The aim of this investigation is to enhance our comprehension of RV circulation and associated diseases.

2. Methods

2.1 Sample collection and RV detection

In this study, a comprehensive analysis was conducted on 19,680 samples obtained from pediatric patients hospitalized with acute lower respiratory tract infections (ARTIs) at the Hainan Maternal and Child Health Hospital. The collection period extended from January 2021 to December 2023. The cohort included in this study comprised patients aged 18 years or younger, all presenting with symptoms indicative of ARTIs upon hospital admission. Nasopharyngeal swabs were systematically collected at the time of admission. Subsequently, these samples were preserved at a temperature of -80 for subsequent examinations. The infection of RV was detected by tNGS, facilitated by KingMed Diagnostics.

2.2 RV classification using VP4/2 sequences

Thirty-two RV-positive samples, representing diverse time points, were randomly selected for genotyping.

Genotyping was performed by amplifying the partial VP4/VP2 region using nested PCR[19].The primer sequences employed are detailed in the Supplementary Material (Table S1). To extract total viral RNA, 200 µl of each sample was processed using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA), following the manufacturer's guidelines. The nested PCR was performed using the SuperScript III One-Step RT-PCR System (Invitrogen, USA) and Go Taq Colorless Master Mix (Promega, USA).

The initial PCR cycle entailed a 5-minute activation at 94°C, followed by 40 cycles of 30 seconds at 95°C for denaturation, 30 seconds at 55°C for annealing, and 5 minutes at 72°C for extension, concluding with an additional 10-minute extension at 72°C. The second round of PCR mirrored the first, with the exception of the annealing temperature, set at 56°C. Each PCR run included a negative control alongside the samples. The resulting 540 bp amplification products were visualized on a 1.5% agarose gel. PCR products from positive samples were forwarded to Beijing Qingke Biotechnology Co., Ltd. for Sanger sequencing. Sequence alignments were conducted using SeqMan software, and the sequences were verified through database comparison using the BLAST tool at the National Center for Biotechnology Information (NCBI).

2.3 Phylogenetic analysis

The VP4/VP2 region reference sequences of RV, consisting a set of 153 sequences, were obtained from the GenBank database and incorporated into the analysis. For phylogenetic tree construction, MEGA X software was employed, utilizing the neighbor-joining method. The robustness of the phylogenetic trees was evaluated through bootstrap analysis with 1000 replicates. Genetic diversity, sequence identity, and amino acid variation were analyzed using BioEdit software. The sequences identified in this study have been submitted to the GenBank database, with accession numbers ranging from OR575561 to OR575592.

2.4 Statistical analysis

The experimental data were analyzed using the IBM SPSS Statistics program (version 26). The data were analyzed using the $\chi 2$ test. P values less than 0.05 were considered statistically significant. Use GraphPad Prism 6.0 software to make statistical analysis graphs.

3. Results

3.1 Sample information and demographics

From January 2021 through December 2023, a total of 19,680 pediatric inpatients diagnosed with ARTIs were admitted to Hainan Maternal and Child Health Hospital. The demographic details of the cases have been summarized in table 1. The age range of these patients spanned from 1 month to 18 years, with an average age of 2.97 ± 2.86 years. The gender distribution exhibited a notable skew towards males, constituting 12,126 (61.62%) of the patient cohort, while females accounted for 7,554 (38.38%) (Table 1).

3.2 Analysis of RV infection trends before and after the ending of Dynamic Zero-COVID Policy

Throughout the study period, 4,242 (21.55%) patients tested positive for RV. RV infections exhibited multiple peaks, with the highest infection rate observed in ARTIs during April 2021 and November 2022 (Figure 1). The overall prevalence of RV infections among pediatric inpatients with ARTIs was 24.36% (1150/4721) in 2021, decreasing to 22.54% (1178/5227) in 2022, and further to 19.67% (1914/9732) in 2023 (Table 2). Statistical analysis revealed significant difference indicating a disparity in the distribution of RV between different years ($\chi^2 = 45.45$, $p < 0.001$). Pairwise comparisons further revealed statistically significant variations in the distribution of RV across these years, indicating that the overall infection rate of RV continues to decline from 2021 to 2023. In 2022, Hainan encountered two significant COVID-19 pandemic in March and August. Subsequently, stringent non-pharmaceutical interventions (NPIs) were implemented and maintained across the year until the repeal of the zero-COVID policies. Despite these measures, a notable suppression of RV infections was not observed, with the infection rate exhibiting only a marginal decrease from 24.36% in 2021. Considering gender divisions, RV infection rates were 22.75% (2759/12126) in males and 17.08% (1290/7554) in females among all inpatients with ARTIs, with a statistically significant difference observed between these rates ($\chi^2 = 91.75$, $p<0.001$). The chi-square test for gender differences across the years did not reveal statistical significance (χ2=0.20, p=0.91), indicating a stable gender distribution over time (Table 2).

Specifically, in the spring of 2021, the RV infection rate was observed to be 37.62%, decreasing to 14.59% in the summer. The rate subsequently increased to 21.81% in the autumn and further to 26.56% in the winter, evidencing significant seasonal variation (χ^2 = 157.88, p < 0.001). The following year, 2022, exhibited a similar pattern with rates of 24.18% in spring, 12.48% in summer, a notable rise to 39.92% in autumn, and 20.77% in winter, again demonstrating significant seasonal differences (χ^2 = 216.25, p < 0.001). In 2023, the infection rates were 23.50% in spring, 16.83% in summer, 20.07% in autumn, and 17.67% in winter, maintaining the trend of significant seasonal variability (χ^2 = 38.74, p < 0.001). These results are in concordance with existing literature, which indicates a higher prevalence of RV in the autumn, winter, and spring months, with a reduced incidence observed during the summer. This consistency reinforces the established understanding of RV seasonal dynamics[20].

3.3 Age-based Analysis of RV Infections

In this investigation, RV-positive patients were categorized into four age groups in alignment with the traditional living habits of Chinese children: 0-1 years (Group 1), 1-3 years (Group 2), 3-7 years (Group 3), and 7-18 years (Group 4). Between 2021 and 2023, in terms of age distribution of patients with RV infection, the highest proportion of RV patients was observed in the 0-1y group, accounting for 36.12% (1532/4242). This was followed by the 3-7y group had a prevalence of 30.46% (1292/4242). The 1-3 year group accounted for 25.93% (1100/4242), while the remaining 7.50% (318/4242) were in the 7-18y group (Table 3). A year-on-year analysis revealed that the proportion of RV infections within the 0-1 years group accounted for 39.5% and 40.24% of total annual positive cases in 2021 and 2022, respectively, but decreased to 31.5% in 2023. Statistical analysis indicated a significant variance in these proportions (χ^2 = 32.24, p< 0.001). For children aged 1-3 years, the proportion of RV infections in the total annual infection decreased from 31.91% in 2021 to 20.12% in 2022, with an increase to 25.91% in 2023. The chi-square test indicated that these variations were significant (χ^2 = 42.15,p < 0.001). Within the 3-7 year age group, the proportion of RV infections in the total annual infection increased exhibited an increase from 25.39% in 2021 to 32.85% in 2022, with a further reduction to 30.03% in 2023. The chi-square test suggested statistical significance (χ^2 = 19.35, p < 0.001). The 7-18 year age group exhibited an increase from 3.13% in 2021 to 6.80% in 2022, with a further rise to 10.55% in 2023. Statistical analysis confirmed the significance of this trend (χ^2 = 58.26, p< 0.001) (Table 3).

Regarding the infection rates of RV among inpatients with ARTIs across various age groups, this study identifies significant fluctuations in the incidence rates among different groups. Between 2021 and 2023, the most pronounced incidence of RV-positive cases was observed within the 0-1 year age group, which accounted for 24.94% (1532 out of 6142) of all cases. This was followed by the 1-3 year age group, accounting for 21.55% (1100 out of 5105). The 3-7 year age group exhibited a prevalence of 21.30% (1292 out of 6066), while the 7-18 year age group comprised 13.43% (318 out of 2367) of cases. The RV infection rates were significantly different between the age groups (χ^2 = 134.24,p <0.001). As shown in figure 2, in the 0-1 year age group, a notable fluctuation in incidence rates was observed in year-on-year analysis, rising from 22.25% in 2021 to 33.55% in 2022, followed by a decrease to 22.47% in 2023. These changes were found to be statistically significant (χ^2 = 72.57, p < 0.001). For children aged 1-3 years, a decrement in the incidence rate from 26.05% in 2021 to 18.16% in 2022 was noted, with a slightly increase to 20.74% in 2023. The chi-square test indicated that these variations were significant (χ^2 = 26.64, p < 0.001). Within the 3-7 year age group, the incidence rate exhibited a decline from 26.14% in 2021 to 21.39% in 2022, with a further reduction to 19.52% in 2023. The chi-square test yielded a value of 21.55, suggesting statistical significance (χ^2 = 21.55, p < 0.001). The 7-18 year age group witnessed a marked decrease in the incidence rate from 24% in 2021 to 11.43% in 2022, with a slight rise to 13.32% in 2023. Statistical analysis confirmed the significance of this trend (χ^2 = 16.84, p < 0.001). Notably, the majority of RV cases occurred in toddlers aged under three years (Table 2).

3.4 Phylogenetic analysis of RV

RV subtypes were identified in 32 RV-positive samples representing diverse time points by analyzing the VP4/VP2 partial regions, comparing them with reference strain sequences of RV-A, B, and C found in the Online 10th Report of the International Committee on Taxonomy of Viruses. As shown in figure 3, phylogenetic analysis of the VP2-VP4 region disclosed the presence of three RV species among the positive samples: RV-A, accounting for 46.88% (15 out of 32), RV-B at 6.25% (2 out of 32), and RV-C, also comprising 46.88% (15 out of 32).

Between 2021 and 2023, there were circulation of 23 distinct confirmed RV subtypes found in this study. RV-A was the most diverse species, encompassing 11 different types (RV-A7, 20, 29, 30, 49, 55, 58, 60, 66, 78, 80). The most prevalent genotypes identified were RV-A66, RV-A80, and RV-A20. In the RV-B category, two types were detected: RV-B3 and RV-B37. As for RV-C, ten different types were noted: RV-C5, C6, C13, C15, C17, C18, C27, C32, C40, C42, and C88, with RV-C15, RV-C6, and RV-C13 being the most common (Figure 3). In this investigation, a notable shift in the dominance between RV-A and RV-C was observed. During the period from April to June 2022, RV-A exhibited a higher prevalence, with a ratio of RV-A to RV-C recorded at 4:1. However, in the latter part of the year, specifically between October and December 2022, the pattern shifted notably, with the prevalence ratio adjusting to a more balanced 1:1.33. This variation suggests a dynamic pattern in the circulation and genetic diversity of RV infections among the pediatric population.

3.5 Impact of RV genotypes on severity of respiratory infections

To explore the relationship between RV subtypes and clinical characteristics, clinical information from the 32 patients with identified subtypes was analyzed (Table 4). Eight patient was diagnosed with asthma among the 32 patients. RV-C was found to be the predominant species in asthma patients, accounting for 50% (4/8) of asthma cases, followed by RV-A for 37.5%(3/8), and RV-B for 12.5%(1/8). There was no significant difference among the positive rate of asthma cases in the group of patients with the same RV species (p =0.667).

In this study, the 32 patients were further divided into non-severe and severe lower respiratory tract infection (LRTI) categories. There was no significant age differences observed between the groups (Table 4). Males demonstrated a higher prevalence of severe RV-associated LRTIs, accounting for 85.71% of severe cases. Asthmatic children showed a greater susceptibility to severe LRTIs and extended hospitalizations, suggesting that asthma may elevate the risk of intensified infections in those with RV.

4. Discussion

Since the emergence of SARS-CoV-2 at the end of 2019, a broad range of strategies, notably NPIs, have been globally implemented to combat the virus. These measures, including city lockdowns, social distancing, use of personal protective equipment, and enhanced hygiene practices, have substantially reduced coronavirus transmission. The application of NPIs has notably interrupted the usual seasonal patterns of common respiratory viruses. A marked decrease in influenza and Respiratory Syncytial Virus (RSV) cases was observed following NPI implementation[21]. In contrast, Rhinovirus (RV) infections exhibited an increasing trend. This is supported by studies from New Zealand, which reported a high incidence of RV infection during the pandemic[22]. Furthermore, a study in Suzhou, China, involving 10,396 viral respiratory infection cases, identified RV as the most frequently detected virus, accounting for 23.3% of infections[23]. Between January 2021 and December 2023, our study included 19,680 pediatric inpatients with ARTIs. Of these, 21.55% tested positive for RV, peaking in April 2021 and November 2022. A year-on-year decrease in RV infections was noted, declining from 24.36% in 2021 to 19.67% in 2023. Seasonal fluctuations in RV infections aligned with existing literature, demonstrating a higher prevalence in autumn, winter, and spring. The study observed that the 0-1 year age group had the highest proportion of RV infections. Significant variations in infection rates across different age groups were noted, predominantly in children under three years. Phylogenetic analysis revealed 23 distinct RV subtypes, with RV-A and RV-C being the most common. A significant shift in the dominance between RV-A and RV-C in 2022 indicated a dynamic pattern in RV circulation and genetic diversity. The study also investigated the correlation between RV subtypes and clinical characteristics, finding a predominance of RV-C in asthmatic patients and a higher incidence of severe RV-associated Lower

Respiratory Tract Infections (LRTIs) in males. This study explores the epidemiological shifts in RV infections among children on Hainan Island, emphasizing the effects of the termination of the zero-COVID policy in a region with distinct climatic and geographical characteristics.

In March and August 2022, Hainan, China, experienced two COVID-19 pandemic. In response, strict NPIs were enforced throughout the year, until the lifting of zero-COVID policies. Despite the implementation of preventive measures, a significant reduction in RV infections was not evident, suggesting that the efficacy of face masks in curbing RV transmission might have diminished during this period. RV is a non-enveloped virus known for its relative resistance to disinfectants containing ethanol and its ability to survive on environmental surfaces for extended periods transmission[4, 5]. While medical masks are effective in blocking large droplets and aerosols, they may not provide adequate protection against smaller particles like RV. This may also be one of the main reasons why RV infection rates remained high during the COVID-19 epidemic. Notably, with the advent of the Omicron variant and a substantial increase in vaccination rates, governments worldwide have begun to roll back these stringent measures, aiming to normalize social activities. This policy shift has led to an increase in reports of respiratory virus resurgence[20]. According to monitoring by the World Health Organization, after a significant reduction in the transmission of respiratory viruses globally during the COVID-19 pandemic, there has been a marked increase in the activity level of respiratory viruses across the world [22]. On 7 December 2022, China's modification of the dynamic zero-COVID-19 policy precipitated a widespread outbreak of COVID-19 in the following months. This alteration, together with the relaxation of non-pharmaceutical interventions (NPIs) and social distancing measures, is presumed to have impacted the epidemiology of other respiratory tract infections. Particularly, an increase in influenza A virus infections among children was noted in Shanghai after the policy change[17]. The concept of viral interference, involving interactions between influenza virus, rhinovirus, and other respiratory viruses, is a plausible factor in the decreased prevalence of RV infections observed in pediatric inpatients with ARTIs in 2023. This suggests a complex interplay of viral interactions within the evolving public health context.

The study demonstrated that, during the COVID-19 epidemic period, RV infection rates on Hainan Island were highest in children under three years old. Furthermore, the prevalence of RV infections diminished progressively with increasing age. This trend is likely due to the development of cumulative immunity from repeated exposure to diverse RV serotypes. Notably, a substantial number of RV cases were recorded in children under three, with toddlers being particularly prone to infection. Given the elevated infection rates in this demographic, our findings underscore the importance of targeted prevention and healthcare strategies. Effective measures include enhancing hygiene practices, advancing vaccination efforts, and educating parents and caregivers about RV risks and preventive methods, aiming to alleviate the disease's impact on young children. Our investigation further identified a notable gender-based disparity in RV infection rates, exhibiting a predominance in male children. This observation contrasts with certain earlier reports. Specifically, Haixia Jiang et al. analyzed 5,832 nasopharyngeal swabs collected from patients with acute respiratory infections spanning 2012 to 2020, finding a RV infection rate of 2.74% (160/5832), with no significant gender disparity in the patient cohort[5]. Similarly, a study by Wanwei Li, Lili et al., which examined nasopharyngeal swabs from 655 patients suffering from ARTIs, also reported no significant gender difference among those infected with rhinovirus [6]. This divergence in findings underscores the complexity of RV transmission dynamics and suggests the influence of additional, possibly region-specific, factors affecting susceptibility and infection rates among different demographics.

Seasonal variation, characterized by changes in humidity, temperature, and climate, serves as an external factor that can significantly influence the severity of viral illnesses. Studies on rhinovirus (RV) patterns in different climatic conditions reveal contrasting findings: while RV detection peaks during the rainy seasons in Malaysia and Latin America, increased RV activity is observed in the dry seasons of Brazil and Cambodia. The RV infections predominantly occurred in autumn, winter, and spring on Hainan Island. Given its tropical setting, the island experiences its rainy season from May to October, suggesting a complex relationship between seasonal patterns and RV infection rates, which may not solely depend on precipitation levels.

Phylogenetic analysis identified three RV species among the positive samples: RV-A (46.88%), RV-B (6.25%), and RV-C (46.88%) in Hainan Island. It was found that RV-A and RV-C are the primary strains transmitting rhinovirus on Hainan Island, with multiple types circulating simultaneously. Our study highlighted a significant temporal transition in RV strain dominance, with RV-C surpassing RV-A to become the predominant strain starting in October 2022. This shift coincided with a peak in RV infection rates in November 2022, the highest observed in the past three years, potentially linked to specific epidemic strain subtypes and variations in annual weather patterns. Additionally, international research highlights significant genotype variations in circulating rhinoviruses, related to both time and geographical factors[24, 25]. The consistent identification and seasonal variation in the dominance of RV-A and RV-C strains across these studies reflect our findings, emphasizing the prevailing influence of these strains.

Previous studies have suggested that RV-C might play a role in severe clinical disease [26]. RV-C was present in the majority of children with acute asthma and was associated with more severe asthma[27, 28]. Wheezing episodes were also more common among individuals with RV-C and RV-A infection than among those with RV-B infection[26]. Another study suggested that RV-C was associated with more severe disease in children <3 years of age[29]. However, some reports found no differences in the clinical characteristics among hospitalized enrolled patients positive for RV-A, RV-B, or RV-C, including wheezing[30]. In our study, we observed that patients infected with RV-A tended to be younger compared to patients infected with RV-C. Additionally, we also noted that children infected with RV-C were more likely to exhibit clinical symptoms of wheezing. These findings suggest that there are differences in age distribution and clinical presentation between RV-A and RV-C. In our analysis, patients were divided into groups based on the severity of Lower Respiratory Tract Infections (LRTIs), showing no significant age-related differences between these categories. Notably, a greater occurrence of severe RV-associated LRTIs was identified in males, with 85.71% of severe cases found in boys, introducing potential gender-specific variations in disease severity. Additionally, the study revealed that children with asthma histories were more susceptible to severe LRTIs and extended hospitalizations, supporting the theory that pre-existing respiratory conditions can amplify RV infection impacts[31, 32]. This emphasizes the importance of vigilant monitoring and possibly specialized treatment approaches for asthmatic children with RV infections.

This study faces several limitations that merit attention. Firstly, all samples were sourced exclusively from Hainan Maternal and Child Health Hospital, the largest tertiary pediatric hospital in Hainan, which predominantly treats severe cases of childhood illnesses. While this facility's prominence lends weight to our findings, the single-center nature of the analysis introduces potential biases, as it may not fully represent the broader pediatric population of Hainan Island. Secondly, RV subtypes were identified in only 32 RV-positive samples collected at different times. This sample size is too limited to comprehensively reflect the variety of RV subtypes circulating across Hainan Island. Future studies that include a wider range of collection sites encompassing more hospitals and regions on Hainan Island, along with an expanded analysis of RV subtypes, are crucial for a more complete understanding of the epidemiology of RV infections in this area.

5. Conclusion

This study comprehensively explores the epidemiological trends and genetic variations of RV infections among hospitalized children on Hainan Island, spanning from before to after the ending of the Dynamic Zero-COVID Policy. It reveals significant insights into the prevalence, diversity, and seasonal fluctuations of RV, as well as the impact of public health policies on these dynamics. Notably, it highlights the changing dominance among RV strains over time and the specific susceptibility patterns seen across different age groups, genders, and conditions like asthma. The study deepens our understanding of RV circulation and diseases, significantly aiding translational research to establish relevant infection models. This essential knowledge facilitates the creation of targeted therapies, particularly aiding those most vulnerable to RV-induced illnesses.

Acknowledgements

The authors wish to thank all the patients for participating in this study, and the support from Emerging Tropical Infectious Diseases Science Innovation Group of Hainan Medical University and the Hainan Tropical Infectious Diseases Biobank.

Funding Statement

This study was supported by the Key Research and Development Plan of Hainan Province (grant No. ZDYF2022SHFZ085); Hainan Provincial Natural Science Foundation of China (grant No. 823RC598, 820QN267, 821QN258, 821QN0896, 821QN262, G20230324004Y and 821RC1070), the National Natural Science Foundation of China (grant No. 82360658, 82060378), the Innovative Scientific Research Project of postgraduates in Hainan Province (grant No. HYYS2021A17, Qhys2021-356, Qhys2021-352, Qhys2023-487 and Qhyb2023-176), and the Hainan Province Clinical Medical Center (QWYH202175). The funding sources had no role in the study design, data collection, analysis, interpretation, or writing of this report.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Ethics Statement

This study received approval from the Ethics Committee of the Hainan Women and Children's Medical Center.

Data Availability Statement

All sequences analyzed during this study are available from the NCBI database (GenBank accession No. OR575561- OR575592).

References

1. Chow EJ, Uyeki TM, Chu HY. The effects of the COVID-19 pandemic on community respiratory virus activity. Nat Rev Microbiol. 2023;21(3):195-210.

2. Poole S, Brendish NJ, Clark TW. SARS-CoV-2 has displaced other seasonal respiratory viruses: Results from a prospective cohort study. J Infect. 2020;81(6):966-972.

3. Takashita E, Kawakami C, Momoki T, Saikusa M, Shimizu K, Ozawa H, et al. Increased risk of rhinovirus infection in children during the coronavirus disease-19 pandemic. Influenza Other Respir Viruses. 2021;15(4):488-494.

4. Leung NHL, Chu DKW, Shiu EYC, Chan KH, McDevitt JJ, Hau BJP, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. Nat Med. 2020;26(5):676-680.

5. Savolainen-Kopra C, Korpela T, Simonen-Tikka ML, Amiryousefi A, Ziegler T, Roivainen M, et al. Single treatment with ethanol hand rub is ineffective against human rhinovirus–hand washing with soap and water removes the virus efficiently. J Med Virol. 2012;84(3):543-547.

6. Ljubin-Sternak S, Meštrović T. Rhinovirus-A True Respiratory Threat or a Common Inconvenience of Childhood?. Viruses. 2023;15(4):825.

7. Atmar RL. Uncommon(ly considered) manifestations of infection with rhinovirus, agent of the common cold. Clin Infect Dis. 2005;41(2):266-267.

8. Ljubin-Sternak S, Meštrović T, Ivković-Jureković I, Kolarić B, Slović A, Forčić D, et al. The Emerging Role of Rhinoviruses in Lower Respiratory Tract Infections in Children - Clinical and Molecular Epidemiological Study From Croatia, 2017-2019. Front Microbiol. 2019;10:2737.

9. Kreger JE, Hershenson MB. Effects of COVID-19 and Social Distancing on Rhinovirus Infections and Asthma Exacerbations. Viruses. 2022;14(11):2340.

10. Kiseleva I, Ksenafontov A. COVID-19 Shuts Doors to Flu but Keeps Them Open to Rhinoviruses. Biology (Basel). 2021;10(8):733.

11. Lee WM, Lemanske RF, Jr., Evans MD, Vang F, Pappas T, Gangnon R, et al. Human rhinovirus species and season of infection determine illness severity. Am J Respir Crit Care Med. 2012;186(9):886-891.

12. McIntyre CL, McWilliam Leitch EC, Savolainen-Kopra C, Hovi T, Simmonds P. Analysis of genetic diversity and sites of recombination in human rhinovirus species C. J Virol. 2010;84(19):10297-10310.

13. Liang WN, Yao JH, Wu J, Liu X, Liu J, Zhou L, et al. Experience and thinking on the normalization stage of prevention and control of COVID-19 in China. Zhonghua Yi Xue Za Zhi. 2021;101(10):695-699.

14. Zhou L, Nie K, Zhao H, Zhao X, Ye B, Wang J, et al. Eleven COVID-19 Outbreaks with Local Transmissions Caused by the Imported SARS-CoV-2 Delta VOC - China, July-August, 2021. China CDC Wkly. 2021;3(41):863-868.

15. Liu J, Liu M, Liang WN. The Dynamic COVID-Zero Strategy in China. China CDC Wkly. 2022;4(4):74-75.

16. Chuang YC, Lin KP, Wang LA, Yeh TK, Liu PY. The Impact of the COVID-19 Pandemic on Respiratory Syncytial Virus Infection: A Narrative Review. Infect Drug Resist. 2023;16:661-675.

17. Liu PC, Cheng FY, Su LY, Ye ZC, Xu MH, Lu LJ, et al. An outbreak of influenza A in Shanghai after ending the zero-COVID policy in February-March 2023. J Infect. 2023;87(2):e33-e35.

18. Piret J, Boivin G. Viral Interference between Respiratory Viruses. Emerg Infect Dis. 2022;28(2):273-281.

19. Wisdom A, Leitch EC, Gaunt E, Harvala H, Simmonds P. Screening respiratory samples for detection of human rhinoviruses (HRVs) and enteroviruses: comprehensive VP4-VP2 typing reveals high incidence and genetic diversity of HRV species C. J Clin Microbiol. 2009;47(12):3958-3967.

20. Al-Romaihi HE, Smatti MK, Al-Khatib HA, Coyle PV, Ganesan N, Nadeem S, et al. Molecular epidemiology of influenza, RSV, and other respiratory infections among children in Qatar: A six years report (2012–2017). International Journal of Infectious Diseases. 2020;95:133-141.

21. Groves HE, Piché-Renaud PP, Peci A, Farrar DS, Buckrell S, Bancej C, et al. The impact of the COVID-19 pandemic on influenza, respiratory syncytial virus, and other seasonal respiratory virus circulation in Canada: A population-based study. Lancet Reg Health Am. 2021;1:100015.

22. Huang QS, Wood T, Jelley L, Jennings T, Jefferies S, Daniells K, et al. Impact of the COVID-19 nonpharmaceutical interventions on influenza and other respiratory viral infections in New Zealand. Nat Commun. 2021;12(1):1001.

23. Shi T, Huang L. Prevalence of respiratory pathogens and risk of developing pneumonia under non-pharmaceutical interventions in Suzhou, China. Epidemiology & Infection. 2023;151:e82.

24. Baillie VL, Moore DP, Mathunjwa A, Morailane P, Simões EAF, Madhi SA. Molecular Subtyping of Human Rhinovirus in Children from Three Sub-Saharan African Countries. J Clin Microbiol. 2019;57(9):e00723-19.

25. Luka MM, Kamau E, Adema I, Munywoki PK, Otieno GP, Gicheru E, et al. Molecular Epidemiology of Human Rhinovirus From 1-Year Surveillance Within a School Setting in Rural Coastal Kenya. Open Forum Infect Dis. 2020;7(10):ofaa385.

26. Lau SK, Yip CC, Lin AW, Lee RA, So LY, Lau YL, et al. Clinical and molecular epidemiology of human rhinovirus C in children and adults in Hong Kong reveals a possible distinct human rhinovirus C subgroup. J Infect Dis. 2009;200(7):1096-1103.

27. Erkkola R, Turunen R, Räisänen K, Waris M, Vuorinen T, Laine M, et al. Rhinovirus C Is Associated With Severe Wheezing and Febrile Respiratory Illness in Young Children. Pediatr Infect Dis J. 2020;39(4):283-286.

28. Sayama A, Okamoto M, Tamaki R, Saito-Obata M, Saito M, Kamigaki T, et al. Comparison of Rhinovirus A-, B-, and C-Associated Respiratory Tract Illness Severity Based on the 5'-Untranslated Region Among Children Younger Than 5 Years. Open Forum Infect Dis. 2022;9(10):ofac387.

29. Lauinger IL, Bible JM, Halligan EP, Bangalore H, Tosas O, Aarons EJ, et al. Patient characteristics and severity of human rhinovirus infections in children. J Clin Virol. 2013;58(1):216-220.

30. Fry AM, Lu XY, Olsen SJ, Chittaganpitch M, Sawatwong P, Chantra S, et al. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. PLoS One. 2011;6(3):e17780.

31. Regina Malveste Ito C, Santos MO, de Oliveira Cunha M, de Araújo KM, de Souza GRL, Rézio GS, et al. Rhinovirus infection and co-infection in children with severe acute respiratory infection during the COVID-19 pandemic period. Virulence. 2024;15(1):2310873.

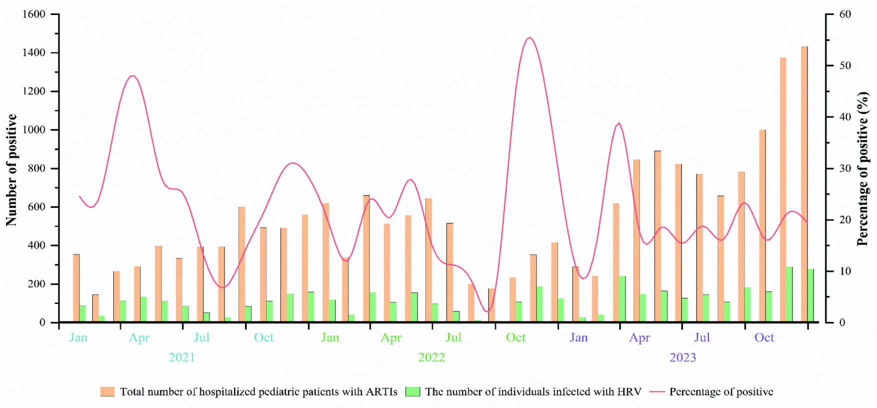
32. Esneau C, Duff AC, Bartlett NW. Understanding Rhinovirus Circulation and Impact on Illness. Viruses. 2022;14(1):141.

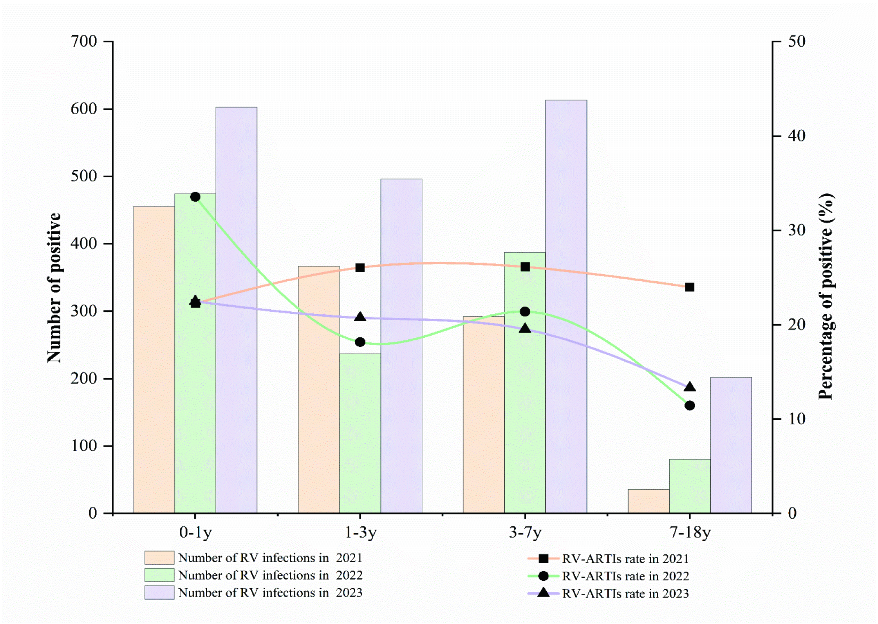
Figure legends

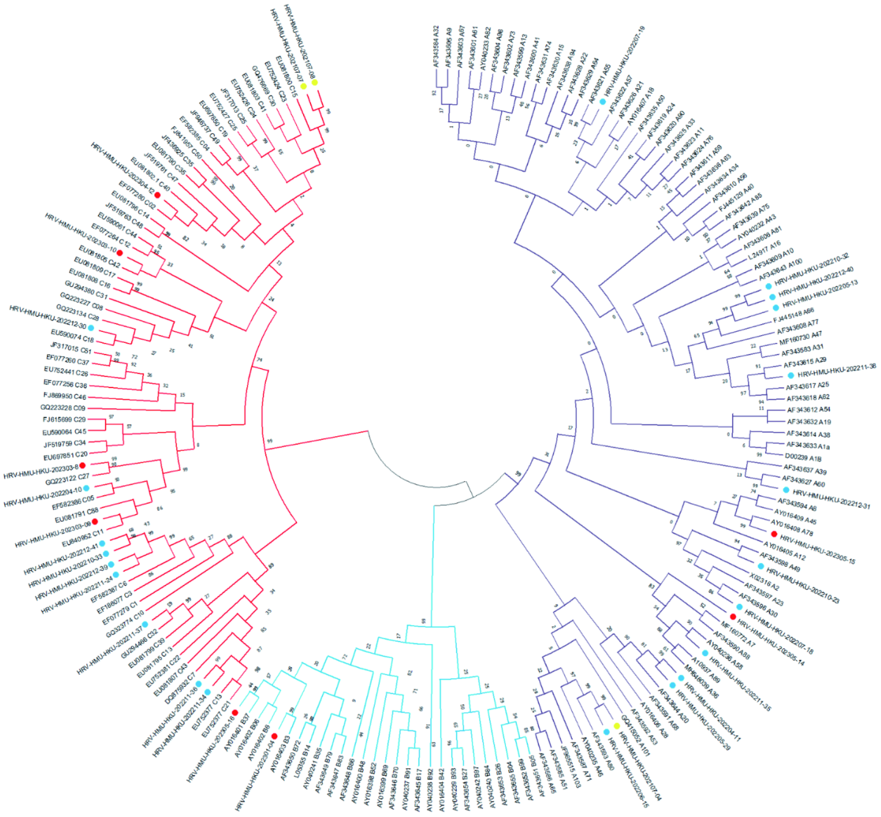
Figure 1. The prevalence of RV in hospitalized pediatric patients with ARTIs from January 2021 to December 2023.

Figure 2. Age-based analysis of RV infections from 2021 to 2023

Figure3. Phylogenetic Analysis of RV Strains. The phylogenetic tree was constructed based on Hainan RV sequences from 2021 to 2023, along with reference sequences with genotype information from around the world. The neighbor-joining method was employed to generate the tree, and branch support was determined through 1000 bootstrap iterations using MEGA software. The resulting tree nodes were annotated with bootstrap values. Sequences from Hainan RV strains, identified by accession numbers, are color-coded by year to indicate prevalent strains: yellow for 2021, blue for 2022, and red for 2023, illustrating the temporal evolution of RV strains.







Hosted file

`Tables.docx` available at https://authorea.com/users/562002/articles/956507-circulation-pattern-and-genetic-variation-of-rhinovirus-infection-among-hospitalized-children-on-hainan-island-before-and-after-the-dynamic-zero-covid-policy-from-2021-to-2023