Postprint of an Epidemiological Study on Non-bacterial Respiratory Pathogens in Children in Northeastern Sichuan

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Abstract

Background Following the lifting of COVID-19 restrictions, surveillance data from disease control and pediatric clinical practice have both indicated that the epidemic pattern of respiratory infections in children has changed compared with previous periods. Regional epidemiological statistics are of directive significance for the diagnosis and treatment of respiratory infections in children. Currently, large-sample epidemiological analyses of respiratory pathogens in children are scarce in northeastern Sichuan.

Objective To investigate the prevalence of 13 non-bacterial respiratory pathogens in children in three regions of northeastern Sichuan Province (Bazhong, Nanchong, and Guang' an) following the lifting of COVID-19 restrictions, and to provide evidence for the prevention and treatment of respiratory infections in children.

Methods A retrospective analysis was conducted on pathogen samples from 15,772 hospitalized children with acute respiratory infection at Bazhong Central Hospital, Nanchong Central Hospital, and Guang' an People' s Hospital from December 7, 2022, to June 30, 2024. The cohort comprised 8,707 males (55.2%) and 7,065 females (44.8%). By age, they were divided into four groups: infant group (<1 year) with 3,938 cases, toddler group (1–<3 years) with 6,434 cases, preschool group (3–<6 years) with 3,231 cases, and school-age group (6–14 years) with 2,169 cases. By season of onset, they were categorized as spring (March–May) with 5,423 cases, summer (June–August) with 2,594 cases, autumn (September–November) with 3,121 cases, and winter (December–February) with 4,634 cases. Multiplex reverse transcription polymerase chain reaction was employed to detect 13 non-bacterial pathogens, including influenza A virus, influenza A H1N1 virus, influenza A H3N2 virus, influenza B virus, parainfluenza virus, Mycoplasma pneumoniae, Chlamydia, metapneumovirus, respiratory syncytial virus, adenovirus, rhinovirus, bocavirus, and coronavirus. The detection status of pathogens in the total sample was examined, and the positive detection rates of each pathogen were compared across different regions, genders, age groups, and seasons.

Results Among 15,772 respiratory samples, 11,618 were positive for pathogens, yielding a detection rate of 73.66%; mixed infections accounted for 3,632 cases (23.03%). The five most frequently detected pathogens were rhinovirus (24.5%), respiratory syncytial virus (16.4%), Mycoplasma pneumoniae (13.8%), influenza A virus (9.4%), and parainfluenza virus (8.9%). The overall detection rates in Bazhong, Nanchong, and Guang' an were 80.9%, 73.7%, and 75.3%, respectively; the difference was statistically significant (^{2}=101.119, P<0.001). The detection rate in male children (72.1%) was lower than that in female children (75.6%) (^{2}=24.539, P<0.001). Except for coronavirus, no statistically significant differences were observed in the detection rates of the remaining pathogens between genders (P>0.05). The overall detection rates among the infant, toddler, preschool, and school-age groups showed statistically significant differences (^{2}=174.613, P<0.001). Except for coronavirus, the detection rates of the remaining pathogens differed significantly across age groups (P<0.05). The overall detection rates among spring, summer, autumn, and winter samples showed statistically significant differences (^{2}=364.584, P<0.001); the highest detection rate occurred in winter (80.0%), and the lowest in spring (72.3%).

Conclusion During the 18 months following the lifting of COVID-19 restrictions, the primary pathogens causing acute respiratory infections in children in the three regions of northeastern Sichuan were rhinovirus, respiratory syncytial virus, Mycoplasma pneumoniae, influenza A virus, and parainfluenza virus, with varying prevalence across regions. Except for coronavirus among the 13 pathogens, no gender-based differences were observed in the detection rates of the remaining pathogens; however, differences existed across age and seasonal groups. COVID-19 has exerted certain influence on the prevalence of other pathogens.

Full Text

Epidemiologic Study of Nonbacterial Respiratory Pathogens in Children in Northeast Sichuan Province

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Abstract

Background Since the end of large-scale COVID-19 epidemic control measures, data from CDC surveillance and pediatric clinics indicate that the prevalence of respiratory infections in children has changed compared with previous patterns. Regional prevalence statistics are directionally significant for diagnosing and treating respiratory infections in children, yet large-sample analyses of pediatric respiratory pathogen epidemiology remain rare in northeast Sichuan Province.

Objective To investigate the prevalence of 13 respiratory non-bacterial pathogens in children across three regions of northeastern Sichuan Province (Bazhong, Nanchong, and Guang' an) following the lifting of COVID-19 restrictions, providing evidence for clinical prevention and treatment of pediatric respiratory infections.

Methods We conducted a retrospective analysis of pathogen samples from 15,772 children hospitalized with acute respiratory tract infections at Bazhong Central Hospital, Nanchong Central Hospital, and Guang' an People' s Hospital between December 7, 2022, and June 30, 2024. The cohort included 8,707 males (55.2%) and 7,065 females (44.8%). Subjects were stratified into four age groups: infant group (<1 year, n=3,938), toddler group (1–<3 years, n=6,434), preschool group (3–<6 years, n=3,231), and school-age group (6–14 years, n=2,169). Seasonal distribution was: spring (March-May, n=5,423), summer (June–August, n=2,594), autumn (September–November, n=3,121), and winter (December–February, n=4,634). Multiplex reverse transcription polymerase chain reaction was used to detect 13 non-bacterial pathogens: influenza A virus, influenza A H1N1, influenza A H3N2, influenza B virus, parainfluenza virus, *Mycoplasma pneumoniae*, chlamydia, metapneumovirus, respiratory syncytial virus, adenovirus, rhinovirus, bocavirus, and coronavirus. We examined overall pathogen detection rates and compared positive detection rates across different regions, genders, age groups, and seasons.

Results Among 15,772 respiratory samples, 11,618 (73.66%) were positive for pathogens, with 3,632 (23.03%) identified as mixed infections. The five most prevalent pathogens were rhinovirus (24.5%), respiratory syncytial virus (16.4%), *Mycoplasma pneumoniae* (13.8%), influenza A virus (9.4%), and parainfluenza virus (8.9%). Total detection rates differed significantly across the three regions: Bazhong (80.9%), Nanchong (73.7%), and Guang' an (75.3%) (^{2}=101.119, P<0.001). The overall detection rate was lower in boys (72.1%) than in girls (75.6%) (^{2}=24.539, P<0.001). Among the 13 pathogens, only coronavirus showed statistically significant gender differences (P<0.05), while the remaining 12 did not (P>0.05). Detection rates varied significantly across age groups (^{2}=174.613, P<0.001), with infants showing the lowest rate (69.3%) compared with toddlers (80.0%), preschoolers (79.2%), and school-age children (75.3%) (all P<0.05). Except for coronavirus, all pathogens showed significant age-related differences in detection rates (P<0.05). Seasonal differences were also significant (^{2}=364.584, P<0.001), with winter showing the highest detection rate (80.0%) and spring the lowest (72.3%). Mixed infections were most common in winter (27.3%).

Conclusion During the 18 months following the lifting of COVID-19 restrictions, the predominant pathogens causing acute respiratory infections in children across the three northeast Sichuan regions were rhinovirus, respiratory syncytial virus, *Mycoplasma pneumoniae*, influenza A virus, and parainfluenza virus, with epidemiological patterns varying by region. Except for coronavirus, pathogen detection rates did not differ by gender but showed significant variation across age and seasonal subgroups. COVID-19 has demonstrably influenced the epidemiology of other respiratory pathogens.

Keywords Respiratory tract infections; Respiratory pathogens; Epidemiology; Child; Respiratory syncytial viruses; Northeast Sichuan

Introduction

Acute respiratory tract infection (ARTI) is the most common disease in pediatrics and a leading cause of hospitalization and mortality among children worldwide [1]. Viral pathogens are the primary causative agents [2], followed by bacterial infections [3], imposing a heavy healthcare burden on society and families. The 2019 Global Burden of Disease Report identified lower respiratory infections as the second leading cause of disease burden in children [4]. The epidemiology of respiratory pathogens is influenced not only by the basic reproduction number (R_0) of the pathogens themselves [5] but also by geographic, climatic, demographic, and economic factors. Since the end of the COVID-19 pandemic, the prevalence patterns of non-COVID-19 respiratory pathogens have shifted [6], with particular attention from researchers focusing on their specific epidemiological characteristics in pediatric populations. This study analyzed respiratory pathogen detection specimens from 15,772 hospitalized children across three hospitals in northeast Sichuan—Bazhong Central Hospital, Nanchong Central Hospital, and Guang' an People' s Hospital—through retrospective analysis. Our objective was to characterize the etiological composition and epidemiological features of pediatric respiratory diseases in this region post-COVID-19, providing targeted references for clinical prevention and seasonal surveillance of respiratory infections in children, and contributing sample data for epidemiological investigations in children residing in western China' s subtropical humid monsoon climate zone.

Methods

Study Design and Subjects

We retrospectively analyzed respiratory specimens from 15,772 children hospitalized with acute respiratory tract infections at Bazhong Central Hospital, Nanchong Central Hospital, and Guang' an People' s Hospital between December 7, 2022, and June 30, 2024. The cohort comprised 8,707 males (55.2%) and 7,065 females (44.8%), aged 28 days to 14 years (mean age 3.08$±$2.95 years). Subjects were divided into four age groups: infant group (<1 year, n=3,938), toddler group (1-<3 years, n=6,434), preschool group (3-<6 years, n=3,231), and school-age group (6-14 years, n=2,169). Seasonal distribution was categorized as spring (March-May, n=5,423), summer (June-August, n=2,594), autumn (September-November, n=3,121), and winter (December-February, n=4,634). This study was approved by the ethics committees of all participating institutions.

Inclusion and Exclusion Criteria

Inclusion criteria: (1) Diagnosis of ARTI according to *Nelson Textbook of Pediatrics* (9th edition) [7]; (2) Age 1 month to 14 years; (3) Pathogen testing performed at admission; (4) Complete medical records available.

Exclusion criteria: (1) Non-infectious hospitalization reasons such as foreign body aspiration, acute asthma exacerbation, or bronchopulmonary dysplasia; (2) Cases of nosocomial infection.

Pathogen Detection Methods

We used the Ningbo Health Gene Technology extraction kit to isolate pathogen samples from pharyngeal secretions. The multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay combined with capillary electrophoresis detected 13 non-bacterial pathogens: influenza A virus, influenza A H1N1, influenza A H3N2, influenza B virus, parainfluenza virus, *Mycoplasma pneumoniae*, chlamydia, metapneumovirus, respiratory syncytial virus, adenovirus, rhinovirus, bocavirus, and coronavirus. The assay included an RT-PCR internal control to monitor the entire detection process, including nucleic acid extraction, RT-PCR, and capillary electrophoresis.

Unified detection protocol across three regions: Single-use flocked swabs were used to collect throat swabs, which were stored in containers with 3 mL cell preservation solution for transport. Specific primers targeting conserved pathogen sequences were designed for single-tube RT-PCR amplification, followed by electrophoretic separation of amplification products. Human RNA and DNA were simultaneously detected to enable dynamic monitoring of sample quality.

Definitions and Statistical Analysis

Detection rate calculation: Detection rate = (Number of positive samples / Total number of samples) × 100%.

Epidemiological definitions: An *epidemic* refers to the distribution and transmission pattern of an infectious disease across different geographic regions, populations, or time periods. An *outbreak* is defined in public health as an abnormal increase in infectious disease cases within a specific time, area, or population [8].

Basic reproduction number (R_0): R_0 describes the transmissibility of a virus, representing the average number of secondary cases generated by one infected individual in a fully susceptible population. Different viruses exhibit varying R_0 values; $R_0>1$ indicates that a pathogen is transmissible and can cause an epidemic [9]. R_0 calculation depends on transmission mechanisms and computational models, with different formulas employed across studies [10].

Statistical methods: Data were analyzed using SPSS Statistics 27.0. A database was constructed to analyze basic pathogen detection patterns and compare detection rates across gender, age groups, and seasons. Categorical data were expressed as rates, and inter-group comparisons of positive rates were performed using ^{2} tests. Statistical significance was defined as $P<0.05$.

Results

Overall Pathogen Detection

Among 15,772 respiratory samples, 11,618 (73.66%) were positive for pathogens. Single infections accounted for 7,986 cases (50.63%), while mixed infections occurred in 3,632 cases (23.03%). The five most frequently detected pathogens were rhinovirus (24.5%), respiratory syncytial virus (16.4%), *Mycoplasma pneumoniae* (13.8%), influenza A virus (9.4%), and parainfluenza virus (8.9%). Rhinovirus showed the highest number of positive cases (3,861), while chlamydia had the fewest (150) .

Regional Variations in Pathogen Detection

Total detection rates differed significantly across regions: Bazhong (80.9%), Nanchong (73.7%), and Guang' an (75.3%) (^{2}=101.119, $P<0.001$). Except for influenza A H1N1, bocavirus, and coronavirus, which showed no significant regional differences ($P>0.05$), all other pathogens exhibited statistically significant regional variation ($P<0.05$) .

Bazhong region: Among 5,919 tested samples, the top three pathogens were rhinovirus (26.5%), *Mycoplasma pneumoniae* (17.4%), and respiratory syncytial virus (15.1%). Rhinovirus maintained high detection rates throughout the year, peaking at 53.9% in May 2024. Influenza A virus experienced outbreak periods in February–April 2023 and December 2023–March 2024, reaching 71.1% in

March 2023. *Mycoplasma pneumoniae* was first detected in December 2022, peaked at 40.8% in July–August 2023, then gradually declined. Respiratory syncytial virus showed elevated detection rates from September 2023 to March 2024, with a maximum of 31.5% [Figure 1: see original paper].

Nanchong region: Among 8,466 samples, the top three pathogens were rhinovirus (22.0%), respiratory syncytial virus (18.3%), and *Mycoplasma pneumoniae* (12.0%). Rhinovirus detection increased from April 2023 and remained elevated, reaching 30.7% in October. Respiratory syncytial virus showed a significant peak of 36% in April 2023, followed by sporadic circulation and another increase during winter (November 2023-February 2024) to 31.4%. Metapneumovirus reached 18.7% in November 2023, higher than in other regions. *Mycoplasma pneumoniae* showed an upward trend from November 2023 to April 2024, with its epidemic peak occurring later than in Bazhong [Figure 2: see original paper].

Guang' an region: Among 1,387 samples, the top three pathogens were rhinovirus (31.2%), adenovirus (11.7%), and respiratory syncytial virus (9.9%). Rhinovirus maintained high detection rates year-round, peaking at 40.5% in September-October 2023. Parainfluenza virus circulated predominantly in July-August 2023 and April–May 2024. Adenovirus detection increased significantly to 18.3% in December 2023 [Figure 3: see original paper].

Gender Differences in Pathogen Detection

Among 15,772 samples, the overall detection rate was lower in boys (72.1%) than in girls (75.6%) (^{2}=24.539, P<0.001). Among the 13 pathogens, only coronavirus showed a significantly higher detection rate in boys (P<0.05), while the remaining 12 pathogens showed no gender differences (P>0.05) .

Age-Related Differences in Pathogen Detection

Overall detection rates differed significantly across age groups (^{2}=174.613, P<0.001): infants (69.3%), toddlers (80.0%), preschoolers (79.2%), and school-age children (75.3%). The infant group had the lowest rate, significantly different from all other groups (P<0.05). Among the 13 pathogens, only coronavirus showed no age-related differences (P>0.05); all others exhibited significant age-related variation (P<0.05). Respiratory syncytial virus was most prevalent in infants (26.2%), bocavirus in toddlers (2.7%), while parainfluenza virus (3.6%), metapneumovirus (4.8%), and rhinovirus (19.6%) were least common in school-age children. *Mycoplasma pneumoniae* (31.3%), chlamydia (3.9%), and influenza B (6.9%) were most prevalent in school-age children .

The top pathogens by age group were: - **Infants:** Respiratory syncytial virus (26.2%), rhinovirus (22.7%), parainfluenza virus (10.2%) - **Toddlers:** Rhinovirus (26.9%), respiratory syncytial virus (18.8%), parainfluenza virus (11.0%) - **Preschoolers:** *Mycoplasma pneumoniae* (25.4%), rhinovirus

(25.2%), influenza A (11.9%) - **School-age:** *Mycoplasma pneumoniae* (31.3%), rhinovirus (19.6%), influenza A (11.3%)

Seasonal Variations in Pathogen Detection

Spring showed the lowest detection rate (72.3%), significantly different from other seasons ($P<0.05$). Autumn and winter had higher rates (79.7% and 80.0%, respectively). Mixed infections were most common in winter (27.3%), significantly higher than other seasons ($P<0.05$). Significant seasonal differences were observed for influenza A, parainfluenza, *Mycoplasma pneumoniae*, respiratory syncytial virus, adenovirus, rhinovirus, bocavirus, chlamydia, influenza B, and coronavirus (all $P<0.05$), but not for influenza A H1N1, influenza A H3N2, or metapneumovirus ($P>0.05$) .

Seasonal patterns included: - Influenza A: higher in spring and winter - Parainfluenza: highest in summer (12.4%) - Influenza B: highest in winter (5.9%) - Adenovirus: lowest in autumn (1.3%) - Rhinovirus: higher in spring and autumn than summer and winter - Respiratory syncytial virus: elevated in autumn and winter - *Mycoplasma pneumoniae*: increased from spring and remained elevated

Pathogen Transmission Characteristics and Regional Context

R_0 values for common respiratory pathogens are summarized in [11-17]. The northeast Sichuan region, located in the northeastern Sichuan Basin, includes five cities (Nanchong, Dazhou, Guang' an, Bazhong, and Guangyuan), covering 64,067.78 km^2 with a population of 19.07 million. The climate is classified as subtropical humid monsoon with large seasonal temperature variations. This study focused on three regions—Bazhong, Nanchong, and Guang' an—which are contiguous from north to south. Background information on population, GDP, climate, and geography is provided in .

Discussion

This study identified 11,618 positive samples (73.66% detection rate) with 3,632 mixed infections (23.03%), both higher than rates reported in other regions during the COVID-19 pandemic [18-19]. Consistent with Luan et al. [20], we observed increased pathogen diversity following pandemic restrictions. No gender differences were found for 12 of 13 pathogens, aligning with Wei et al. [21], though coronavirus showed male predominance. Regional detection rates differed from those reported in Shanghai and Hefei [18-19], with distinct epidemic patterns across our three study regions: Bazhong experienced an influenza A outbreak in March 2023 and a *Mycoplasma pneumoniae* peak in July–August 2023; Nanchong showed high metapneumovirus detection and delayed *Mycoplasma pneumoniae* circulation; Guang' an had high rhinovirus and adenovirus detection rates.

Pathogen prevalence varied significantly by age, with toddlers showing the highest overall detection rate (80.0%) and infants the lowest (69.3%). Respiratory syncytial virus predominated in infants, while *Mycoplasma pneumoniae* prevalence increased with age. These findings align with Lu' s observations [6] during the pandemic, though recent international reports indicate rising respiratory syncytial virus detection rates among toddlers and school-age children [22], highlighting age-specific susceptibility patterns and the need for enhanced infection prevention in infants.

Seasonal patterns also differed from pre-pandemic observations. While influenza historically peaked in winter-spring and declined in summer [23], our study showed influenza A elevation in spring and winter, parainfluenza peaking in summer, and adenovirus lowest in autumn. Nine pathogens showed significant seasonal variation, with winter having the highest overall detection rate (80.0%) and mixed infection rate (27.3%). Rhinovirus remained highly prevalent across all seasons, while respiratory syncytial virus increased in autumn-winter, and *Mycoplasma pneumoniae* maintained high detection rates from spring onward. These shifts may reflect changes in population mobility, pathogen activity, and specific transmission characteristics (e.g., influenza's high R_0 and short epidemic period), resulting in distinct regional epidemic patterns.

Three common epidemiological features emerged across northeast Sichuan: (1) year-round high rhinovirus circulation; (2) increased *Mycoplasma pneumoniae* detection compared with pandemic levels [24], coinciding with a nationwide epidemic [25]; and (3) distinct temporal peaks for different pathogens. Potential explanations include reduced adherence to non-pharmacological interventions (NPIs) post-lockdown [26], limited effectiveness of surgical masks against non-enveloped viruses like rhinovirus, diminished influenza activity in southern China during the pandemic [27], increased population aggregation after restrictions eased, and accumulation of susceptible populations due to immunity gaps during the pandemic. Additionally, the post-lockdown period coincided with natural epidemic cycles for several pathogens, including *Mycoplasma pneumoniae.*

Geographic and socioeconomic factors influenced pathogen transmission. Nanchong contributed the largest sample size, consistent with its population and GDP; Guang' an, despite moderate population and GDP, had the smallest sample, possibly due to proximity to medically advanced Chongqing and high population mobility; Bazhong, though lowest in GDP, contributed more samples than Guang'an, potentially reflecting geographic and transportation constraints. This demonstrates the entropic nature of pathogen transmission and epidemic evolution, shaped by multiple geographic, economic, demographic, and climatic factors.

Limitations

This study has several limitations. First, it focused exclusively on non-bacterial pathogens, excluding bacterial cultures from sputum or bronchoalveolar lavage fluid, limiting comprehensive understanding of bacterial pathogen epidemiology. Second, sample sizes were not adjusted to match regional population proportions, potentially affecting statistical power. Future multicenter, continuous surveillance studies are needed to provide more comprehensive epidemiological data on pediatric respiratory infections in this region, informing more precise prevention and treatment strategies.

Conclusion

During the 18 months following the lifting of COVID-19 restrictions, ARTI remained prevalent among children in northeast Sichuan, with rhinovirus, respiratory syncytial virus, *Mycoplasma pneumoniae*, influenza A virus, and parainfluenza virus as the predominant pathogens. Epidemiological patterns varied by region, age, and season but not by gender. The epidemic characteristics changed compared with the pandemic period, with higher positive detection rates and altered seasonal peaks. COVID-19 has demonstrably impacted the epidemiology of other respiratory pathogens. This study provides representative data from three major hospitals in northeast Sichuan using standardized detection methods, minimizing sample bias. Our findings can inform regional pediatric respiratory disease prevention and control efforts and provide sample data for epidemiological investigations in children residing in western China’ s subtropical humid monsoon climate zone.

Author Contributions

LUO Jing conceptualized the study, designed the research, implemented the investigation, and drafted the manuscript. LIU Juan and KUANG Jianhua collected and organized the data. ZHOU Juan and LUO Yanqing performed statistical analysis and created figures and tables. FU Qiang revised the manuscript, provided quality control, and supervised the project.

Conflicts of Interest

The authors declare no conflicts of interest.

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Note: Figure translations are in progress. See original paper for figures.