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Analysis of Common Respiratory Infected Pathogens in 3100 Children after the Coronavirus Disease 2019 Pandemic*

Jun-e MA[1†], Qing-feng MA[2†], Wei WANG[3†], Yan ZENG[4†], Xiang-hua CUI[1], Guo-lei WANG[1], Si-yu LIU[5], Zhen WANG[1], Xuan XIAO[6#], Zu-neng XIA[1#], Heng-cheng ZHU[4#], Cheng-liang ZHU[1#]

[1]*Department of Clinical Laboratory, Renmin Hospital of Wuhan University, Wuhan 430060, China*

[2]*Department of Clinical Laboratory, Liyuan Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430077, China*

[3]*Department of Clinical Laboratory, Wuhan Fourth Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430077,China*

[4]*Department of Urology, Renmin Hospital of Wuhan University, Wuhan 430060, China*

[5]*State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan 430060, China*

[6]*Department of Ophthalmology, Renmin Hospital of Wuhan University, Wuhan 430060, China*

[Abstract] Objective: To investigate the epidemiological features in children after the coronavirus disease 2019 (COVID-19) pandemic. **Methods:** This study collected throat swabs and serum samples from hospitalized pediatric patients of Renmin Hospital of Wuhan University, Wuhan, Hubei province, China before and after the COVID-19 pandemic. Respiratory infected pathogens [adenovirus (ADV), influenza virus A/B (Flu A/B), parainfluenza virus 1/2/3 (PIV1/2/3), respiratory syncytial virus (RSV), *Mycoplasma pneumoniae* (MP), and *Chlamydia pneumoniae* (CP)] were detected. The pathogens, age, and gender were used to analyze the epidemiological features in children after the COVID-19 pandemic. **Results:** The pathogen detection rate was significantly higher in females than in males ($P<0.05$), and the infection of PIV1 and MP was mainly manifested. After the COVID-19 pandemic, PIV1, PIV3, RSV, and MP had statistically different detection rates among the age groups ($P<0.05$), and was mainly detected in patients aged 0–6 years, 0–3 years, 0–3 years, and 1–6 years, respectively. When comparing before the COVID-19 pandemic, the total detection rate of common respiratory pathogens was lower ($P<0.05$). Except for the increase in the detection rate of PIV1 and CP, the infection rate of other pathogens had almost decreased. **Conclusion:** The prevention and control measures for the COVID-19 pandemic effectively changed the epidemiological features of common respiratory tract infectious diseases in pediatric children.

Key words: before and after the COVID-19 pandemic; common respiratory infectious pathogens; pediatric children

Respiratory infections are the main cause of morbidity and mortality in children younger than 5 years old worldwide[1], and mainly caused by infection from bacteria, viruses, and fungi. In addition, bacteria and viruses have been the major pathogens detected in children with respiratory infections[2, 3]. Respiratory viruses are the most common causes of acute respiratory infections (ARIs)[4] with influenza (flu) being one of the commonest causes of viral respiratory infection and loss of school days[5]. Furthermore, the Global Burden of Disease (GBD) project has estimated that 10% of deaths caused by respiratory tract infection were attributed to a flu infection[6]. Children's respiratory infection with respiratory syncytial virus (RSV) was also the most important risk factor for the onset of wheezing[7]. About 4.5% of viral respiratory infections were caused by adenoviruses (ADV), which could cause serious consequences of admission to a

Jun-e MA, E-mail: mje222@whu.edu.cn; Qing-feng MA, E-mail: 2002ly0714@hust.edu.cn; Wei WANG, E-mail: ww8950@sina.com; Yan ZENG, E-mail: zengyanwuhan@163.com

†The authors contributed equally to this work.

#Corresponding authors, Cheng-liang ZHU, E-mail: xinchengzhu@163.com; Heng-cheng ZHU, E-mail: zhuhcgj@aliyun.com; Zu-neng XIA, E-mail: xiazunen9908@163.com; Xuan XIAO, E-mail: xiaoxuan1111@163.com

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pediatric intensive care unit (PICU) and mechanical ventilation[8]; moreover, the infected titer of ADV was associated with the disease severity in patients younger than 9 years old and served as a predictor of the respiratory disease course[9]. *Mycoplasma pneumoniae* (MP) was the prominent respiratory pathogen of pediatric community-acquired pneumonia[10–12], and caused epidemics every 3–7 years in South Korea[13].

Respiratory infections caused by viruses like MP and *Chlamydia pneumoniae* (CP) have high contagiousness and basic principles of management include prompt adoption of infection control measures and preventive vaccination. The coronavirus disease 2019 (COVID-19), which broke out in 2019, is a novel highly contagious respiratory infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)[14]. As such, the Chinese government adopted a series of protective and preventive measures, such as wearing masks, reducing crowd gathering and mobility, paying attention to hand hygiene, implementing isolation measures for patients or suspected patients, and rapidly controlling the spread of the SARS-CoV-2 pandemic. However, it was not clear whether these protective and preventive measures for COVID-19 were effective against pediatric respiratory infections. This study analyzed the respiratory infectious pathogens in hospitalized pediatric children before and after the COVID-19 pandemic and explored whether the epidemiological features changed with the effective protective and preventive measures for the pandemic.

1 MATERIALS AND METHODS

1.1 Patients and Materials

Totally, 10 918 pediatric children (7818 cases were collected from September 1 to December 31, 2018 and May 1 to August 31, 2019, before the COVID-19 pandemic; 3100 cases were collected from May 1 to December 31, 2020, after the pandemic), younger than 12 years old, were diagnosed with acute respiratory infection in Renmin Hospital of Wuhan University, Wuhan, Hubei province, China and enrolled in this study. The results of seven respiratory virus antigens, and IgM antibody results of MP and CP were collected. According to their age, the 3100 pediatric children (1851 males; 1249 females) hospitalized after the COVID-19 pandemic were divided into four groups: younger than 1 year old (521 cases), 1 year to younger than 4 years old (1484 cases), 4 years to younger than 7 years old (698 cases), and 7 years to younger than 14 years old (397 cases). Compared with the pediatric children hospitalized before the pandemic, gender and age had no significant difference in the pediatric patients hospitalized after the pandemic (P>0.05).

This study was approved by the Health Research Ethics Board of Renmin Hospital of Wuhan University (No. WDRY2020-K066).

1.2 Methods

Throat swab samples were obtained from pediatric children. Seven direct detections for fluorescent antigens for RSV, Flu A and B, and parainfluenza virus (PIV) were performed using a D3 Ultra TM DFA Respiratory Virus Screening ID Kit (Diagnostic Hybrids Inc., USA) and fluorescence microscope (EUROstar III Plus, Germany). Approximately 3 mL of venous blood was collected from each child and placed in a vacuum coagulation tube and centrifuged at 4000 r/min for 10 min to separate the serum. The IgM antibody of MP and CP was detected using a Pneumoslide IgM kit (Vircell Microbiologists, Spain) and fluorescence microscope (EUROstar Ⅲ plus, Germany).

1.3 Statistical Analysis

Statistical analysis was performed using IBM SPSS 25 (USA). Non-normal distribution data were expressed as the median and interquartile, and analyzed by a U test. The count data were expressed as a percentage and analyzed by a χ^2 test. A P value <0.05 indicated the statistical significance.

2 RESULTS

2.1 Detection of Respiratory Pathogens in Pediatric Children

Among 3100 pediatric children with a common respiratory infection after the COVID-19 pandemic, a total of 1112 (35.87%) children were infected with pathogens. Among the 9 pathogens, the highest detection rate was 28.19% in the MP infection, and influenza virus A and B (Flu A and B) were not detected. However, the detection rate of the six other pathogens was as follows: PIV1 (4.00%), RSV (3.35%), PIV3 (1.94%), CP (0.77%), ADV (0.32%), and PIV2 (0.03%). Among the 1112 pediatric children, 1028 cases were infected with a single pathogen, 83 cases were infected with two pathogens, and one case was infected with three pathogens.

2.2 Relationship between the Infected Respiratory Pathogens and Gender

Among the 3100 pediatric children after the COVID-19 pandemic, 611 out of 1851 male cases (33.01%) and 501 out of 1249 female cases (40.11%) were infected with at least one pathogen. Therefore, the total detection rate of the pathogens differed statistically between the different genders (P<0.05). Among the cases infected with a single pathogen, the detection rate of PIV1 and MP in female children was higher than that in male children (P<0.05), the detection rate of PIV3 in male children was higher than that in female children (P<0.05), and the detection rate of other pathogens displayed no statistical difference between the genders (P>0.05). The details of the results are shown in table 1.

Table 1 Relationship between infected respiratory pathogens and gender

Pathogen	Positive cases (%)		χ^2	P
	Male (n=1851)	Female (n=1249)		
ADV	8 (0.43)	2 (0.16)	0.975	0.190
FluA	0 (0.00)	0 (0.00)	<0.001	1.000
FluB	0 (0.00)	0 (0.00)	<0.001	1.000
PIV1	63 (3.40)	61 (4.88)	4.256	0.039
PIV2	1 (0.05)	0 (0.00)	<0.001	1.000
PIV3	44 (2.38)	16 (1.28)	4.720	0.030
RSV	59 (3.19)	45 (3.60)	0.397	0.529
CP	13 (0.70)	11 (0.88)	0.309	0.578
MP	468 (25.28)	406 (32.51)	19.216	<0.001
≥1 pathogen	611 (33.01)	501 (40.11)	16.356	<0.001

ADV: adenovirus; Flu A: influenza virus A; Flu B: influenza virus B; PIV1: parainfluenza virus 1; PIV2: parainfluenza virus 2; PIV3: parainfluenza virus 3; RSV: respiratory syncytial virus; CP: *Chlamydia pneumoniae*; MP: *Mycoplasma pneumoniae*

2.3 Relationship between Respiratory Pathogen Infection and Age

According to age, 3100 pediatric children who were enrolled after the COVID-19 pandemic were divided into four age groups: 0– year, 1– year, 4– years, and 7–14 years. The detection rate of the pathogens in the four age groups with a respiratory tract infection was as follows: 23.03% (120/521), 40.63% (477/1484), 38.97% (272/698), and 29.47% (117/397), respectively. Furthermore, the total detection rate of the pathogens was statistically different among the four age groups (P<0.05), in which pediatric children aged between 1 to 6 years were the main infected cases. Among the cases infected with a single pathogen, the detection rate of PIV1, PIV3, RSV, and MP was statistically different among the four age groups (P<0.05), and these pathogens mainly infected pediatric children aged 0–6 years, 0–3 years, 0–3 years, and 1–6 years, respectively. The details of the results are shown in table 2.

2.4 Difference of Respiratory Pathogens' Detection Rate between Pediatric Patients Enrolled before and after the COVID-19 Pandemic

There was no statistical difference in the gender and age between cases enrolled before and after the COVID-19 pandemic (P>0.05; table3). Compared with the detection rate of the respiratory pathogens before the pandemic, the detection rate of a common respiratory infectious pathogen was lower after the pandemic (P<0.05). Among these pathogens, the detection rate of PIV1 and CP was significantly increased (P<0.05), the detection rates of ADV, Flu A, Flu B, PIV 3, and RSV were significantly decreased (P<0.05), and the detection rate of PIV2 did not show any change. The details of the results are shown in table 4.

3 DISCUSSION

With the improvement of social welfare and medical standards, ARIs are still the main cause of the under-five mortality worldwide[15]. In addition, mortality due to ARIs is significantly varied across regions with mortality remaining higher in middle- and low-income countries[16]. Moreover, ARIs account for approximately 70% of the morbidity of pediatric

Table 2 Relationship between respiratory pathogen infection and age

Pathogen	Age group				χ^2	P
	0–	1–	4–	7–14		
	(n=521)	(n=1484)	(n=698)	(n=397)		
ADV	1	8	0	1	4.760	0.190
Flu A	0	0	0	0	<0.001	1.000
Flu B	0	0	0	0	<0.001	1.000
PIV1	21	65	34	4	11.197	0.011
PIV2	0	1	0	0	1.089	0.780
PIV3	17	36	7	0	17.752	<0.001
RSV	27	63	13	1	25.580	<0.001
CP	7	10	5	2	2.801	0.423
MP	53	477	235	109	105.446	<0.001
≥1 pathogen	120	603	272	117	61.942	<0.001

ADV: adenovirus; Flu A: influenza virus A; Flu B: influenza virus B; PIV1: parainfluenza virus 1; PIV2: parainfluenza virus 2; PIV3: parainfluenza virus 3; RSV: respiratory syncytial virus; CP: *Chlamydia pneumoniae*; MP: *Mycoplasma pneumoniae*

Table 3 The difference in gender and age between pediatric patients who were enrolled before and after the COVID-19 pandemic

COVID-19 pandemic	Gender	n	Age (years)
Before	Male	4647	3 (1–4)
	Female	3171	
After	Male	1851	3 (1–4)
	Female	1249	
		χ^2=0.067	z=–0.128
		P=0.796	P=0.898

Table 4 The difference in the respiratory pathogens' detection rate between pediatric patients enrolled before and after the COVID-19 pandemic

Pathogens	COVID-19 pandemic		χ^2	P
	Before (n=7818)	After (n=3100)		
ADV	221 (2.83)	10 (0.32)	62.217	<0.001
FluA	27 (0.35)	0 (0.00)	10.773	0.001
FluB	60 (0.77)	0 (0.00)	23.923	<0.001
PIV1	106 (1.36)	124 (4.00)	75.258	<0.001
PIV2	14 (0.18)	1 (0.03)	3.487	0.062
PIV3	301 (3.85)	60 (1.94)	25.451	<0.001
RSV	537 (6.87)	104 (3.35)	49.598	<0.001
CP	5 (0.06)	24 (0.77)	42.269	<0.001
MP	2510 (32.11)	874 (28.19)	15.882	<0.001
≥1 pathogen	3416 (43.69)	1112 (35.87)	55.969	<0.001

ADV: adenovirus; Flu A: influenza virus A; Flu B: influenza virus B; PIV1: parainfluenza virus 1; PIV2: parainfluenza virus 2; PIV3: parainfluenza virus 3; RSV: respiratory syncytial virus; CP: *Chlamydia pneumoniae*; MP: *Mycoplasma pneumoniae* children in developing countries[17], which has greatly increased the respective society's medical and economic burden. As the prevalence of ARIs is regional, this is very important for empirical therapy and for the provision of evidence for the respective country's health department to take active prevention and control measures[18]. Additionally, the COVID-19 pandemic has promoted the public's prevention and control of respiratory-borne diseases in Wuhan[19], which has had an impact on the epidemiological features of respiratory tract infectious diseases; as a consequence, it would be necessary to reassess the distribution of pathogens and formulate new prevention and control measures to contol the pathogens' spread in children.

Our study analyzed the epidemiological features of common respiratory infectious diseases for pediatric children in Wuhan before and after the COVID-19 pandemic, and the results showed that the total detection rate of pathogens in pediatric children was 35.87% after the pandemic. The detection rates of the pathogens from high to low were as follows: MP (28.19%), PIV1 (4.00%), RSV (3.35%), PIV3 (1.94%), CP (0.77%), ADV (0.32%), and PIV2 (0.03%). Similar to Liu Rui's report[20], MP, PIV1, and RSV were the most susceptible pathogens for children in Wuhan during this period, and the infection rates of other pathogens were not consistent. Therefore, two factors may account for the difference in the prevalence of the respiratory pathogens: (1) After the pandemic, the prevention and control measures (especially wearing a mask) had changed the infection rate of the pathogens. (2) Compared with other researchers, few cases enrolled in our study caused biased results. Our results also showed that the mixed infection rate was relatively low in Wuhan with the dual infection of MP and other pathogens that were the most common type of mixed infection.

From the results, we knew that the total detection rate of pathogens in male pediatric children was significantly lower than in female pediatric patients, which was consistent with conclusion of Na *et al*[21]. Males usually prefer sports and exercise longer time than females, and the number of males pursuing regular sporting activities was significantly greater than that of females[22]. Likewise, the personality would make immunity and resistance to infection stronger in males than in females; nevertheless, this would need big data to validate in the future. Additionally, no children were detected being infected with the flu, which confirmed that the protective and preventive measures for the pandemic effectively controlled the spreading of the flu. Through the comparison of the detection rate of pathogens in pediatric children among the different ages, we found that the total infection rates were different among the age groups (P<0.05). The detection rates of PIV1, PIV3, RSV, and MP were statistically different, and these pathogens were mainly detected in the 0–6 years, 0–3 years, 0–3 years, and 1–6 years age groups, respectively. Therefore, it would be very important for medical and health institutions to implement key prevention and management measures based on the prevalence of the infectious pathogens in different aged children.

After the outbreak of COVID-19, a series of prevention and control measures effectively reduced the spread of the virus in Wuhan. The pandemic also greatly improved the people's hygiene habits and protection awareness against respiratory infectious diseases. Except for PIV1, PIV2, and CP, our study showed that the detection rate of other pathogens for hospitalized pediatric children in Wuhan was significantly lower than thatbefore the pandemic. The study confirmed that the isolation of the presumed positive patients, reducing crowd gathering and mobility, wearing masks, and paying attention to hand hygiene could effectively reduce the infection of respiratory infectious pathogens[23]. For the prevention of PIV1, PIV2, and CP pandemics, more preventive measures would be needed.

This study also had the following limitations. Firstly, the number of hospitalized pediatric patients with respiratory infection in Wuhan had decreased significantly after the COVID-19 pandemic, so the number of cases enrolled in this study was relatively small. Secondly, the number of hospitalized pediatric children with COVID-19 returned to zero since May 1, 2020, which was why an analysis of seasonal epidemiology could not be performed. Finally, before the pandemic, the infection status of common respiratory infectious pathogens in hospitalized pediatric children was not analyzed, but the related results could be found in Liu's report[20]. Consequently, more data should be used for further research.

Conflict of Interest Statement

The authors declared no conflict of interest.

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