Pathogen spectrum changes of respiratory tract infections in children in Chaoshan area under the influence of COVID-19

Chuang-Xing Lin1*, Hao-bin Lian1*, Guang-Yu Lin1, Dan-gui Zhang2, Xiao-Ying Cai1, Zhi-Wei Cai1 and Fei-qi Wen3;

1Department of Pediatrics, The Second Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China

2Translational Medicine Research Center, The Second Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China

3Shenzhen Children's Hospital, Shenzhen, Guangdong, China

*Chuang-Xing Lin and Hao-bin Lian should be considered joint first author

Correspondence to: Fei-qi Wen, Shenzhen Children's Hospital, Shenzhen, Guangdong, China

E-mail: 272816142@qq.com

Conflicts of Interest: None.

Keywords: Virology (human) and epidemiology, Paediatrics, Respiratory infections

Word counts:

Abstract: 270 words

Text: 2528 words

Total number of figures: 4 figures and 3 tables
Abstract

From January 24, 2020 to May 18, 2020, Chaoshan took measures to limit the spread of COVID-19, such as restricting public gatherings, wearing masks, and suspending classes. We explored the effects of these measures on the pathogen spectrum of pediatric respiratory tract infections in Chaoshan.

Pharyngeal swab samples were collected from 4075 children hospitalized for respiratory tract infection before (May–December 2019) and after (January–August 2020) the COVID-19 outbreak. We used liquid chip technology to analyze 14 respiratory pathogens. The data were used to explore between-group differences, age-related differences, and seasonal variations in respiratory pathogens.

The number of cases in the outbreak group (1222) was 42.8% of that in the pre-outbreak group (2853). Virus-detection rates were similar in the outbreak (48.3%, 590/1222) and pre-outbreak groups (51.5%, 1468/2853; $\chi^2 = 3.446; P = 0.065$), while the bacteria-detection rate was significantly lower in the outbreak group (26.2%, 320/1222) than in the pre-outbreak group (44.1%, 1258/2853; $\chi^2 = 115.621, P < 0.05$). With increasing age, the proportions of respiratory syncytial virus (RSV) and cytomegalovirus (CMV) infections decreased, while those of Mycoplasma pneumoniae (MP), and adenovirus (ADV) infections increased. Streptococcus pneumoniae (SP), CMV, and rhinovirus infections peaked in autumn and winter, while RSV infections peaked in summer and winter. We found that the proportion of virus-only detection decreased with age, while the proportion of bacteria-only detection increased with age (Table 2).

Anti-COVID-19 measures significantly reduced the number of pediatric hospitalizations for respiratory tract infections, significantly altered the pathogen spectrum of such infections,
and decreased the overall detection rates of 14 common respiratory pathogens. The proportion of bacterial, but not viral, infections decreased.

**Background**

As one of the most common pediatric diseases, respiratory tract infection is a major public health problem in both developing and developed countries, and poses a massive economic burden globally[1-4]. The pathogen spectrum of epidemic respiratory tract infections differs among different geographic locations according to the climatic environment[5-8]. The Chaoshan area, located in Southeastern China, includes four cities, namely, Shantou, Shanwei, Chaozhou, and Jieyang, and has a large population. This area has a typical subtropical monsoon climate with hot and humid summers and cold and humid winters. Therefore, the pathogen spectrum of respiratory infections in children in this area has its own unique characteristics. The spread and prevalence of respiratory pathogens in 2020 has been considerably different from that in previous years owing to the prevention and control measures taken against coronavirus infectious disease 2019 (COVID-19), such as the suspension of classes, restrictions on public gatherings, and wearing of masks. The purpose of this study was to explore the effects of the anti-COVID-19 measures on the pathogen spectrum of respiratory tract infections in children in the Chaoshan area.

1. Materials and Methods

1.1 **Study subjects and ethics statement**

In the present study, pharyngeal swabs were collected from 4075 children (ages, 1 month to 14 years, mean age: 2 years and 6 months, median age: 1 year) who were treated for...
respiratory tract infections in Children’s Hospital of the Second Affiliated Hospital of Shantou University Medical College, between May 2019 and August 2020. This hospital is the largest children’s medical center in Chaoshan area. The inclusion criteria were pediatric in-patients with clinically diagnosed respiratory tract infection. The diagnostic criteria for respiratory tract infection consisted of cough, expectoration, and any one of the following three conditions: (1) fever, (2) increase in the total leukocyte count and/or proportion of neutrophils, and (3) wet rales appearing in the lungs and/or inflammatory infiltrative lesions in the lungs on X-ray examination. The exclusion criteria were as follows: (1) newborns less than 28 days old, (2) inadequate pharyngeal swab samples, i.e., too few sampled cells or insufficient nucleic acid extraction, (3) incomplete clinical data, for example, lack of medical records or clinical diagnosis, (4) chest X-ray changes in respiratory tract caused by non-infectious causes such as pulmonary embolism, heart failure, pulmonary edema, and lung cancer. With January 1, 2020 as the boundary, the children included in the study were divided into two groups: the pre-outbreak group (May 2019 to December 2019) and the outbreak group (January 2020 to August 2020).

All the specimens were collected with the written consent of the parents, and the study protocol was approved by the ethics committee of the Second Affiliated Hospital of Shantou University Medical College.

1.2 Specimen collection

Upon admission, patients aged greater than 2 years were instructed to gargle with warm saline twice, and then, disposable aseptic pharyngeal swabs were used to gently and rapidly wipe the secretions on the palatine arch, pharynx, and tonsils on both the right and left sides. After sampling, the pharyngeal swabs were quickly placed in collection tubes, which were
immediately sealed using the tube covers, marked with the sampling date, and patient name and number, stored in a refrigerator at 2°C–8°C, and analyzed within 24 h.

1.3 Nucleic acid-based pathogen detection

The respiratory swabs were sent to Guangzhou Da’an Clinical Examination Center, where they were subjected to total viral nucleic acid extraction via the magnetic bead method by using the KingFisher automated nucleic acid extraction instrument and the viral total nucleic acid extraction kit from Guangzhou Meiji Biotechnology Co. Ltd. [9], according to the manufacturers’ instructions. After the extraction, nucleic acids of respiratory pathogens were detected using multiplex polymerase chain reaction (PCR) [10] and Luminex suspension liquid chip technology [11]. This technology integrates flow cytometry, encoding microspheres, lasers, and digital signal processing, enabling the rapid, sensitive, and specific detection of different pathogens. The diagnostic sensitivity and specificity of liquid chip technology are similar to those of PCR. Simultaneous detection of the following 14 main respiratory pathogens was performed: influenza A virus (FluA), influenza B virus (FluB), parainfluenza virus (PIV), human rhinovirus (HRV), adenovirus (ADV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), bocavirus (BoV), cytomegalovirus (CMV), Haemophilus influenzae (HI), Streptococcus pneumoniae (SP), Moraxella catarrhalis (MC), Mycoplasma pneumoniae (MP), and Bordetella pertussis (BP).

1.4 Statistical methods

Data were statistically analyzed using SPSS v23.0. Count data were expressed as cases and percentages. The chi-square test was used to compare the rate of pathogen detection in
each group. Double-tailed probability was used in all tests, and a P value below 0.05 was considered statistically significant.

2. Results

2.1 General information

Of the 4075 cases in the present study, 2853 cases were included in the pre-outbreak group, and 1222 cases were included in the outbreak group (Table 1). The number of cases in the outbreak group was lower than that in the pre-outbreak group (at 57.2%). The positive rate of pathogen detection was significantly higher in the pre-outbreak group (74.5%, 2125/2853) than in the outbreak group (61.0%, 746/1222; \( \chi^2 = 74.191, P < 0.05 \)). The virus detection rate did not significantly differ between the outbreak (48.3%, 590/1222) and pre-outbreak groups (51.5%, 1468/2853; \( \chi^2 = 3.446, P = 0.065 \)). In contrast, the bacterial detection rate was significantly lower in the outbreak group (26.2%, 320/1222) than in the pre-outbreak group (44.1%, 1258/2853; \( \chi^2 = 115.621, P < 0.05 \)).

2.2. Respiratory pathogen detection

The top five pathogens detected in the outbreak group were CMV (27.1%, 331/1222), SP (19.1%, 234/1222), RSV (16.5%, 202/1222), HRV (6.6%, 81/1222), FluA (4.0%, 49/1222; Figure 1A), while the top five pathogens in the pre-outbreak group were SP (22.1%, 630/2853), CMV (20.6%, 587/2853), MP (12.3%, 352/2853), RSV (11.6%, 332/2853) and HRV (10.6%, 301/2853; Figure 1B). The positive rates of detection of CMV, SP, RSV, MP, HRV and FluA significantly differed between the outbreak and pre-outbreak groups (\( \chi^2 = 20.787, 4.405, 17.991, 109.929, 15.489 \) and 26.219 respectively; P < 0.05 for all). The other
pathogens also statistically differed between the two groups (P < 0.05 for all), except hMPV (P > 0.05). Only 7 cases of hMPV were detected in total; no case of hMPV was detected in the outbreak group.

2.3. Respiratory pathogens and age

To study the changes in the respiratory pathogen spectrum with age, we divided all the study subjects into the following age groups: 0 to ≤6 months, >6 months to ≤1 year, >1 year to ≤3 years, >3 years to ≤6 years, and >6 years. We assessed the proportions of the detection of a viral pathogen only, a bacterial pathogen only, both bacterial and viral pathogens, and no pathogen at all in different age groups (Figure 2). We found that the proportion of virus-only detection decreased with age, while the proportion of bacteria-only detection increased with age (Table 2).

We also assessed the proportion of the detection of individual pathogens in each age group (Figure 3 and Table 3). With increase in age, the detection rates of RSV and CMV gradually decreased (χ² = 196.668, 330.742, respectively; P < 0.005 for all), while those of MP and ADV increased (χ² = 214.657, 65.691 respectively; P < 0.005 for all).

2.4. Seasonal variations in respiratory pathogens

In this study, the four seasons are defined as follows: spring is March, April, and May, summer is June, July, and August, autumn is September, October, and November, and winter is December, January, and February. We found that SP, CMV, and HRV infections peaked in autumn and winter in 2019, and decreased after the COVID-19 outbreak (Figure 4). RSV infections peaked in summer and winter in 2019 and summer in 2020, and the proportion of
RSV infections decreased in the spring of 2020, i.e., after the outbreak. MP and ADV infections peaked in the summer of 2019 and then gradually decreased.

3. Discussion

The present study showed that the prevention and control measures taken against COVID-19 significantly reduced the number of children hospitalized because of respiratory tract infections, changed the pathogen spectrum of respiratory tract infections, and decreased the proportion of bacterial infections, but did not significantly change the proportion of viral infections.

Acute respiratory infection is an important cause of morbidity and mortality in children under 5 years of age [12,13]. Furthermore, over a period of more than 50 years, there has been no significant change in the mortality rate of acute respiratory infections [4,14,15]. Therefore, in developing countries, including China, acute respiratory infection remains an important cause of death in children [16]. From January 24, 2020 to May 18, 2020, the Chaoshan area took measures to limit the spread of COVID-19, such as restricting public gatherings, wearing masks in public places, and suspending classes. This paper studied the impact of these measures on the pathogen spectrum of respiratory tract infections in children in the Chaoshan area. A total of 14 respiratory pathogens were investigated during a period of 16 months. We found that the number of cases of respiratory tract infections during the 8 months after the COVID-19 outbreak (January to August 2020) was only 57.2% of the total number of cases in the 8 months before the outbreak (May to December 2019). Hence, we believe that the prevention and control measures taken during the COVID-19 pandemic significantly reduced the number of children who needed to be hospitalized due to respiratory tract infections.
In the outbreak group, respiratory pathogens were detected in 61.0% of the subjects. This rate is similar to the respiratory pathogen-detection rate (60.3%) among children in Dongguan City, another subtropical area [17], but it is lower than the rate in the pre-outbreak group (74.5%). These results imply that the respiratory pathogen spectrum of children in the Chaoshan area changed during the COVID-19 outbreak, and the proportion of respiratory infections caused by 14 common pathogens decreased. Notably, the proportion of viral pathogens did not change significantly, with virus detection rates of 48.3% in the outbreak group and 51.5% in the pre-outbreak group. Moreover, the virus detection rate in our study is similar to that found in our hospital in previous years (54.9%)[4].

The top five pathogens detected in the outbreak group were CMV (27.1%), SP (19.1%), RSV (16.5%), HRV (6.6%) and FluA (4.0%). The top five pathogens detected in the pre-outbreak group were SP (22.1%), CMV (20.6%), MP (12.3%), RSV (11.6%) and HRV (10.6%). The rate detection of most pathogens decreased in the outbreak group, compared with the pre-outbreak group. However, the rate detection of CMV and RSV increased in the outbreak group, compared with the pre-outbreak group. The rate detection of RSV increased from 11.6% (332/2853) in the pre-outbreak group to 16.5% (202/1222) in the outbreak group. Nevertheless, the anti-COVID-19 measures did appear to reduce the spread of RSV infections, as the number of actual cases of RSV infection reduced from 332 in the pre-outbreak group to 202 in the outbreak group, amounting to a decrease of 39.2%. RSV is mainly transmitted by respiratory droplets [18]. The virus particles are small, and ordinary masks cannot completely block its transmission, so the prevention and control measures may have had less of an impact on RSV infections than on other pathogens, such as bacteria. CMV is an opportunistic pathogen that is transmitted through a variety of routes, including sexual transmission, blood contact, and inhalation of or contact with body fluids [19], so the transmission of CMV is also less affected by epidemic prevention and control measures. SP
is a commensal bacterium in the human respiratory tract, with a high carrying rate among the general population. It can be transmitted by droplets and aerosols, so limiting contact with people and wearing masks can significantly reduce its incidence. Another important reason is that there is no routine vaccination for SP in the Chaoshan area.

This study showed that with increase in age, the proportions of RSV and CMV infections gradually decreased, while the proportions of MP, SP, and ADV infections increased. Consistent with our findings, a study in the United States found that RSV is the pathogen with the highest detection rate among children under 5 years old with pneumonia; the detection rate of RSV was as high as 37%, and it gradually decreased with age. Some authors believe that the gradual decrease in the infection rates of RSV and CMV with age may be related to the low autoimmunity of very young children. The proportions of MP infection gradually increased with age, which is consistent with a study from Dongguan, and may be related to the development of the normal respiratory microflora. In this study, the proportion of ADV infections increased with age. However, other studies have shown that the proportion of ADV infections does not increase with age, and there is no inevitable relationship between the two. Our result may be related to the small number of ADV infection cases detected in this study and the great difference in the number of children in the different age groups.

SP, CMV, and HRV infections peaked in autumn and winter in 2019, and the proportions of these infections decreased after the pandemic prevention and control measures were put in place. RSV infections peaked in summer and winter in 2019 and summer in 2020, and decreased in spring in 2020, probably due to the prevention and control measures. MP and ADV infections peaked in the summer of 2019 and then decreased gradually. Of course, infections by various pathogens are also related to the climate of Chaoshan area, which is a typical subtropical monsoon climate with hot and humid summers and cold and humid
winters. This environment with high humidity may be beneficial to the transmission, colonization, and invasion of RSV, ADV, and MP [17].

This study has some limitations. First, the study lasted for only 16 months, which is a relatively short time. A total of 4075 cases were included in this study, with January 1, 2020 as the boundary to divide the cases into two groups: the pre-outbreak group with 2853 cases and the outbreak group with 1222 cases. Thus, the data for each group were collected over a period of 8 months. Because the study period was less than 2 years, our data cannot be compared with data from the same region collected at the same time in different years, but can only be compared with data from the same region during the same length of time. Therefore, extending the research time may lead to more meaningful results. Second, this study only tested for the 14 most common respiratory pathogens in this region, and did not analyze other pathogens.

4. Conclusion

This study shows that the prevention and control measures taken against the COVID-19 pandemic significantly reduced the number of children who required to be hospitalized because of respiratory tract infections, significantly altered the pathogen spectrum of respiratory tract infections, and decreased the positive rate of detection of respiratory tract pathogens. Among the 14 most common respiratory pathogens, the proportion of bacterial infections decreased, but the proportion of viral infections did not decrease significantly.

5. Acknowledgements
We are very grateful to the doctors and nurses who helped collect the pharyngeal samples. This work was supported by the Guangdong Science and Technology Special Fund (project approval numbers: 49 and 135) and Guangdong Medical Research Fund (project number: A2020551).

6. Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Fei-qiu Wen, upon reasonable request.

References

1. Leung, D. T., Chisti, M. J., & Pavia, A. T. (2016). Prevention and Control of Childhood Pneumonia and Diarrhea. *Pediatric clinics of North America, 63*(1), 67–79. doi:10.1016/j.pcl.2015.08.003

2. Yao, K.-H., et al. (2011). Pneumococcal serotype distribution and antimicrobial resistance in Chinese children hospitalized for pneumonia. *Vaccine, 29*(12), 2296-2301. doi:10.1016/j.vaccine.2011.01.027

3. Nair, H., et al. (2010). Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet (London, England), 375*(9725), 1545-1555. doi:10.1016/s0140-6736(10)60206-1

4. Cai, X. Y., et al. (2014). Respiratory virus infections among children in South China. *Journal of medical virology, 86*(7), 1249-1255. doi:10.1002/jmv.23931
5. Yu, X., et al. (2012). Etiology and clinical characterization of respiratory virus infections in adult patients attending an emergency department in Beijing. *PLoS One, 7*(2), e32174. doi:10.1371/journal.pone.0032174

6. Zhao, Y., et al. (2019). Comparison of viral and epidemiological profiles of hospitalized children with severe acute respiratory infection in Beijing and Shanghai, China. *BMC Infectious Diseases, 19*(1), 729. doi:10.1186/s12879-019-4385-5

7. Liu, T., et al. (2015). Viral Etiology of acute respiratory tract infections in hospitalized children and adults in Shandong Province, China. *Virology Journal, 12*, 168. doi:10.1186/s12985-015-0388-z

8. Liu, J., et al. (2020). Viral and bacterial coinfection among hospitalized children with respiratory tract infections. *American Journal of Infection Control, 48*(10), 1231-1236. doi:10.1016/j.ajic.2020.01.013

9. Yang, G., et al. (2011). Comparison of commercial systems for extraction of nucleic acids from DNA/RNA respiratory pathogens. *Journal of Virological Methods, 171*(1), 195-199. doi:10.1016/j.jviromet.2010.10.024

10. Huang, H. S., et al. (2018). Multiplex PCR system for the rapid diagnosis of respiratory virus infection: systematic review and meta-analysis. *Clinical Microbiology and Infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 24*(10), 1055–1063. https://doi.org/10.1016/j.cmi.2017.11.018

11. Yan, Y., et al. (2017). A multiplex liquid-chip assay based on Luminex xMAP technology for simultaneous detection of six common respiratory viruses. *Oncotarget, 8*(57), 96913–96923. https://doi.org/10.18632/oncotarget.18533
12. Williams, B. G., et al. (2002). Estimates of world-wide distribution of child deaths from acute respiratory infections. *The Lancet Infectious Diseases*, 2(1), 25-32. doi:10.1016/s1473-3099(01)00170-0

13. Liu, L., et al. (2012). Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet (London, England)*, 379(9832), 2151-2161. doi:10.1016/s0140-6736(12)60560-1

14. Jain, S., et al. (2015). Community-acquired pneumonia requiring hospitalization among U.S. children. *The New England journal of medicine*, 372(9), 835-845. doi:10.1056/NEJMoa1405870

15. Mizgerd, J. P. (2006). Lung infection--a public health priority. *PLoS medicine*, 3(2), e76. doi:10.1371/journal.pmed.0030076

16. Wang, H., et al. (2016). Prevalence of respiratory viruses among children hospitalized from respiratory infections in Shenzhen, China. *Virology journal*, 13, 39. doi:10.1186/s12985-016-0493-7

17. Sun, Z., Zhang, R., & Zhong, C. (2019). A surveillance of children lower respiratory tract infection cases in Dongguan City, from 2017 to 2018. *Journal of Molecular Diagnostics and Therapy*, 011(004), 329-337. doi:CNKI:SUN:YXYQ.0.2019-04-014

18. Hall, C. B., Simoes, E. A., & Anderson, L. J. (2013). Clinical and epidemiologic features of respiratory syncytial virus. *Current topics in microbiology and immunology*, 372, 39-57. doi:10.1007/978-3-642-38919-1_2

19. Fonseca Brito, L., Brune, W., & Stahl, F. R. (2019). Cytomegalovirus (CMV) Pneumonitis: Cell Tropism, Inflammation, and Immunity. *International journal of molecular sciences*, 20(16). doi:10.3390/ijms20163865
20. El Moujaber, G., et al. (2017). Molecular mechanisms and epidemiology of resistance in Streptococcus pneumoniae in the Middle East region. *Journal of medical microbiology, 66*(7), 847-858. doi:10.1099/jmm.0.000503

21. Obaro, S., & Adegbola, R. (2002). The pneumococcus: carriage, disease and conjugate vaccines. *Journal of medical microbiology, 51*(2), 98–104. https://doi.org/10.1099/0022-1317-51-2-98

22. Subramanian, K., Henriques-Normark, B., & Normark, S. (2019). Emerging concepts in the pathogenesis of the Streptococcus pneumoniae: From nasopharyngeal colonizer to intracellular pathogen. *Cellular microbiology, 21*(11), e13077. doi:10.1111/cmi.13077

23. He, Y., et al. (2014). A 3-year prospective study of the epidemiology of acute respiratory viral infections in hospitalized children in Shenzhen, China. *Influenza and other respiratory viruses, 8*(4), 443-451. doi:10.1111/irv.12257

**Figure 1.** Distribution of pathogens detected in the outbreak and pre-outbreak groups.
Figure 2. Pathogen detection results among children with respiratory tract infections stratified by age.
Figure 3. Distribution of detected pathogens among different age groups

Figure 4. Seasonal variations in the detection of respiratory pathogens
Table 1. Comparison of infectious pathogens between the outbreak and pre-outbreak groups

| Pathogen detected          | Outbreak group (%) | Pre-outbreak group (%) | \( \chi^2 \) | P      |
|----------------------------|--------------------|------------------------|-------------|--------|
| None                       | 476 (39.0)         | 728 (25.5)             | 74.191      | <0.05  |
| Viral only                 | 426 (34.9)         | 867 (30.4)             | 7.898       | <0.05  |
| Bacterial only             | 156 (12.8)         | 657 (23.0)             | 56.419      | <0.05  |
| Bacterial and viral        | 164 (13.4)         | 601 (21.1)             | 32.791      | <0.05  |
| Total                      | 1222 (100)         | 2853 (100)             |             |        |

Table 2. Pathogen detection results among children with respiratory tract infections stratified by age

| Pathogen                  | 0 to ≤6 mo (%) | >6 mo to ≤1 yr (%) | >1 to ≤3 yrs (%) | >3 to ≤6 yrs (%) | >6 yrs (%) | \( \chi^2 \) | P      |
|---------------------------|----------------|-------------------|------------------|-----------------|-----------|-------------|--------|
| No                        | 306 (30.5)     | 287 (26.1)        | 262 (26.7)       | 194 (34.0)      | 155 (36.8)| 26.643      | <0.005 |
| Viral pathogen only       | 475 (47.3)     | 419 (38.1)        | 218 (22.2)       | 105 (18.4)      | 76 (18.1) | 257.172     | <0.005 |
| Bacterial pathogen only   | 91 (9.1)       | 142 (12.9)        | 247 (25.2)       | 181 (31.8)      | 152 (36.1)| 243.821     | <0.005 |
| Bacterial-viral co- detection | 132 (13.1)     | 251 (22.8)        | 254 (25.9)       | 90 (15.8)       | 38 (9.0)  | 94.912      | <0.005 |
| Total                     | 1004 (100.0)   | 1099 (100.0)      | 981 (100.0)      | 570 (100.0)     | 421 (100.0)|            |        |
Table 3. Distribution of detected pathogens among different age groups

| Pathogen | 0 to ≤6 mo (%) (N = 1004) | >6 mo to ≤1 yr (%) (N = 1099) | >1 to ≤3 yrs (%) (N = 981) | >3 to ≤6 yrs (%) (N = 570) | >6 yrs (%) (N = 421) | χ² | P |
|----------|-----------------------------|-------------------------------|-----------------------------|----------------------------|----------------------|----|----|
| FluA     | 11 (1.1)                    | 15 (1.4)                      | 25 (2.5)                    | 22 (3.9)                   | 17 (4.0)             | 23.623 | <0.005 |
| FluB     | 4 (0.4)                     | 8 (0.7)                       | 16 (1.6)                    | 14 (2.5)                   | 21 (5.0)             | 49.438 | <0.005 |
| PIV      | 65 (6.5)                    | 77 (7.0)                      | 42 (4.3)                    | 18 (3.2)                   | 12 (2.9)             | 21.573 | <0.005 |
| HRV      | 98 (9.8)                    | 114 (10.4)                    | 103 (10.5)                  | 47 (8.2)                   | 20 (4.8)             | 14.378 | <0.005 |
| ADV      | 13 (1.3)                    | 38 (3.5)                      | 70 (7.1)                    | 45 (7.9)                   | 36 (8.6)             | 65.691 | <0.005 |
| RSV*     | 234 (23.3)                  | 162 (14.7)                    | 113 (11.5)                  | 23 (4.0)                   | 2 (0.5)              | 196.668 | <0.005 |
| hMPV     | 2 (0.2)                     | 4 (0.4)                       | 1 (0.1)                     | 0 (0.0)                    | 0 (0.0)              | 4.395  | >0.005 |
| BoV      | 6 (0.6)                     | 49 (4.5)                      | 35 (3.6)                    | 2 (0.4)                    | 0 (0.0)              | 63.410 | <0.005 |
| CMV*     | 321 (32.0)                  | 383 (34.8)                    | 155 (15.8)                  | 44 (7.7)                   | 15 (3.6)             | 330.742 | <0.005 |
| HI       | 28 (2.8)                    | 49 (4.5)                      | 39 (4.0)                    | 20 (3.5)                   | 10 (2.4)             | 6.496  | >0.005 |
| SP*      | 72 (7.2)                    | 224 (20.4)                    | 325 (33.1)                  | 143 (25.1)                 | 100 (23.8)           | 209.070 | <0.005 |
| MC       | 42 (4.2)                    | 81 (7.4)                      | 73 (7.4)                    | 32 (5.6)                   | 13 (3.1)             | 19.842 | <0.005 |
| MP*      | 20 (2.0)                    | 57 (5.2)                      | 107 (10.9)                  | 100 (17.5)                 | 92 (21.9)            | 214.657 | <0.005 |
| BP*      | 90 (9.0)                    | 31 (2.8)                      | 10 (1.0)                    | 17 (3.0)                   | 2 (0.5)              | 113.924 | <0.005 |
| Total    | 1006 (100.2)                | 1292 (117.6)                  | 1114 (113.6)                | 527 (92.5)                 | 340 (80.8)           |        |     |

Pathogens labeled (*) showed significant differences among different age groups.