Open Journal of Pediatrics, 2024, 14, 851-864
https://www.scirp.org/journal/ojped
ISSN Online: 2160-8776
ISSN Print: 2160-8741

Epidemiological and Subtype Characterization of Influenza Viruses Infection in Children in Shenzhen, China during Three Consecutive Seasons (January 2016-December 2018)

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How to cite this paper: Kuang, Y.X., Ma, R.H., Jia, L., Yao, Q., Zhang, C.H. and Fu, X.Y. (2024) Epidemiological and Subtype Characterization of Influenza Viruses Infection in Children in Shenzhen, China during Three Consecutive Seasons (January 2016-December 2018). *Open Journal of Pediatrics*, **14**, 851-864.
https://doi.org/10.4236/ojped.2024.145080

Received: August 8, 2024
Accepted: September 3, 2024
Published: September 6, 2024

Abstract

Background: Children with seasonal influenza infection cause a significant burden of disease each year in the pediatric clinic. Influenza A and B viruses are the major types responsible for illness. A better understanding of the periodicity facilitates the prevention and control of influenza in children. **Objective:** This study aims to analyze the epidemiological patterns and subtype characterization of influenza viruses among children in Shenzhen, China. **Methods:** Influenza samples were collected by nasopharyngeal swabs from influenza like illness patients in Shenzhen Children's Hospital from January 2016 to December 2018. The positive cases and influenza subtypes were determined by gold labeled antigen detection and reverse transcriptase polymerase chain reaction. The influenza periodicity and age, subtype distribution as well as the association between climate parameters and different influenza subtypes were analyzed by SPSS 22.0. **Results:** The influenza positive rate during 2016-2018 was 21.0%, with a highest positive rate in the year 2018. The positive rate varied by month, season, and year describing a sequence of peaks presenting primarily in all year including spring, summer and winter. The characteristics of influenza peak were different in each year, with a spring peak in 2016 and a summer plus a winter-spring peaks in 2017 and 2018. In addition, influenza B exhibited a winter-spring seasonal pattern while influenza A displayed a more variable seasonality, highlighting influenza B rather than

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influenza A which had a negative association with climate parameters. Influenza-positive cases were older than influenza-negative cases ($P < 0.05$). Among those positive cases, inpatients were younger than outpatients ($P < 0.05$), and the age of influenza A patients was younger than those influenza B patients, highlighting hospitalization with influenza often occurred in younger individuals infected with influenza A. **Conclusion:** Influenza activity in children from Shenzhen typically displays both winter-spring and summer peaks. Influenza A epidemic occurred separately or co-circulated with influenza B, with a winter-spring pattern for influenza B and a much more variable seasonality for influenza A. Influenza B had a negative association with climate parameters. In addition, hospitalization with influenza often occurs in younger individuals infected with influenza A.

Keywords

Influenza, Influenza Like Illness, Gold Labeled Antigen Detection, Reverse Transcriptase Polymerase Chain Reaction, Influenza A, Influenza B

1. Introduction

Influenza, an acute respiratory infectious disease caused by influenza virus, has become a global concern with considerable levels of morbidity and mortality and exhibits a regular seasonal occurrence worldwide, causing devastating pandemics and significant economic burden [1]-[3]. The World Health Organization (WHO) estimates that influenza severely affects three to five million individuals each year, causing 250,000 to 500,000 deaths worldwide [4]; of these, up to 84,000 to 92,000 deaths were associated with influenza in China [5]. It affects people of all ages, especially children [6]-[8], among whom severe disease is most likely to occur, combined with high burden in influenza-related costs.

Consequently, it is essential to have a better understanding of the seasonality of influenza, as the distinct and predictable seasonality facilitates timing, appropriate resource allocation, and the implementation of annual public health interventions for the effective prevention and control of influenza [9]. The seasonality of influenza has been well described in temperate regions both in northern and southern hemisphere and where influenza activity typically coincides with winter or early spring months [10]-[13]. However, it is still largely unknown on the timing of influenza activity in tropical or subtropical regions with respect to seasonal climate factors that may predict such activity [14]. A few pieces of literature indicated that unlike temperate regions, some tropical regions have influenza peaks in rainy season throughout the year [15] [16], while others have two distinct influenza seasons during the winter/spring and summer months within a year, such as Singapore and Hong Kong SAR [17] [18]. In Chinese mainland, there are two seasonal patterns according to the influenza surveillance data [19], with a regular winter peak in the northern part which is consistent with other countries and regions with temperate climate, and both winter/spring and summer peak in some southern provinces [20]. Yet it is reported that the influenza activity in Guangdong province tends to have a seasonal epidemic period in summer [21]. Shenzhen, as a window city in Guangdong province, connects Chinese Mainland to Hong Kong SAR and to other counties in Southeast Asia, situated at the border of subtropical and tropical regions in China. The humid subtropical marine weather affected by the East Asian monsoon in this region and large population migration reinforced the heterogeneity in the epidemiology of influenza. Thus, it is necessary to study the seasonal characteristics of influenza activity in Shenzhen.

To describe the detailed epidemiological dynamics of influenza virus in children in Shenzhen and explore whether subtropical areas had year-round multi-stage or significantly random influenza activities compared to temperate areas, we collected and analyzed influenza samples from Shenzhen Children's Hospital during the period 2016 to 2018.

2. Materials And Methods

2.1. Ethics Statement

This study was approved by Shenzhen Children's Hospital. It was also approved by the Ethics Committee of Shenzhen Children's Hospital. Written informed consent was obtained from the parents of every child participant enrolled in this study.

2.2. Study Area

Shenzhen, the fourth biggest city in China, located in the southern part of China and situated in the north hemisphere from 114˚03' E longitude and 22˚32' N latitude. The total area under the city's administration is 1996 square kilometers. The total population of the city amounted to 13.02 million by the end of 2018. Shenzhen has a humid subtropical weather influenced by the East Asian monsoon. The average temperature was 23.4˚C. The average rainfall was 178 mm, with an average relative humidity of 77%. Shenzhen Children's Hospital, the largest comprehensive children's hospital in Shenzhen, receiving most pediatric patients in Shenzhen, represents the epidemiological pattern and characteristics of influenza in children in Shenzhen.

2.3. Meteorological Data

We obtained data for the meteorological variables at daily intervals from the National Meteorological Information Center (http://cdc.cma.gov.cn). T, Average temperature (˚C); TM, maximum temperature (˚C); Minimum temperature (˚C); H, Humidity (%) and precipitation (mm). The humidity was collected from a meteorological station in Shenzhen city. Daily diurnal variation in temperature was calculated by subtracting the maximum and minimum temperature. These data were available for the period from January 2016 to December 2018 without any missing values.

2.4. Diagnosis Criteria and Specimen Collection

Influenza samples were collected from influenza like illness patients in Shenzhen Children's Hospital for the period from 2016 to 2018 (Total number: 70699). The flocked plastic/polyester swabs (BeiKe biological company, China) were used to collect nasopharyngeal samples from patients. The positive cases and influenza subtypes were determined by influenza antigen colloidal gold detection and reverse transcriptase polymerase chain reaction. The positive cases and influenza subtypes were determined by gold labeled antigen detection and reverse transcriptase polymerase chain reaction. As there is a high coincidence rate (87.5%) between the two methods and the amount of specimens detected by gold labeled antigen detection was much larger than that detected by reverse transcriptase polymerase chain reaction, we chose the former method and specimens for the present study.

2.5. Laboratory Testing for Influenzas

RT-PCR was used for nucleic acid detection: Influenza A RNA was extracted from the nasopharyngeal samples using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). Amplification of the hemagglutin in (HA) and neuraminidase (NA) genes occurred in a one-step RT-PCR reaction using One-step RT-PCR kit (Qiagen, Hilden, Germany). Complete HA and NA ORFs were basically amplified using primer sets recommended by WHO [6]. Hereby, two primer pairs were used to amplify each segment. The primer pair H3N2R 1104 and N2F257 for A (H3N2) amplification were modified during this research based on initial sequencing results to enhance the yield of the RT-PCR product as following H3N2 R1104 (ATCCACACGTCATTTC CATCATCA) AND N2F257 (AAACCAGCAGAATACAGAAATTGGTC). Screening for A (H1N1) pdm09 and A (H3N2) was performed using the N1F401/NARUc and H3A1F3/HARUc primer pairs respectively. Influenza antigen was detected by Influenza antigen colloidal gold detection kit (Guangzhou Wanfu biological Co. Ltd. China) according to the instructions. The specimen type was nasopharyngeal swab. The processed samples were directly added to the gold standard reagent for detection. The results were observed after 15 minutes.

2.6. Statistical Analyses

The influenza periodicity and age, subtype distribution as well as the association between climate parameters and different influenza subtypes were analyzed by SPSS 22.0. We used linear regression models to determine whether the mean proportion of samples that tested positive for influenza each month was associated with mean monthly temperature, humidity and solar radiation as well as precipitations in Shenzhen. In a linear regression model, coefficients represent the relationship strength between the independent variables including temperature, humidity, solar radiation, and precipitation and the dependent variable (the monthly proportion of positive influenza virus tests for A or B). The t-test is used for individual coefficients in a regression model to determine if they are significantly different from zero. The *P*-value is used to assess whether a coefficient is significantly different from zero. If the *P*-value < 0.05, the coefficient is considered statistically significant.

3. Results

3.1. Time Distribution of Influenza Positive Samples

The influenza positive rate for the period from January 2016 to December 2018 was 21.0% with 14,763 positives out of a total of 70,699 samples. And it was 18.1% in the year 2016 with 3096 positives out of 17,073 samples, and 17.6% in the year 2017 with 3987 positives out of 22,698 samples while 24.8% in the year 2018 with 7680 positives out of 30,928 samples. The frequency of influenza positive case was highest in year 2018 (24.8%) as compared to 2017(17.6%) and 2016 (18.1%). The number of influenza patients in the year 2018 was largest, accounting for 52.0% of the total (**Table 1**).

Table 1. Time distribution of Influenza positive samples.

Year	Pos. No.	Neg. No.	Rate (%)	Total	Pearson Chi-square χ^2	*P* value
2016	3096	13,977	18.1	17,073	2.151	0.142[#]
2017	3987	18,711	17.6	22,698	406.045	0.000[*]
2018	7680	23,248	24.8	30,928	283.469	0.000[△]
Total	14,763	55,936	21.0	70,699	521.229	0.000[☆]

Note: The divided inspection standard was $\alpha = 0.05/(3(3-1)/2+1) = 0.0125$, and $P <$ 0.0125 was regarded as statistically significant; # meant the comparison between the year 2016 and 2017, * meant the comparison between the year 2017 and 2018, △ meant the comparison between the year 2016 and 2018 while ☆ meant the total comparison among the three year.

3.2. The Epidemic Pattern and Influenza Subtypes

73.3% of the influenza samples were from outpatients, and 26.7% of those were from inpatients. Yet there were identical influenza epidemic peaks for outpatients and inpatients. The positive rate varied by month, season, and year describing a sequence of peaks presenting primarily in all year including spring, summer and winter. The characteristics of influenza peak was different in three consecutive year (**Figure 1**, **Figure 2**).

There was only one influenza peak in the year 2016, while two peaks in the year 2017 and 2018. A major peak appeared in Mar. and Apr. in the year 2016 with a prevalence rate of 40.2% and 26.2% respectively. And in the year 2017, there was a major peak in July and Aug. with a prevalence rate of 41.4% and 23.5% and a minor peak in Dec. with a prevalence rate of 18.8%. In addition, there were two major peaks in the year 2018, which appeared in Jan., Feb., Mar. with a prevalence of 35.2%, 30.7% and 21.5% and Nov., Dec. with a prevalence rate of 22.8%, 40.1% respectively. Influenza A and B epidemics occurred each year, influenza A was dominant in summer, winter and spring, accounting 75.2% of the total for the period, while influenza B was dominant in spring and winter, accounting 75.7%. Influenza A epidemic might occur separately, such as summer in 2017 and winter in 2018, or co-circulated actively with influenza B, such as spring in 2016 and 2018, with peaks overlapped each other.

In 2016, The frequency of influenza was higher in March (40.2%) and April (26.2%) than others. During the peak months, from March to April, the incidence of influenza A and influenza B were almost similar, following slightly higher frequency of influenza B occurred at May. In 2017, the incidence of new cases of influenza A and B infections reached to peak at July (41.4%) and Aug (23.5%), with a second minor peak at the end of the year (18.8%). Notably, the incidence of influenza A cases were shown in every month, while influenza B showed a gradual increase at July and a peak at the end of the year. The distribution of cases of influenza in 2018 had a bimodal shape with two peaks, one peak in the beginning of the year (January 35.2%, February 30.7%, March 21.5%) and second near the end of the year (November 22.8%, December 40.1%). Influenza A was detected in each month and reached to peak the end of the year, and influenza B predominantly showed up in January-March (**Figure 1**).

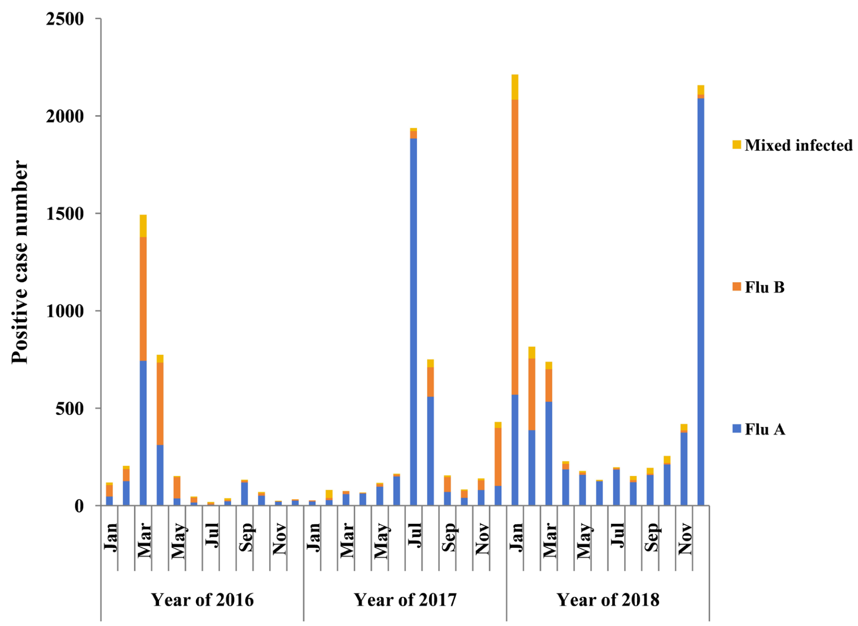

Figure 1. The subtype distribution of influenza patients for the period from 2016 to 2018: The bar chart presents data on the prevalence of different influenza types over a three-year period from 2016 to 2018. The chart categorizes the influenza types into three groups: Flu A (blue bar), Flu B (red bar), and Mixed infection (green bar).

Figure 2 shows that there were seasonal variations in the distribution of influenza cases. The incidence of laboratory-confirmed influenza increased rapidly from January 2016 in outpatients, followed by a rapid increase among inpatients the following month. Both groups showed a peak at March. In year 2017, the incidence of influenza showed a gradual increase among outpatients at June and a peak at July. The peak of inpatients occurred later in July-August. Notably, from November 2017 through January 2018 there was a substantial increase in outpatients, with peaks in inpatients the following month. A second peak in 2018 was in December among outpatients while inpatients were still under tracking.

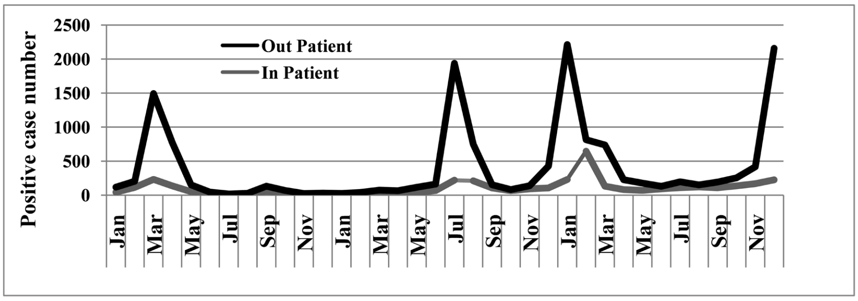

Figure 2. The influenza epidemic peaks for the period from 2016 to 2018: The line graph illustrates the trends in the number of influenza epidemic peaks over the period from 2016 to 2018, categorized by two patient groups: Out-Patient (black line) and In-Patient (grey line).

3.3. The Gender, Age Distribution of In/Out Patients with Influenza A and B

No significant difference in gender was observed in the distribution of cases of influenza A and B between 2016 and 2018. Compared with influenza-negative cases, influenza-positive cases were older ($P < 0.05$). Among all the age groups, the positive cases are were the most in the 3 - 6 years group, accounting for 35.4%, and the least in the 0 - 1 years group, accounting for 19.4%, while and the negative cases are were the most in the 0 - 1 years age group, accounting for 47.1%, and least in the 6 - 17 years group, accounting for 8.9% (**Table 2**). In addition, the age of inpatients was younger than those outpatients ($P < 0.05$, **Table 3**). The frequency of inpatients was higher than that of outpatients among children aged 0 - 3 years. On the other hand, the frequency of inpatients was lower among aged 3 - 17 years. Notably, approximately 70% of the included positive inpatients were infected with influenza A. What is more, age distribution of influenza A and B was different, influenza A affected younger children more frequently and B affected relatively older children. Among all the positive cases, influenza A patients accounted for a higher proportion in the age group of 1 - 3 and 3 - 6 years and a lower proportion in the age group of 0 - 1 and 6 - 17 years, while on the other hand, the frequency of influenza B accounted for a higher proportion among school-aged children of 3 - 6 years and 6 - 17 years. Influenza A patients were younger than those influenza B patients overall ($P < 0.05$).

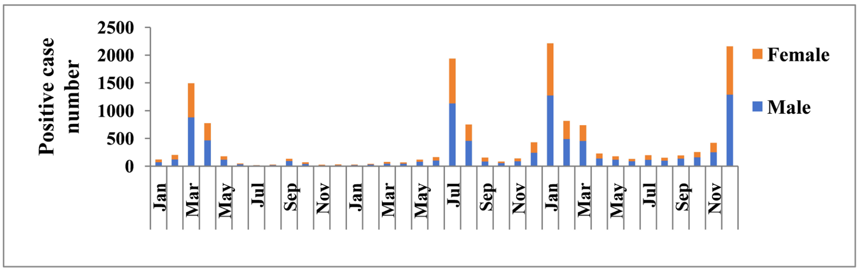

Figure 3. Age distribution of influenza patients for the period from 2016 to 2018: The figure is a bar chart illustrating the number of individuals categorized by gender over three consecutive years from 2016 to 2018. The data is segregated by two primary groups: female (red bar) and male (blue bar).

Table 2. The Age distribution of Influenza patients in the year of 2016 to 2018.

Age group	Total Number	Pos. No. & Pro. (%)	Influ. A & Pro. (%)	Influ. B & Pro. (%)	Mixed infected & Pro. (%)	Neg. No. & Pro. (%)	*P* value
0 - 1 years	29210	2859 (19.4)	1928 (19.7)	769 (18.3)	162 (20.7)	26,351 (47.1)	
1 - 3 years	17029	3538 (24.0)	2583 (26.4)	760 (18.0)	195 (25.0)	13,491 (24.1)	
3 - 6 years	16355	5220 (35.4)	3471 (35.5)	1446 (34.3)	303 (38.8)	11,135 (19.9)	
6 - 17 years	8105	3146 (21.3)	1788 (18.3)	1237 (29.4)	121 (15.5)	4959 (8.9)	
Total	70699	14763 (100.0)	9770 (100.0)	4212 (100.0)	781 (100.0)	55,936 (100.0)	0.000

Table 3. The Age distribution of outpatients and inpatients in the year of 2016 to 2018.

Age group	Total No.	Outpatients No. & Pro (%).	Inpatients No. & Pro. (%)	*P* value
0~1 years	2859	1463 (13.6)	1396 (35.0)	
1~3 years	3538	2438 (22.6)	1100 (27.6)	
3~6 years	5220	4256 (39.5)	964 (24.2)	
6~17 years	3146	2618 (24.3)	528 (13.2)	
Total	14,763	10,775 (100.0)	3988 (100.0)	0.000

3.4. Association of Influenza Subtypes with Climate Parameters

According to the data, monthly proportion positive for influenza virus A was not associated with temperature, humidity and solar radiation (coefficient −0.071, $P = 0.681$; coefficient 0.026, $P = 0.880$; coefficient −0.126, $P = 0.463$ respectively), while monthly proportion positive for influenza virus B had an intimate association with temperature, humidity as well as solar radiation (coefficient −0.500, $P = 0.002$; coefficient −0.362, $P = 0.030$; coefficient −0.353, $P = 0.035$ respectively), which exhibited a consistently negative correlation. Additionally, the association between monthly proportion positive for both influenza virus A and B and precipitation was not statistically significant (coefficient 0.065, $P = 0.705$; coefficient −0.213, $P = 0.213$ respectively).

4. Discussion

The epidemiological patterns of influenza A and B observed among children in Shenzhen displayed a year-round and multi-stage characteristic rather than randomly. It increased not only in colder months (December-March), which coincided with the distinct winter-spring peak in temperate areas [13], but also in summer months (July-August), which exhibited a frequent occurrence in tropical or subtropical areas [15] [16] [22]. In addition, influenza virus that occurred through 2017 and 2018 presented a bimodal curve shape with two distinct peaks in summer and winter-spring, not consistently with a previous study in Guangdong [21]. Various factors could be involved in the seasonal variation. The most important contributing factor for these epidemic characteristics may be the climate, a humid subtropical marine weather influenced by the East Asian monsoon due to the geographical location of the border of China's subtropical and tropical regions. Another potential factor may be population movements and local social contacts [23]. Over 70% of the Shenzhen population consists of migrant workers, and the large-scale migration after Chinese New Year may contribute to the initiation of influenza epidemics.

In addition to the environmental factors, properties of the virus itself may play a role either. The data demonstrated that there were two major peaks caused by seasonal influenza A and another two major peaks attributed to both influenza A and B as well as a minor peak owing to influenza B. Besides the influenza A epidemics, these two types of viruses co-circulated actively during the same epidemic durations, with peaks overlapped each other. Nevertheless, the same virus subtype never predominant in more than two consecutive influenza peaks, with a gradual replacement from one subtype to another. Importantly, seasonality of influenza A was much more variable than influenza B.

In this study, we also noted that there was an intimate association between influenza B and climate parameters, such as temperature, humidity and solar radiation, which displayed a negative correlation, being consistent with its winter-spring seasonal activity [24] [25]. A number of factors such as seasonal crowding, influenza virus survival in respiratory droplets, vitamin D deficiency due to cold weather and less solar radiation may influence host susceptibility and drive influenza circulation patterns [12]. On the other hand, the association between influenza A and climate parameters was not significant, elucidating its occurrence at any time of the year [25]-[28]. Besides the environmental factors and host susceptibility, another remarkable factor maybe properties of influenza A, including its various subtypes, and mutation rates as well as immune escape [29]. Influenza virus A is subtyped according to the type and antigenicity of its surface glycoproteins, HA and NA. Up to 18 HA and 11 NA subtypes have been described so far, of which H1 - H3, H5, H7 and H10 have been found to circulate in human [30] [31]. Virus mutations in HA and NA, mainly caused by antigenic drift, lead to the antigenic diversity and intraspecies transmission as well as interspecies transmission [32].

According to our study, about 56.7% influenza-positive cases were school-aged children, suggesting high social contact rates at school may promote the infection. In the present analysis of influenza positive cases, children with influenza A showed a tendency with younger ages than children with influenza B. Additionally, hospitalization with influenza often occurred in younger individuals infected with influenza A. In other words, young children are infected with Influenza A more frequently than influenza B. Moreover, young individuals are more susceptible to severe influenza than those older children. Several factors may be involved in these epidemic characteristics. Firstly, the pre-existing immunity in young children is weak due to the low vaccination rate [33] and narrow antibody response to natural infection with influenza virus [34]. The next, their respiratory tract may not be well-developed and their own immune system is in a developing state, which leads to their susceptibility to influenza virus attack and severe symptoms. Finally but not lastly, children's personal hygiene habits are not so good, coughing without cover their mouth and nose, washing hands not frequently, jointly increase the risk of influenza transmission and infection. Another essential factor may be the properties of influenza A, with much more various subtypes and frequent mutations than influenza B.

Influenza vaccination is likely to be more cost-effective to mitigate influenza-associated socioeconomic burden [35] [36]. In China, annual immunization campaigns are implemented based on the Northern Hemisphere winter season [37] [38]. Influenza vaccination coverage was very low, about 1% - 2%, even in developed provinces [39]. Such a coverage level could be attributed to the lower baseline awareness against seasonal influenza and payment requirement in most regions in China [40] [41]. By contrast, free immunization programs accompanied a higher vaccination coverage and a prospective reduced influenza-related morbidity, mortality, and costs [42] [43]. The findings of our present study highlight the need for enhanced protection against influenza A and B through the use of influenza vaccine in children, twice a year in Shenzhen area.

5. Conclusion

Shenzhen, owing to its geographical location, climate factors, environmental conditions, social and demographic characteristics etc., displayed a unique epidemiological pattern and characteristics, better understanding of which may avail the prevention and control of influenza. The present study revealed that influenza epidemic among children in Shenzhen displayed year-round and multi-stage characteristic rather than randomly, with winter-spring and summer peaks. Influenza A epidemic occurred separately or co-circulated actively with influenza B, with a winter-spring pattern for influenza B and a much more variable seasonality for influenza A. Influenza B rather than influenza A had a negative correlation with climate parameters. In addition, hospitalization with influenza often occurs in younger individuals infected with influenza A, which may be associated with the susceptibility of young children and the high variability of influenza A virus. Influenza vaccination coverage should be promoted especially in young children, twice a year in Shenzhen maybe advisable.

Acknowledgements

This work was supported by Guangdong High-level Hospital Construction Fund and Shenzhen Science and Technology Research Fund (No. JCYJ2021032414220-1004).

Ethical Approval Statement

This study was approved by the ethics committee of Shenzhen Children's Hospital. Informed consent for publication was obtained from her parents of the individual participant in the study.

Informed Consent

All guardians of the subjects included in this study provided appropriate informed consent.

Authors' Contributions

YXK, RHM and LJ contributed to the collection of the data, data analysis, and manuscript drafting and revising; QY, YXK and CHL contributed to the data collection. XYF contributed to the design and administrative support. All authors approved the final manuscript as submitted.

Conflicts of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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