



# Evaluation and control strategy analysis of influenza cases in Jiujiang City, Jiangxi Province, China from 2018 to 2022

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## Abstract

According to World Trade Organization (WTO) statistics, the incidence of seasonal influenza in China has been on the rise since 2018. The aim of this study was to identify and investigate the influence of factors related to the incidence of four common types of influenza viruses. Data of patients with common cold and associated virus infections are described, and a logistic regression model based on gender, age and season was established. The relationship between virus type and the above three factors was analyzed in depth and significant ( $p < 0.05$ ) associations noted. We noted a fluctuation trend, with the infection rate of influenza virus showing an upward trend from 2018 to 2019 and from 2021 to 2022 and a downward trend from 2019 to 2021. The total number of cases in adolescents aged 18-30 years was higher than that in the elderly. The impact of different types of influenza virus on the population ranked from large to small, with special roles played by Influenza B/Victoria, H3N2, Influenza A/H1N1 pdm and Influenza B/Yamagata.

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Key words: influenza; viral infections; statistical analysis; logistic regression, China

Conflict of interest: the authors declare no potential conflict of interest, and all authors confirm accuracy.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Received: 1 April 2024.

Accepted: 22 July 2024.

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Licensee PAGEPress, Italy  
Geospatial Health 2024; 19:1294  
doi:10.4081/gh.2024.1294

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## Introduction

Influenza outbreaks are caused by distinct types, such as seasonal, pandemic and zoonotic viruses, each possessing unique characteristics (Li *et al.*, 2023). Humans are primarily infected by Influenza A and Influenza B, with the most common types including A/H1N1, A/H3N2, B/Victoria and B/Yamagata (Li *et al.*, 2021; Plans Rubio *et al.*, 2022). Particular influenza pandemics include the 'Spanish flu' in 1918 caused by the H1N1; the 'Asian flu' in 1957 caused by H2N2; the 'Hong Kong flu' in 1968 caused by H3N2; the 'bird flu' in 2003 and 2013 caused by H5N1 and H7N9, respectively; and the 'swine flu' in 2009 caused by H1N1 pandemic disease Mexico (pdm) (Tate, 2018; Berche, 2022). If zoonosis occurs with these new, re-assorted viruses, as it did with the 2009 H1N1 influenza pandemic (pdm09), the risk of pandemic spread would be significant. The concern is that seasonal, pandemic and zoonotic influenza viruses have different characteristics that contribute to human infection (Qian *et al.*, 2023; Zhu *et al.*, 2023).

According to the World Health Organization (WHO), atypical influenza epidemic infects about several million people, with 3.5 million people affected severely including 290,000-650,000 deaths; Dalmau Llorca *et al.*, 2022). The influenza season of 2017-2018 witnessed exceptional activity, with approximately 48.8 million reported cases and 959,000 hospitalizations, leading to 79,400 deaths (Kondratiuk *et al.*, 2022). Influenza outbreaks pose a significant health risk during both the seasonally epidemics and during pandemics. WHO estimates (Peck *et al.*, 2023) that the annual seasonal influenza epidemic results in approximately 3 to 5 million infections and from 250,000 to 500,000 deaths, so early detection is critical to effectively allocate public health resources and plan appropriate control measures. It is necessary to analyze the types and factors of influenza, and predictive modelling can be made, which is of great significance for the prevention and control of the influenza virus. Estimates of influenza-associated mortality can help policymakers determine the effectiveness of influenza prevention and control measures (Hennessy *et al.*, 2016). Estimation is further valuable in assessing the overall burden of influenza disease, identifying high-risk populations and assessing the impact of influenza on public health (Brunner *et al.*, 2019).

This study monitored the incidence of influenza cases admitted to Nanchang University Affiliated Hospital of Jiujiang City in Jiangxi Province, China from 2018 to 2022. The distribution characteristics were examined based on gender, age and season and aimed to identify the influence of these factors on the incidence of four types of influenza viruses based on logistic multivariate regression analysis. These research results should provide reference materials and assist strategies for influenza health management, with consideration of key factors to achieve prevention

and control. Logistic regression model has three functions: finding risk factors, forecasting and discrimination. Based on this knowledge, the theme of this paper was to establish a model based on the existing data set.

## Material and Methods

### Data source

The influenza sampling method originally proposed by Chatterjee *et al.* (2020) was used in this study. From 2018 to 2020, 15-30 throat swabs from patients with typical influenza symptoms were collected weekly throughout the year in the outpatient department of the First Affiliated Hospital of Nanchang University in Jiujiang City. The samples were refrigerated and sent for laboratory examination within 24 hours, with those not possible to be examined immediately stored at -80°C.

### Virus identification

The reverse transcription polymerase chain reaction (RT-PCR) according to the modification proposed by El Feghaly *et al.* (2021) was applied for the diagnosis of influenza virus. Madin-Darby canine kidney (MDCK) cells were provided by the Beijing Municipal Center for Disease Control and Prevention. Anti-A/H1N1 pdm subtype; anti-A/H3N2 subtype; anti-B Yamagata lineage; and anti-B Victoria lineage virus sera were provided by the Chinese Center for Disease Control and Prevention (China CDC). The Qiagen RNeasy Mini Kit extraction kit for RNA (<https://www.qiagen.com/>) was utilized together with the Taqmanone step real-time (RT) PCR system kit from ABI/Thermo Fisher, USA (<https://www.thermofisher.com>).

The MDCK culture conditions were done according to Byrd-Leotis *et al.* (2022) with slight differences introduced by Byrd-Leotis *et al.* (2022). Briefly, the throat swab specimens were used to inoculate confluent MDCK cells and incubated at 34°C with 5% CO<sub>2</sub> for 2 hours for viral adsorption. The cells were then incubated at 37°C with 5% CO<sub>2</sub> in an incubator. Observations for cyto-pathological effects were made daily. After 3-4 days of cultivation, a haemagglutination test was performed with 1% guinea pig erythrocytes to detect the presence and titre of the virus. The RT-PCR system was operated in strict accordance with the kit instructions, including lysis, adsorption, washing and amplification. When using the fluorescence PCR method for detection, 1 µL of enzyme mix, 19 µL of mix, 5 µL of processed final sample and 25 µL of reaction system were prepared. The PCR reaction cap was tightly sealed during the amplification process and the reaction conditions were first kept at 45°C for 10 minutes, then at 95°C for 15 minutes,

with each cycle lasting 15 seconds. Next, it was kept at 60°C for 60 seconds, with the amplification repeated for 50 cycles. The fluorescent signal values were obtained for 3 to 15 cycles in conjunction with the baseline. Results were considered negative based on a ct threshold (2<sup>ΔΔCT</sup>) of >40 and positive based on <40.

### Statistical analysis

A multivariate model was established based on logistic regression analysis. Various attribute data related to influenza, such as age, sex and season were collected as independent variables. Dependent variables representing the prevalence of influenza caused by four different viruses were considered, *i.e.* H1N1, Yamagata, H3N2 and Victoria. The attribute data were used to identify potential factors that may be associated with influenza. The assignment of factors and the model output is presented in Table 1.

EXCEL software was used to analyze the data distribution and assign factors from the statistical results and IBM's SPSS Statistics 23 software was employed for data analysis. Chi-square tests were conducted to determine associations. Multivariate models, including binary logistic regression and multinomial logistic regression, were used to analyze the data. Binary logistic regression was applied to assess the influence of gender, age and season on the incidence of individual influenza cases. Multinomial logistic regression was used to evaluate the impact of these factors on the incidence of the four types of influenza viruses. These models helped summarize the characteristics influencing influenza incidence and provided strategies for influenza health management.

### Logistic regression

Binary logistic regression model predicts the probability of the event occurring as a function of the independent variables. The coefficients (β's) indicate the strength and direction of the association between each independent variable and the probability of the event. We used the following equation to express this:

$$\text{logit}(p) = \ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k$$

where the logit function ( $\text{logit}(p)$ ) is the natural logarithm of the odds that the dependent variable  $YYY$  equals 1 (*i.e.* that the event of interest occurs);  $p$  the probability of the event occurring (*e.g.*, incidence of influenza);  $\beta_0$  the intercept; and  $\beta_1, \beta_2, \dots, \beta_k$  the coefficients for the independent variables  $X_1, X_2, \dots, X_k$  that represent the factors gender, age and season.

**Table 1.** Multinomial binary logistic regression of the assignment of the factors.

Item (factor)	Variable (type)	Assignment
Sex (X1)	Classified	Male=0, Female=1
Age (X2)	Classified	>18=0; 18~30=1; 31~39=2; 40~49=3; ≥50=4
Season (X3)	Classified	3~5=0; 6~8=1; 9~11=2; 12~2=3
Diagnosis (Y1)	Classified	New influenza A H1N1 = 0; Yamagata=1; Seasonal H3N2=2; Victoria=3



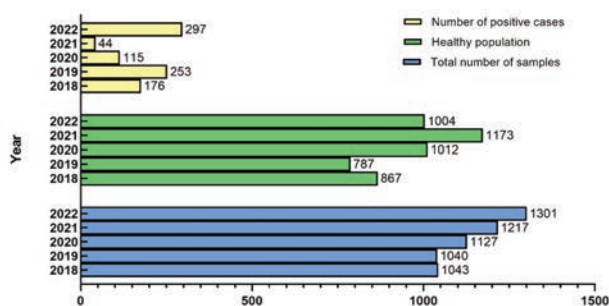
Multinomial logistic regression extends binary logistic regression to handle dependent variables with more than two categories. It provides a set of regression equations, one for each category of the dependent variable (excluding the reference category). The coefficients ( $\beta$ 's) describe the relationship between the independent variables and the odds of being in each category relative to the reference category. For a multinomial logistic regression with a dependent variable having  $J$  categories, the equations are:

$$\log\left(\frac{P(Y=j)}{P(Y=J)}\right) = \beta_{j0} + \beta_{j1}X_1 + \beta_{j2}X_2 + \dots + \beta_{jk}X_k$$

where  $P(Y=j)$  represents the probability that the dependent variable  $Y$  falls into category  $j$  (with  $j=1,2,\dots,J-1$ );  $P(Y=J)$  the probability that the dependent variable  $Y$  falls into the reference category  $J$  (used as the baseline);  $\beta_{j0}, \beta_{j1}, \beta_{j2}, \dots, \beta_{jk}$  are the coefficients for the independent variables  $X_1, X_2, \dots, X_k$  for each category  $j$  compared to the reference category  $J$ ; and  $X_1, X_2, \dots, X_k$  represent the independent variables gender, age and season.

**Wald test**

We applied the Wald formula to test the significance of individual coefficients in the model. It is generally used to assess constraints of parameters based on their weighted distances (Fahrmeir *et al.*, 2013). It is calculated as the square of the ratio of a coefficient (in this case  $B$ ) to its standard error (SE) that measures the level of accuracy with which a sample represents a population, *i.e.*  $(B/SE)^2$ . We also utilized the  $\text{Exp}(B)$  expression, which represents the exponentiation of the  $B$  coefficient, also known as the odds ratio. It indicates the change in odds of the outcome occurring for a one-unit increase in the predictor variable. Values greater than 1 indicate increased odds and values less than 1 indicate decreased odds.



**Figure 1.** Results of influenza tests carried out in Jiujiang City in Jiangxi Province, China from 2018 to 2022.

**Results**

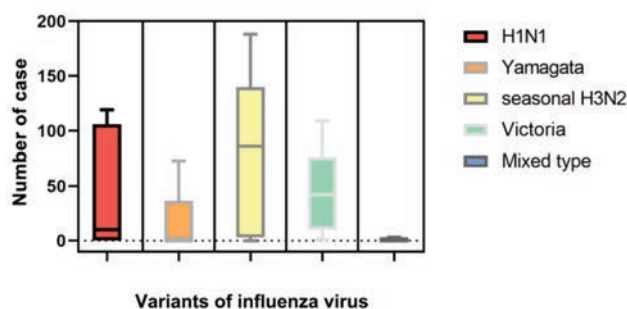
**Trend of virus distributions**

As seen in Figure 1, the throat swab samples gathered during the 5-year influenza outbreak surveillance from 2018 to 2022 accumulated to 5,728, with more than one thousand samples collected each year. These samples included influenza patients, healthy individuals, and close contacts of related cases. The year with the highest infection rate was 2019, with a total of 24.3% and 253 cases of influenza. The infection rate showed an increasing trend from 2018 to 2019 and from 2021 to 2022, while a decreasing trend was observed from 2019 to 2021. Therefore, the infection rate during the 5-year period exhibited a fluctuating trend ranging from 3.6% to 26.3%. During the three years (2020-2022) of the COVID-19 pandemic in China, the number of influenza virus infections in Jiujiang City showed a downward trend from 2020 to 2021, followed by an upward trend from 2021 to 2022, exhibiting an overall fluctuating pattern.

Figure 2 shows that the highest number (188 cases) of seasonal H3N2 virus infections during the 5-year period occurred in 2022. This was followed by the H1N1 pdm virus in 2019, with a total of 119 cases. Other influenza viruses with more than 90 cases each year included Victoria virus (2022), seasonal H3N2 virus (2019) and H1N1 pdm virus (2018). In conclusion, Victoria virus, seasonal H3N2 virus and H1N1 pdm virus showed the highest occurrence.

**Distribution of influenza virus positive cases**

The distribution statistics of seasonal influenza from 2018 to 2022 based on sex, age and season are shown in Table 2. Except for 2019, when the number of male cases exceeded that of female ones, the remaining years showed a higher number of female cases. Based on the age distribution, the highest number of cases was 90 (8.7%), which occurred in the year 2019 in the 18-30 age group. It is important to note that the number of cases in the 18-30 age group was consistently the highest over the five-year period, despite the common belief that older adults have a higher incidence rate.



**Figure 2.** Distribution of the four main types of influenza viruses in Jiujiang City in Jiangxi Province, China in 2018-2022.

Regarding the seasonal distribution, the 2018-2019 winter seasons and the summer season of 2022 had higher numbers of cases, with 169 (16.2%), 156 (15.0%) and 186 (14.3%) cases respectively. In contrast, the number of cases in all four seasons of 2021 was relatively low, ranging from 3 to 23 cases (0.3 to 1.9%). Influenza viruses with more than 100 cases included H1N1 pdm in 2019 (119 cases), seasonal H3N2 in 2022 (188 cases) and Victoria in 2022 (109 cases). The corresponding incidence rates were 11.4%, 14.5%, and 8.4% respectively. The Yamagata virus had only one occurrence in the five-year period, specifically in 2018 with 73 cases (7.00%). Similarly, the mixed influenza virus appeared in three cases (0.3%) in the same year.

### Logistic regression analysis

Non-collinearity among the independent variables was validated using the variance inflation factor (VIF). The fact that they all were below the threshold indicates that multicollinearity was not a concern in the regression analyses. Multinomial logistic regression further elucidated the impact of these factors on the incidence of the four distinct types of influenza viruses. In fact, the input parameters of each factor could be assigned and replaced into the model to determine the probability of influenza as shown in Table 3.

Table 4 shows that significant associations with regard to certain ages and seasons had  $p$ -values less than 0.0001 indicating a

**Table 2.** The distribution statistics of sex, age and season affecting influenza virus positive cases in Jiujiang City from 2018 to 2022.

Item	Number (%) of pos. cases 2018	Number (%) of pos. cases 2019	Number (%) of pos. cases 2020	Number (%) of pos. cases 2021	Number (%) of pos. cases 2022
<b>Sex</b>					
Male	94 (9.0)	132 (12.7)	45 (4.0)	23 (1.9)	85 (6.5)
Female	82 (7.9)	121 (11.6)	70 (6.2)	174 (14.3)	212 (16.3)
<b>Age (years old)</b>					
< 18	43 (4.1)	51 (4.9)	39 (3.5)	6 (0.5)	23 (1.8)
18~30	54 (5.2)	90 (8.7)	40 (3.6)	17 (1.4)	85 (6.5)
31~39	20 (1.9)	50 (4.8)	6 (0.5)	8 (0.7)	73 (5.6)
40~49	26 (2.5)	27 (2.6)	12 (1.1)	7 (0.6)	40 (3.1)
≥50	33 (3.2)	35 (3.4)	18 (1.6)	6 (0.5)	76 (5.8)
<b>Season (month)</b>					
Spring (3~5)	6 (0.6)	72 (6.9)	0 (0.0)	10 (0.8)	65 (5.0)
Summer (6~8)	0 (0.0)	19 (1.8)	0 (0.0)	8 (0.7)	186 (14.3)
Autumn (9~11)	1 (0.1)	6 (0.6)	1 (0.1)	3 (0.3)	2 (0.2)
Winter (12~2)	169 (16.2)	156 (15.0)	14 (0.1)	23 (1.9)	44 (3.4)
<b>Influenza type</b>					
H1N1pdm	93 (8.9)	119 (11.4)	10 (0.9)	0 (0.0)	0 (0.0)
Yamagata	73 (7.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Seasonal H3N2	6 (0.6)	92 (8.9)	86 (7.6)	0 (0.0)	188 (14.5)
Victoria	1 (0.1)	42 (4.0)	19 (1.7)	44 (3.6)	109 (8.4)
Mixed type	3 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

**Table 3.** Factors investigated.

Item (factor)	Variable (type)	Assignment
Sex (X1)	Classified	Male=0, Female=1
Age (X2)	Classified	>18=0;18~30=1; 31~39=2; 40~49=3;≥50=4
Season (X3)	Classified	3~5=;6~8=1; 9~11=2;12~2=3
Diagnosis (Y1)	Classified	Negative=0;Positive=1

**Table 4.** Likelihood-ratio test.

Effect	Model fitting criteria		Likelihood ratio tests	
	Reduced model likelihood (-2Log)	Chi-square	df	$p$
Intercept	215.850	<0.0001	0	Not done
Sex	216.856	1.006	1	0.316
Age	306.582	90.732	4	<0.0001***
Season	931.724	715.874	3	<0.0001***

\*\*\*statistically significant ( $p < 0.0001$ );  $df$  =degrees of freedom.



highly significant relationship. The chi-square test values for these factors, as well as sex, turned out positive, though not strongly so for the latter. Based on the *p*-values and chi-square test results, the likelihood-ratio test results for age and season were deemed good and that further analysis could be conducted using this model.

Table 5 presents the results of the logistic regression model. The risk of developing influenza in people younger than 18 years old and 18~30 years old was found to be clearly higher compared

to those >50 years old, while it was lower for the December-February period compared to any other time of the year. Thus, it was concluded that age and season were the main factors influencing the prevalence of influenza during this period in China.

### Logistic multiple regression analysis

Table 6 shows that the logistic multiple regression analysis model had a chi-square value of 263.496 and a highly significant

**Table 6.** Logistic multiple regression analysis on likelihood-ratio test

Effect	Model fitting criteria Reduced model likelihood (-2Log )	Chi-square	Likelihood ratio tests df	<i>p</i>
Intercept	263.496	507.935	24	<0.001***
Sex	269.725	6.230	3	0.101
Age	329.849	66.435	12	<0.001***
Season	715.474	451.947	9	<0.001***

\*\*\*statistically significant (*p*<0.0001); *df*, degrees of freedom.

**Table 7.** Logical multiple regression analysis results of the target variables

Diagnosis	Variable	B	SE	Wald	<i>p</i>	Exp(B)	95% CI for Exp(B) Lower Upper		
Yamagata	Intercept	-1.012	0.339	8.934	0.003				
	Gender	Male	Reference						
		Female	-0.454	0.284	2.553	0.110	0.635	0.364	1.108
	Age	>= 50 years old	Reference						
		< 18 years old	1.082	0.415	6.805	0.009	2.949	1.309	6.647
		18~30 years old	0.001	0.413	0.000	0.998	1.001	0.446	2.248
		31~39 years old	-0.815	0.569	2.052	0.152	0.443	0.145	1.350
	Season	40~49 years old	0.059	0.467	0.016	0.899	1.061	0.425	2.649
		Dec.-Feb.	Reference						
		Mars-May	-1.007	0.641	2.467	0.116	0.365	0.104	1.283
June-August		0.232	3.931.193	0.000	1.000	1.262	0.000	.c	
Sep.-Nov.	-18.003	0.000			0.000	0.000	0.000		
Seasonal H3N2	Intercept	-0.415	0.256	2.621	0.105				
	Gender	Male	Reference						
		Female	-0.468	0.206	5.177	0.023	0.626	0.418	0.937
	Age	≥ 50 years old	Reference						
		< 18 years old	1.104	0.324	11.580	0.001	3.016	1.597	5.696
		18~30 years old	0.446	0.294	2.300	0.129	1.561	0.878	2.777
		31~39 years old	-0.231	0.348	0.441	0.507	0.794	0.401	1.570
	Season	40~49 years old	-0.582	0.387	2.257	0.133	0.559	0.262	1.194
		Dec-Feb.	Reference						
		Mars-May	0.807	0.307	6.889	0.009	2.241	1.227	4.094
June-August		21.351	0.261	6.67E+03	<0.001	1.87E+09	1.12E+09	3.13E+09	
Sep.-Nov.	1.962	1.133	2.997	0.083	7.112	0.772	65.560		
Victoria	Intercept	-1.527	0.313	23.742	<0.001				
	Gender	Male	Reference						
		Female	-0.201	0.214	0.884	0.347	0.818	0.537	1.244
	Age	≥ 50 years old	Reference						
		< 18 years old	0.698	0.397	3.095	0.079	2.010	0.923	4.375
		18~30 years old	1.180	0.336	12.348	<0.001	3.256	1.686	6.290
		31~39 years old	1.010	0.365	7.671	0.006	2.745	1.343	5.609
	Season	40~49 years old	0.534	0.402	1.769	0.184	1.706	0.776	3.748
		Dec.-Feb.	Reference						
		Mars-May	2.364	0.283	69.928	<0.001	10.637	6.111	18.513
June-August		19.617	0.000						
Sep.-Nov.	2.803	1.088	6.639	0.010	16.489	1.956	139.023		

*B*, coefficient of the predictor variable (positive values suggest a positive relationship, while negative values suggest a negative relationship); *SE*, standard error; *Wald*,  $(B/SE)^2$  where high values indicates strong significance; *Exp(B)*, exponentiation of the *B* coefficient, where values >1 indicate increased odds and values <1 lower odds.; *CI*; confidence interval.

likelihood-ratio test result with a  $p$ -value  $<0.0001$ . Furthermore, the significance of the age and season factors were statistically significant at  $p < 0.001$ , indicating a good fit of these two factors in the model. However, the season factor had a higher significance than that of age. Table 7 presents the results of the logistic multiple regression model. The risk of developing the new influenza A/H1N1 in people less than 18 years old compared to those above 50 years was 2.949 times higher than the risk of infection by the Yamagata strain, while the risk of developing seasonal H3N2 in people under 18 years was 3.016 times higher than that for people above 50 years. Additionally, the risk of developing the Victoria strain in people under 18 years old was 2.745 times that of developing influenza A/H1N1. During the period from March to May, the risk of developing seasonal H3N2 was 2.241 times higher than in any other period. The risk of developing the Victoria strain was 10.637 times higher during the same period for new influenza A/H1N1 and 16.489 times higher during the September–November period.

## Discussion

One of the key problems in the field of communicable diseases is how to control the spread of influenza virus quickly and effectively. The importance and role of distributional statistics applied here lie primarily in revealing the transmission patterns of the disease, predicting epidemic trends, guiding public health interventions as well as evaluating prevention and control measures as discussed by Moriarty *et al.* (2014) and Palache *et al.* (2017). Binary logistic regression was employed to identify the risk of age, sex and season. This approach helped analyzing the spread of the disease across different seasons and demographic groups (such as age and sex) and assisted the targeting of preventive measures and intervention strategies can be developed to proactively prepare preventive actions at the right time and for specific age groups as mentioned by Ursin *et al.* (2020).

The finding that the number of influenza cases fluctuates in a wave-like pattern, with an upward trend of infection rates in the first two years of the COVID-19 pandemic (2018 and 2019) contrasts with the common belief that the COVID-19 pandemic led to a decline in global influenza infection rates. Notably, the number of cases among adolescents aged 18–30 was higher than that among the elderly, a fact that warrants significant attention. This may be attributed to factors such as high work pressure, irregular sleep patterns, and unhealthy diets leading to weakened immune function (Yu *et al.*, 2022), but we still do not have final answer to this question. The validation by VIF of non-collinearity among the independent variables ensures the robustness of these models and confirms that multicollinearity did not adversely affect the regression analysis. Indeed, the absence of multicollinearity highlighted the mechanisms driving the incidence of influenza and enhanced the comprehension of the dynamics. It also provides practically useful information for targeted health management strategies. Multivariate logistic regression analysis plays a crucial role in the study of influenza. These models enabled us to both identify and quantify the risk factors associated with influenza, providing valuable insights into the impact of different interventions on its incidence. Furthermore, multivariate logistic regression analysis elucidated the underlying mechanisms of influenza occurrences. By analyzing the weights and interactions of different risk factors, it can be said that the relationships between various factors are now better understood.

## Conclusions

The theme of this paper was to establish a model based on existing data sets. For future data, the input parameters of each factor can straightforwardly be assigned into the model (replacing our data) to determine the probability of future influenza epidemics. Importantly, the Victoria influenza virus has shown an upward trend over the past five years, so precautions should be taken against infection by this virus. Understanding the distribution and determinants of influenza through sophisticated statistical methods provides a foundation for developing effective public health strategies and interventions to manage and mitigate influenza outbreaks.

Targeted control strategies for influenza should be successful if the following actions are carried out: i) planned physical exercise and nutrition intake should be encouraged as this is beneficial for the general immunity status; ii) when one year's incidence rate is low, influenza prevention and control measures should be strengthened in preparation for a potentially uptick in the following year; and iii) more attention should be paid to the impact of viral infections in the winter, especially for H1N1 pdm, and we should be vigilant about the risk of seasonal H3N2 in the summer.

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