




# Dynamic Shifts in Respiratory Pathogens During the First Post-COVID-19 Autumn-Winter in Beijing

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**Background:** The COVID-19 pandemic and its associated nonpharmaceutical interventions have profoundly changed the epidemiology of respiratory pathogens. Following the lifting of COVID-19 restrictions in December 2022 and its adjustment to Class B management, respiratory infections have resurged in China. This study aimed to understand the infection spectrum, mixed infection pattern and temporal dynamic changes of respiratory pathogens in the first autumn-winter after the outbreak of COVID-19 in Beijing.

**Methods:** A total of 864 unique throat swab specimens from patients with respiratory infections at Beijing Tongren Hospital were analyzed, with no repeated testing. Respiratory pathogens were detected by multiplex PCR against 31 viral, bacterial, and atypical pathogens. Detection rates, co-infection patterns, and temporal trends were analyzed across age groups and clinical settings.

**Results:** The total detection rate of respiratory bacteria was 79.63% (688/864), the highest was in children (86.67%), and the lowest was in elderly patients (70.49%). The detection rate of outpatients and emergency patients was significantly higher than that of inpatients ( $p < 0.001$ ). Co-infections were found in 59.16% of the positive cases, mainly viral-bacterial and bacterial-bacterial combinations. The highest detection rates of viruses were influenza A virus (27.31%), influenza B virus (10.42%) and human adenovirus (5.09%). Among bacterial pathogens, *Haemophilus influenzae* (26.62%), *Acinetobacter baumannii* (18.75%) and *Klebsiella pneumoniae* (14.47%) were most frequently detected. Weekly analyses showed alternating circulation of influenza A and B viruses, with an increase in bacterial load late in the influenza season.

**Conclusion:** The first autumn-winter after COVID-19 outbreak in Beijing was characterized by extensive co-circulation of multiple respiratory pathogens, with high viral infection burden and frequent bacterial co-infection. These findings highlight the importance of continued molecular surveillance and integrated pathogen testing strategies for clinical management and public health response in a post-COVID-19 era.

**Keywords:** respiratory infection, multiplex PCR, pathogen spectrum, co-infection, post-COVID, Beijing

## Introduction

Respiratory infections remain a major cause of morbidity in all age groups, especially in children, the elderly, and those with underlying medical conditions. The COVID-19 pandemic and the widespread implementation of non-pharmaceutical interventions (NPIs) have dramatically changed the transmission patterns of common respiratory pathogens, resulting in reduced long-term exposure and immunity gaps at the population level.

Following the relaxation of COVID-19 control measures, multiple respiratory pathogens, including influenza viruses, respiratory syncytial virus,<sup>1,2</sup> and *Mycoplasma pneumoniae*,<sup>3,4</sup> have shown notable re-emergence worldwide. In China, the 2022–2023 influenza season was postponed, and a sharp increase in activity was observed in early 2023, accompanied by localized outbreaks of other respiratory pathogens.<sup>5</sup> These changes suggest that the post-COVID-19 period may be characterized by altered pathogen dynamics and increased clinical complexity.<sup>6,7</sup>

In the autumn-winter of 2023, the number of patients presenting with respiratory symptoms increased significantly in Beijing, raising concerns about co-epidemics of viral and bacterial pathogens and the possibility of co-infections.<sup>8</sup>

However, comprehensive data describing pathogen distribution, co-infection patterns, and temporal trends during this transition period remain limited.

Based on the data of routine clinical diagnosis, this study analyzed the spectrum of respiratory pathogens, mixed infection patterns and weekly dynamics of patients with respiratory infections in a tertiary hospital in Beijing in the first autumn-winter after COVID-19.

## Materials and Methods

### Study Population

Patients with respiratory infections who underwent routine clinical care in the inpatient (IP), outpatient (OP), and emergency department (ED) of Beijing Tongren Hospital, Capital Medical University, between October and December 2023 were included in this study. This was a retrospective observational study based on routine clinical diagnostic data.

Patients were eligible for inclusion if they presented with at least one symptom of a respiratory infection (eg, fever, cough, sore throat, headache, fatigue, nasal congestion, or runny nose) at or before the clinical visit. Key exclusion criteria included a confirmed diagnosis of a non-infectious condition explaining the symptoms (eg, tumors).

This study was approved by the Institutional Review Board (IRB) of Beijing Tongren Hospital (approval number: TREC2024-KY196). Written informed consent was obtained from the legal guardians of pediatric participants. For adult participants, informed consent was waived by the Ethics Committee due to the retrospective nature of the study and the use of anonymized clinical data.

Clinical and demographic data were extracted retrospectively from electronic medical records. Pharyngeal swab specimens had been collected as part of routine clinical care, and laboratory processing and testing followed the hospital's standardized diagnostic protocol.

### Specimens and Detection of Pathogens

For specimen collection prior to therapeutic intervention, pharyngeal swabs were utilized. The swabs were immediately transferred, without delay, into individual sterile centrifuge tubes pre-filled with 3 mL of sterile physiological saline solution. After pre-processing with physical disruption, genomic DNA/RNA was isolated using a commercial DNA/RNA extraction kit as described in the manual. Respiratory PCR samples were tested using the Coyote Respiratory Panel (for scientific research), which detects 31 common respiratory pathogens, including: (i) 16 respiratory RNA viruses—influenza A virus (FLUAV), influenza A/H1-2009 virus, influenza A/H3 virus (FLUAV-H3), influenza B virus (FLUBV), human parainfluenza virus 1 (HPIV1), human parainfluenza virus 2 (HPIV2), human parainfluenza virus 3 (HPIV3), human parainfluenza virus 4 (HPIV4), human coronavirus 229E (HCoV\_229E), human coronavirus HKU1 (HCoV\_HKU1), human coronavirus NL63 (HCoV\_NL63), human coronavirus OC43 (HCoV\_OC43), human respiratory syncytial virus (HRSV), human metapneumovirus (HMPV), human rhinovirus (HRV), and measles virus (MeV); (ii) two respiratory DNA viruses—human adenovirus (HAdV) and human bocavirus (HBoV); (iii) ten common respiratory bacteria—*Haemophilus influenzae* (*H. influenzae*), *Streptococcus pyogenes* (Group A *Streptococcus*, GAS), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Moraxella catarrhalis* (*M. catarrhalis*), *Escherichia coli* (*E. coli*), *Acinetobacter baumannii* (*A. baumannii*), *Bordetella pertussis* (*B. pertussis*), and *Legionella pneumophila* (*L. pneumophila*); and (iv) two atypical bacteria—*Mycoplasma pneumoniae* (*M. pneumoniae*) and *Chlamydia pneumoniae* (*C. pneumoniae*). Abbreviations are listed in [Table S1](#).

Real-time fluorescence reverse transcription polymerase chain reaction (RT-PCR) was performed on Gentier 48E Real-Time PCR System (Xi'an Tianlong science and technology CO., LTD). Quantitative real-time RT-PCR analysis was performed according to standard procedures.

### Statistical Analysis

Statistical analyses were performed using R (version 4.2.1). Data are presented as percentages, mean  $\pm$  SD, or median (interquartile range, IQR), as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact

test. Continuous variables were compared using Student's *t*-test or the Mann–Whitney *U*-test, as appropriate. A two-sided *P* value < 0.05 was considered statistically significant.

## Results

### Study Population

Between October and December 2023, a total of 864 patients met the inclusion criteria and were included in the analysis. The median age was 34.0 years (IQR, 25.0–57.0), and 51.16% (442/864) were female. Patients were stratified into four age groups: children (0–18 years), young adults (18–35 years), middle-aged adults (36–60 years), and older adults (≥61 years). Specimens from IP and OPED accounted for 30.56% (264/864) and 69.44% (600/864), respectively. Table 1 summarizes the demographic characteristics of the study cohort.

### Dominant Respiratory Pathogens Identified During the Autumn-Winter Season in Beijing, 2023

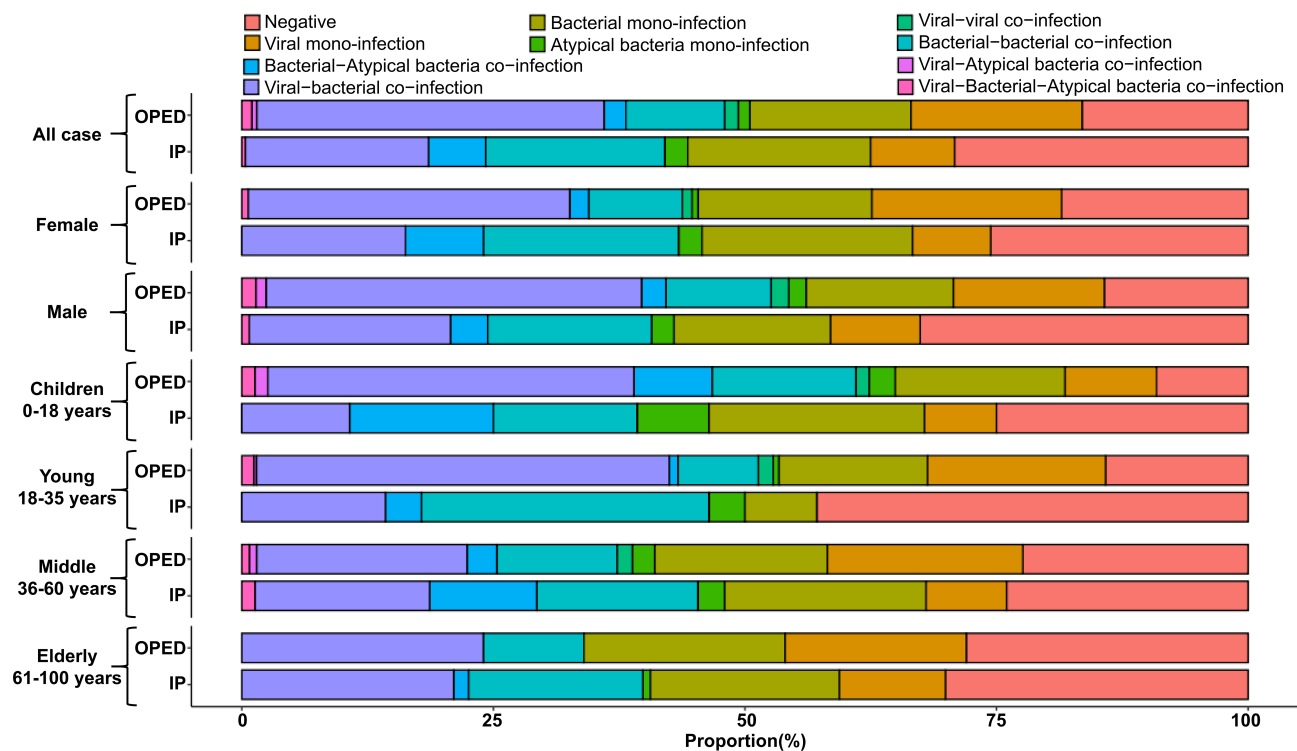
To assess the overall burden of respiratory pathogens, we next analyzed pathogen detection rates across different age groups and clinical settings. In this study of 864 patients, 688 (79.63%) were found to be positive for respiratory pathogens. There was no statistically significant difference in the pathogen positivity rate between males (79.86%, 337/422) and females (79.41%, 351/442). However, a chi-squared test revealed significant differences in pathogen positivity rates among various age groups (*p* = 0.001). The highest positivity rate was observed in children (0–18 years) at 86.67% (91/105). Rates were subsequently lower in young adults (18–35 years, 83.65%, 307/367) and middle-aged individuals (36–60 years, 77.03%, 161/209), with the elderly (≥61 years) showing the lowest rate at 70.49% (129/183). In comparison, the pathogen positivity rate was significantly higher in OPED (83.50%, 501/600) than IP (70.83%, 187/264) (*p* < 0.001).

Based on the proportion of positive detections, the most frequently identified viral pathogen was FLUAV (27.31%, 236/864), followed by FLUBV (10.42%, 90/864) and HAdV (5.09%, 44/864). Among bacterial pathogens, *H. influenzae* was the most prevalent (26.62%, 230/864), followed by *A. baumannii* (18.75%, 162/864) and *K. pneumoniae* (14.47%, 125/864). Six pathogens, including MeV, HPIV1, HPIV2, HCoV\_NL63, HCoV\_OC43 and *L. pneumophila*, were not detected in all samples. Further analysis revealed that there was no obvious difference of pathogens between different genders (Table S2). However, across age groups, there were statistically significant positivity differences in five viruses (FLUAV, FLUBV, FLUAV-H3, HRSV and HAdV), two specific bacteria (*H. influenzae* and *GAS*), as well as one typical bacterium (*M. pneumoniae*) (*p*<0.01) (Table S3). Notably, FLUAV was the dominant virus in all age groups, with 33.79% in the young, 25.68% in the elderly, 22.49% in the middle and 17.14% in children. The positivity rate for *H. influenzae* was highest in children (48.57%) and progressively declined with age: 29.97% in young adults, 22.49% in middle-aged adults, and 12.02% in the elderly.

**Table 1** The Characteristics of the Study Population

Characteristics	Number	Percentage (%)
<b>Gender</b>		
Male	422	48.84%
Female	442	51.16%
<b>Age (years)</b>		
Children (0–18 years)	105	12.15%
Young (18–35 years)	367	42.48%
Middle (36–60 years)	209	24.19%
Elderly (≥61 years)	183	21.18%
<b>Sources</b>		
OPED	600	69.44%
Hospital inpatient (IP)	264	30.56%

**Note:** Bold text in the table represents category headings.



**Figure 1** Prevalence of pathogens in mono-infection and co-infection in OPED and IP patients. Positive proportion of viruses, viral-viral co-infections, virus-bacteria co-infections, bacterial-bacterial co-infections, and bacteria in different groups.

**Abbreviations:** OPED, Outpatient and Emergency Departments; IP, Hospital inpatient.

Given the differences in disease severity and healthcare utilization, pathogen distributions were further compared between outpatient/emergency department patients and hospitalized patients. Compared to IP, OPED cases showed significantly higher detection rates for three respiratory viruses (FLUAV: 31.33% vs 18.18%,  $p < 0.001$ ; FLUBV: 14.33% vs 1.52%,  $p < 0.001$ ; HAdV: 6.67% vs 1.52%,  $p = 0.003$ ) and *H. influenzae* (31.83% vs 14.77%,  $p < 0.001$ ), while *A. baumannii* (15.17% vs 26.89%) and *P. aeruginosa* (9% vs 18.18%) were more prevalent in IP (both  $p < 0.001$ ) (Figure 1 and Table S4). Age-stratified analysis revealed higher OPED detection of FLUAV in children (22.08% vs 3.57%,  $p = 0.026$ ) and young adults (35.69% vs 10.71%,  $p = 0.007$ ), FLUBV in young (14.33% vs 1.52%,  $p < 0.001$ ) and middle-aged adults (18.88% vs 3.57%,  $p = 0.041$ ), and HAdV in children (6.67% vs 1.52%,  $p = 0.003$ ). *H. influenzae* was more frequent in OPED among children (54.55% vs 32.14%,  $p = 0.042$ ) and elderly (20.00% vs 9.02%,  $p = 0.042$ ). Conversely, *P. aeruginosa* was significantly more common in IP patients who were young (25.00% vs 9.14%,  $p = 0.008$ ) or middle-aged (16.00% vs 6.72%,  $p = 0.032$ ). In contrast, *A. baumannii* was more prevalent among IP patients who were middle-aged (28.00% vs 11.19%,  $p = 0.002$ ) or elderly (28.57% vs 10.00%,  $p = 0.008$ ).

## High Co-Infection Burden: Co-Infection Rates Exceed Single Infection Rates

To further explore infection complexity, we examined the frequency and patterns of co-infections among pathogen-positive patients. Among the 688 pathogen-positive patients, 40.84% (281) had mono-infections, while the majority, 59.16% (407), had co-infections, highlighting a high co-infection burden. Among the 407 patients with co-infections, viral-bacterial and bacterial-bacterial co-infections were predominant, accounting for a combined 88.70% of cases. Specifically, viral-bacterial co-infections occurred in 62.65% (255/407) of patients, while bacterial-bacterial co-infections were found in 26.04% (106/407). Virus-atypical and virus-virus co-infections were relatively uncommon. The age-stratified analysis revealed significant variations in co-infection patterns ( $p < 0.001$ ): viral-bacterial co-infections predominated in young adults (38.96%), followed by children (29.52%), elderly (21.86%), and middle-aged adults (19.62%). In contrast, bacterial-atypical bacterial co-infections were most prevalent in children (9.52%), with

substantially lower rates in other age groups (middle-aged: 5.74%; young and elderly: 1.09% each). As illustrated in [Figure 2](#), viral-bacterial co-infections were significantly more common in OPED than in IP (18.18% vs 4.50%,  $p < 0.001$ ). This difference was most pronounced in children (36.36% vs 10.71%,  $p = 0.011$ ) and young adults (41.00% vs 10.71%,  $p = 0.005$ ). Conversely, IP showed higher bacterial-bacterial (17.80% vs 9.83%,  $p = 0.001$ ) and bacterial-atypical co-infections (5.68% vs 2.17%,  $p = 0.007$ ), with significant age-specific variations: young inpatients had elevated bacterial-bacterial rates (41.00% vs 10.71%,  $p = 0.005$ ), while middle-aged inpatients showed increased bacterial-atypical infections (10.67% vs 2.99%,  $p = 0.022$ ).

The predominant viral-bacterial co-infections were FLUAV–*H. influenzae*, FLUAV–*A. baumannii*, FLUAV–*K. pneumoniae*, and FLUBV–*H. influenzae*. Bacterial-bacterial co-infections primarily involved *H. influenzae*–*A. baumannii*, *H. influenzae*–*E. coli*, and *S. aureus*–*A. baumannii*, while bacteria–atypical bacteria co-infections were mainly observed for *H. influenzae*–*M. pneumoniae*.

Among the above-mentioned co-infected pathogens, *S. aureus*–*A. baumannii* was significantly more prevalent in females (male vs female: 0.47% vs 3.06%,  $p = 0.045$ ), and the positive rate increased with age (children 0%, young adults 0.52%, middle-aged 1.16%, elderly 7.14%,  $p = 0.002$ ). Furthermore, the prevalence of *H. influenzae*–*M. pneumoniae* differed significantly across age groups ( $p = 0.004$ ), and FLUBV–*H. influenzae* was particularly prevalent in OPED patients (OPED vs IP: 4.05% vs 0%,  $p = 0.031$ ). Notably, although *K. pneumoniae*–*P. aeruginosa* was not among the most frequent co-infection pairs overall, it was detected more frequently in elderly patients and inpatients.

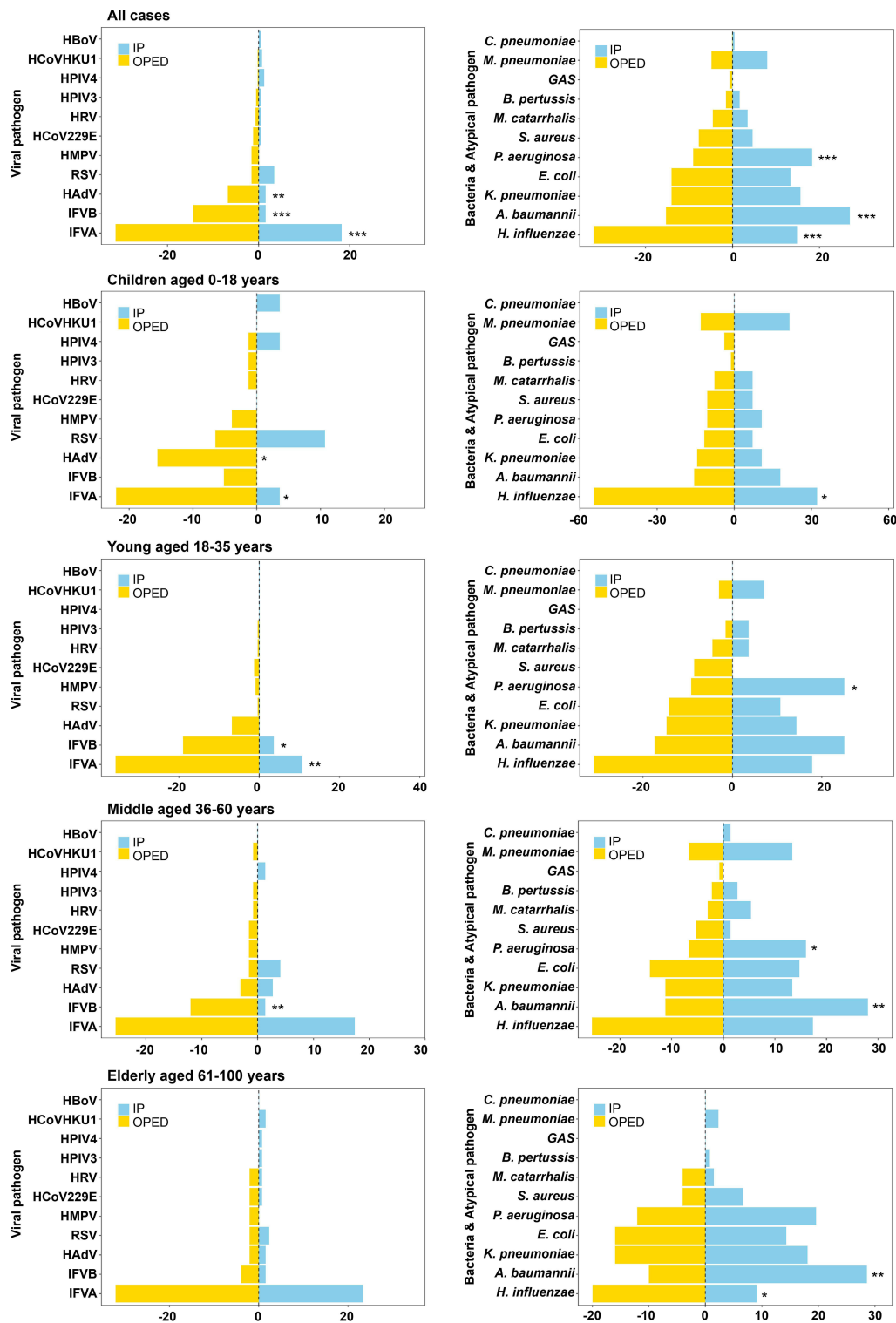
## Weekly Positive Rate Trends of 22 Pathogens

To characterize temporal variation during the study period, weekly detection rates of respiratory pathogens were analyzed. In addition, the positivity rates of five bacterial pathogens (*H. influenzae*, *A. baumannii*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*) exceeded 10% from week 50 onward, which substantially increased the risk of co-infections during peak influenza activity. Over a 12-week period from October to December 2023, the overall pathogen detection rate was 79.63%. However, weekly analysis revealed a statistically significant difference in positivity rates across the weeks ( $P < 0.001$ ). Prior to week 49, the positive detection rate for each week was lower than the overall positive detection rate; In contrast, data from after week 49 show that the positivity rate for each individual week was higher than the average rate for the entire period. Specifically, weekly positivity rates ranged from a minimum of 58.54% (98/168) in week 45 (Nov. 6–12, 2023) to a maximum of 91.67% (154/168) in week 52 (Dec. 25–31, 2023). We identified 12 pathogens with varying positive detection rates ( $p < 0.05$ ) across different weeks, which included 7 viruses, 5 bacteria, and 2 atypical bacteria. The identified viruses were FLUAV, FLUBV, HRV, HPIV4, HCoV-HKU1, HBoV and FLUAV-H3. The bacterial and atypical pathogens included *A. baumannii*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*, along with *M. pneumoniae* and *C. pneumoniae* ([Tables S5](#) and [S6](#)). Among these, the positive rate of FLUAV showed an increasing trend starting in week 48, peaking in week 50. Following week 50, although the detection rate of FLUAV declined, the detection rate of FLUBV increased, demonstrating an alternating epidemiologic pattern between FLUAV and FLUBV ([Figure 3](#)). At the same time, we found that the positive detection rate of five bacterial pathogens (*H. influenzae*, *A. baumannii*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*) exceeded 10% since week 50, which greatly increases the risk of co-infections during an influenza pandemic.

## Discussion

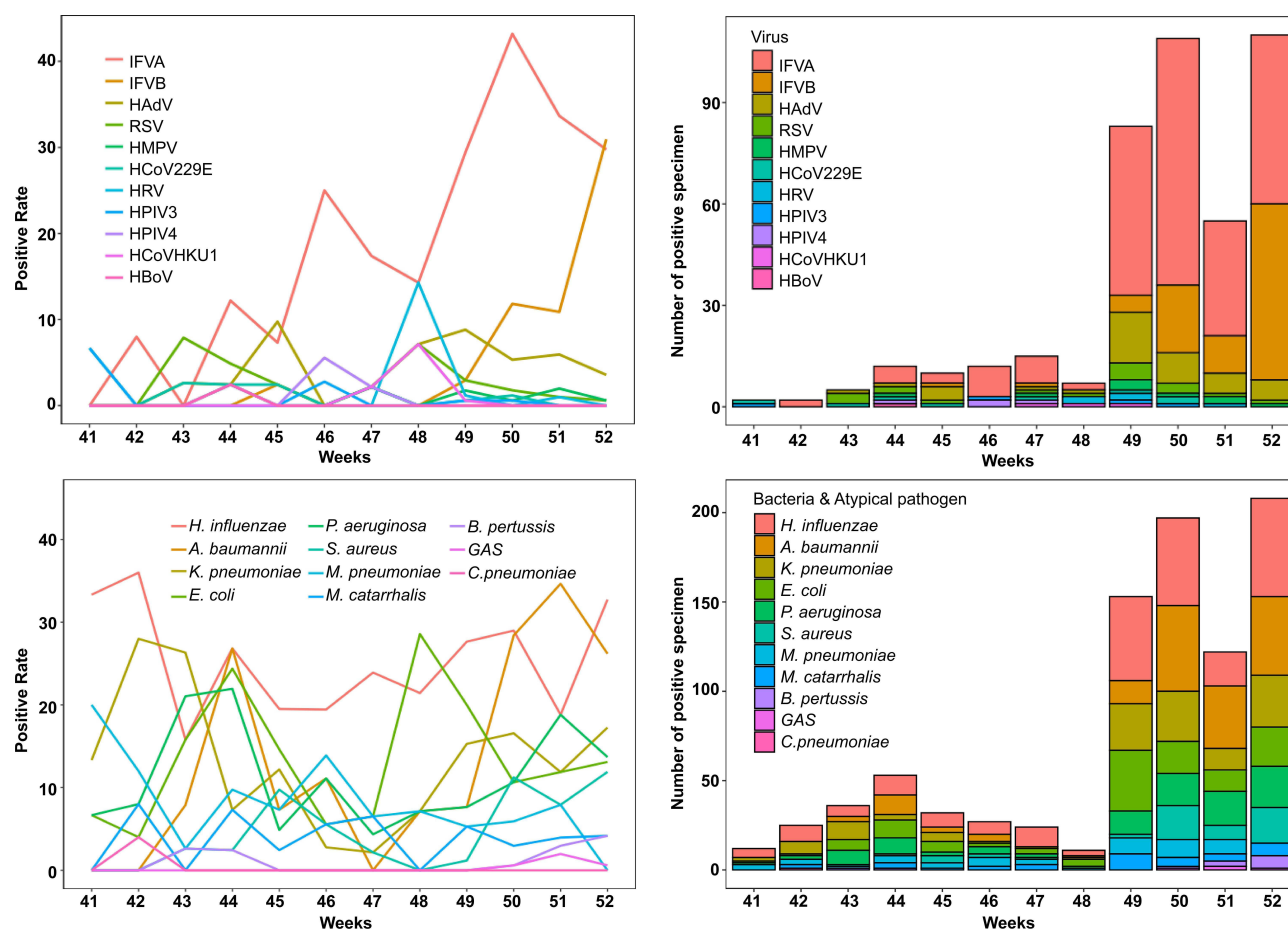
In this study, we identified 31 respiratory pathogens in pharyngeal swab specimens from 864 patients with respiratory infections, who were recruited at Beijing Tongren Hospital, Capital Medical University, between October and December 2023. The overall pathogen detection rate was 79.63%, with co-infections (59.16%) outnumbering mono-infections (40.84%), reflecting the increased complexity of respiratory disease epidemiology in the post-COVID-19 era. The predominant viral pathogens—FLUAV, FLUBV, and HAdV—followed established seasonal trends,<sup>9,10</sup> while the dominant bacterial pathogens such as *H. influenzae*, *A. baumannii*, and *K. pneumoniae* were consistent with known clinical significance in respiratory infections.<sup>11–14</sup>

Children and young adults had higher detection rates of pathogens (86.67% and 83.65%, respectively) and higher co-infection rates (56.19% and 52.32%, respectively). Of note, there was a clear difference in the pathogen spectrum among



**Figure 2** Comparison of the positivity rates of 22 respiratory pathogens between OPED and IP patients. The figure shows the positivity rates of 11 viral pathogens (left column) and 11 bacterial pathogens (right column) in OPED and IP patients. The horizontal bars represent pathogen positivity rates (%), with OPED and IP groups shown for comparison. (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

**Abbreviations:** FLUAV, influenza A virus; FLUBV, influenza B virus; HPIV3, human parainfluenza virus 3; HPIV4, human parainfluenza virus 4; HCoV\_229E, human coronavirus 229E; HCoV\_HKU1, human coronavirus HKU1; HRSV, human respiratory syncytial virus; HMPV, human metapneumovirus; HRV, human rhinovirus; HAdV, human adenovirus; HBoV, human bocavirus; *M. pneumoniae*, *Mycoplasma pneumoniae*; *C. pneumoniae*, *Chlamydia pneumoniae*; GAS, *Streptococcus pyogenes* (group A *Streptococcus*); *H. influenzae*, *Haemophilus influenzae*; *K. pneumoniae*, *Klebsiella pneumoniae*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*; *M. catarrhalis*, *Moraxella catarrhalis*; *E. coli*, *Escherichia coli*; *A. baumannii*, *Acinetobacter baumannii*; *B. pertussis*, *Bordetella pertussis*.



**Figure 3** Temporal trends in the positive rates of 22 pathogens from week 41 to 52, 2023. The top figures illustrated the weekly dynamics of viral pathogens, while the bottom figures showed those of bacterial over weeks 41–52. The left figures displayed the weekly positive rate (%) for each pathogen, and the right figures showed the corresponding number of positive specimens.

patients of different ages. Children were mainly colonized or infected with *Haemophilus influenzae*, *Mycoplasma pneumoniae*, HAdV, and HRSV, while young adults showed a higher prevalence of influenza virus (FLUAV/FLUBV). We observed a sequential influenza activity, with FLUAV cases rising from week 48 and peaking at week 50, followed by a FLUBV surge—a pattern consistent with Beijing surveillance data and national reports.<sup>15,16</sup> This variation suggests an earlier seasonal pattern in the post-NPI period. Meanwhile, five bacterial pathogens (*Haemophilus influenzae*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*) remained >10% positive from week 50, significantly increasing the risk of co-infection during the peak period of respiratory virus activity.

*Mycoplasma pneumoniae* played an important role in children's infection, with a detection rate of 15.24%, which was significantly higher than the overall detection rate (5.79%). The persistent circulation (>5% positivity at weeks 44 to 51) is consistent with reports of a surge in pediatric pneumonia in Beijing.<sup>17</sup> Alarmingly, a recent study in Beijing found a 77.3% positive rate of pneumobronchitis among pediatric pneumonia cases and widespread macrolide resistance, underscoring the growing treatment challenge.<sup>18</sup> Similarly, HAdV re-emerged as a key pathogen. A multicenter prospective study in Beijing revealed that its positivity rate had fallen to a low of 0.16% during the COVID-19 pandemic (February 1, 2020, to December 31, 2021).<sup>19</sup> In addition, the dominant HAdV type shifted from HAdV-B to HAdV-C.<sup>20</sup> The overall detection rate of HAdV was 5.09%. The detection rate of HAdV in children (11.43%) and outpatients (6.67%) was higher than that in inpatients (1.52%). This rebound is consistent with the data from Peking Union Medical College Hospital (October through December 2023: 8.82%-14.19% prevalence),<sup>21</sup> confirming HAdV's return as a major pediatric ARTI pathogen following its decline during the pandemic.

HRSV is the main pathogen of acute lower respiratory tract infections (ALRTIs) in children under 5 years old in Beijing, with obvious seasonal epidemic characteristics.<sup>22</sup> The detection rate of HRSV in children increased from 2.08% to 7.62%, which was the primary cause of respiratory tract infection in children. Notably, the surveillance data showed that the 2023 autumn-winter peak (10.04%) was the highest in nine years (January 2015 to January 2024),<sup>23</sup> reflecting significant post-NPI epidemiological disruption through combined local and imported virus circulation.<sup>24</sup>

Collectively, the 2023 autumn-winter season in Beijing was marked by the co-circulation of multiple pathogens (FLUAV, *M. pneumoniae*, HAdV, HRSV), presenting significant challenges for infection management.<sup>25</sup> We observed distinct epidemiological patterns: (1) alternating influenza A/B epidemics with heightened susceptibility among young adults (FLUAV: 33.79%; FLUBV: 17.71%), potentially linked to behavioral and immunological factors;<sup>26</sup> (2) higher pathogen detection in OPED (83.5%) than inpatients (70.83%,  $p < 0.001$ ), reflecting age-related care-seeking behaviors (elderly hospitalization: 72.68%; young adult OPED: 92.37%); (3) increased co-infection risk.<sup>27</sup> These findings highlight the critical role of multiplex PCR in both clinical management and public health surveillance,<sup>28,29</sup> particularly during this transitional epidemiological period.<sup>30</sup> In addition, the age stratification and distribution of different health care settings observed in this study suggest the need for targeted prevention strategies during epidemiological transitions.

There are differences in the distribution of pathogens between outpatients, emergency patients and inpatients. The higher viral detection rates in OPED Settings may reflect patient health care-seeking behavior and illness severity, whereas the increased prevalence of certain bacterial pathogens among hospitalized patients may be related to underlying illness or hospital-related factors. These observations highlight the need to tailor diagnostic and therapeutic approaches in different clinical Settings.

Taken together, these findings suggest that the first post-COVID-19 autumn-winter in Beijing was characterized by a complex multi-pathogen epidemic and a large burden of mixed infections. Multiplex PCR assays enable timely and comprehensive detection of pathogens, support clinical decision making, and inform public health surveillance during periods of dynamic epidemiological changes.

## Conclusions

In the first autumn-winter after the COVID-19 outbreak in Beijing, respiratory viruses and bacteria co-existed widely, and the frequency of co-infection was high. These findings underscore the importance of comprehensive molecular surveillance and integrated diagnostic strategies to support clinical management and public health responses in the post-COVID-19 era.

## Declarations

The study protocol was approved by the Ethics Committee of Beijing Tongren Hospital, Capital Medical University (Approval No. TREC2024-KY196), and all procedures were performed in accordance with the ethical principles of the Declaration of Helsinki. All data analyzed in this study were derived from routine clinical diagnostic procedures and were anonymized prior to analysis. Written informed consent was obtained from the legal guardians of pediatric participants. For adult participants, informed consent was waived by the Ethics Committee due to the retrospective nature of the study and the use of anonymized clinical data.

## Abbreviations

COVID-19, Coronavirus Disease 2019; NPI, non-pharmaceutical interventions; HRSV, Human respiratory syncytial virus; *M. pneumoniae*, *Mycoplasma pneumoniae*; IP, Inpatient; OPED, Outpatient/emergency departments; FLUAV, Influenza A virus; FLUAV-H3, Influenza A/H3 virus; FLUBV, Influenza B virus; HPIV1, Human parainfluenza virus 1; HPIV2, Human parainfluenza virus 2; HPIV4, Human parainfluenza virus 4; HCoV\_NL63, Human coronavirus NL63; HCoV\_OC43, Human coronavirus OC43; HRV, Human rhinovirus; MeV, Measles virus; HAdV, Human adenovirus; HBoV, Human bocavirus; *H. influenzae*, *Haemophilus influenzae*; *K. pneumoniae*, *Klebsiella pneumoniae*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *A. baumannii*, *Acinetobacter baumannii*; *L. pneumophila*, *Legionella pneumophila*; *C. pneumoniae*, *Chlamydia pneumoniae*; RT-PCR, Reverse transcription polymerase chain reaction; IQR, Interquartile range.

## Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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## Disclosure

The authors declare no conflicts of interest in this work.

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