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Increased circulation of adenovirus in China during 2023-2024: Association with an increased prevalence of species B and school-associated transmission

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SUMMARY

Objectives: We report on the surge in human adenovirus (HAdV) cases in China starting in October 2023 and analyze the key drivers behind this increased circulation in the post-COVID-19 period.
Methods: We analyzed targeted next-generation sequencing (tNGS) data from 1,875,862 hospitalized acute respiratory infection (ARI) cases across 4758 hospitals in 314 cities throughout all 31 provinces of mainland China. An in-house script was used to analyze the positivity rates of different HAdV species nationwide and across various provinces. We also assessed the age-specific infection risk for HAdV species B and C using restricted cubic splines (RCS), and we tested differences in HAdV infection rates between vacation periods and school terms using the Kruskal–Wallis test.
Results: We identified an increased prevalence of HAdV species B replacing circulating species C, and this increase was associated with elevated HAdV activity in China from October 2023 to August 2024. Age-specific analysis indicates that, compared to HAdV species C, HAdV species B has a higher infection rate in school-aged children. Comparison of HAdV incidence rates during school terms and vacations showed that schools are the primary transmission setting for HAdV species B. These findings strongly support the conclusion that school-associated cluster infections caused by HAdV species B are the drivers of the ongoing increased circulation of HAdV in China.
Conclusion: This study found that changes in susceptible populations and transmission settings due to an increased prevalence of HAdV species B were the key factors driving the elevated HAdV activity in China starting in October 2023.

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Introduction

Human adenoviruses (HAdV) belong to seven species within the genus *Masadenovirus*, family *Adenoviridae*. HAdV is associated with a spectrum of diseases that includes respiratory infections, keratoconjunctivitis, and gastroenteritis.[1] HAdVs are classified into seven species, HAdV-A to HAdV-G, which may be further classified into different serotypes using traditional methods, such as hemagglutination and serum neutralization reactions, or more advanced methods, such as genomic sequencing and bioinformatic analysis.[2,3] HAdV is recognized as a major causative agent of acute respiratory tract infections (ARTIs) in children, accounting for at least 3–7% of ARTI cases.[4,5] HAdV species B (HAdV-3, 7, 11, 14, and 21), C (HAdV-1, 2, 5, and 6) and E are most commonly associated with respiratory infections.[1,6,7]

The major HAdV subtypes detected in various countries and regions differ and change over time.[2,8,9] In China, HAdV-3 and -7 are the major

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types, and the dominant species alternates from year to year.[2,10] The COVID-19 pandemic has affected the epidemiological characteristics of many respiratory pathogens. For example, during the COVID-19 pandemic, HAdV species B (HAdV-3 and -7) nearly disappeared in China, while species C became the predominant strain.[11,12] This situation was also observed in the United States.[13] After the pandemic, with the relaxation of recommendations and mandates for the use of non-pharmaceutical interventions (NPIs), an increase in HAdV epidemic activity has been observed in many countries, including Japan, South Korea, Italy, and the United States, and there was a shift in the predominant HAdV species in the United States.[14–17] However, the epidemiological characteristics of HAdVs and the circulating species in China following the relaxation of NPIs remain unclear.

In this study, we analyzed tNGS data from the nationwide hospital laboratory network of KingMed Diagnostics, which has established 39 medical center laboratories across China, serving over 25,000 hospitals or healthcare centers. This network covers regions in which over 90% of the population resides. Based on the 1.8 million ARTI cases diagnosed by tNGS nationwide from January 1, 2023, to August 18, 2024, we identified a marked increase in the prevalence of HAdV species B (replacing previously dominant species C) in China, which may have contributed to increased circulation of HAdV observed from October 2023 onwards. Analyses of patient age and transmission setting suggest that cluster infections of species B among school-aged children in school settings are the primary factors contributing to the ongoing increased circulation of HAdV.

Methods

Data source

This study used targeted next-generation sequencing (tNGS) diagnostic data from hospitalized patients with ARTIs, provided by KingMed (Guangzhou, Guangdong, China), a diagnostic company offering third-party testing services to over 25,000 healthcare institutions across China. The data were collected from January 2022 to August 2024, covering 4758 hospitals in 314 cities across all 31 provinces of mainland China, representing over 90% of the country's total population. The tNGS platform used in this study can identify different species of HAdV. Detailed methodologies and data quality control standards are provided in the supplementary materials (Appendix pp 1–2).

Procedures

For the tNGS samples, data on sex, name, date of birth, sampling date, location (e.g., Guangdong, Guangzhou), sampling site (e.g., nasopharyngeal), and type of infection were obtained from sample submission forms. Samples missing any of that information were excluded from the study. Samples with infection types classified as ARTI were included in this study. Samples with the same sex, name, date of birth, and sampling location were identified as duplicates and excluded from the study. Sputum and bronchoalveolar lavage fluid samples were classified as lower respiratory tract (LRT) samples and were analyzed using RP100™ Respiratory Pathogen Microorganisms Multiplex Testing Kits (KingCreate, China) (Appendix pp 3–5). Nasopharyngeal and oropharyngeal swabs were classified as upper respiratory tract (URT) samples and were analyzed using URP50™ Respiratory Pathogen Microorganisms Multiplex Testing Kits (KingCreate, China) (Appendix pp 5–7).

Statistical analysis

The data analysis protocol was reviewed and approved by the ethics review committees of KingMed (2024158). The HAdV positivity rates were calculated across different provinces, weeks, ages, and sexes. Chi-square tests were used to analyze differences in positivity rates across sex and age groups. The positivity rate of MP for each age was calculated, and the risk of MP infection across various ages was assessed using the restricted cubic spline (RCS) method.[18] Winter and summer vacation dates for all 31 provinces in mainland China were obtained from local education authorities. Differences in HAdV infection rates between vacation periods and school terms were tested using the Kruskal–Wallis test[19] (Appendix pp 2).

Results

Data overview

The tNGS data of 1,875,862 ARTI patients from 4758 hospitals in mainland China were collected from January 1, 2023, to August 18, 2024. The number of HAdV-positive cases was 148,465, with a positivity rate of 7.91%. Among HAdV-positive cases, there were 85,742 male cases, accounting for 57.75%, and 62,723 female cases, accounting for 42.25%. Males had a significantly higher incidence than females ($\chi^2 = 9.856$, $P = 0.0017$), which aligns with findings for lower respiratory infections.[20] In terms of age distribution, there were 88,324 cases in children aged 0–6 years (59.491%), 43,443 cases in individuals aged 6–18 years (29.26%), 8901 cases in adults aged 18–60 years (6.00%), and 7797 cases in those over 60 years (5.25%).

Increased circulation of HAdV

Examining the trend in national HAdV case numbers and positivity rates, starting from week 44 of 2023, both the number of HAdV-positive cases and the HAdV positivity rate among patients with ARTI surged (Fig. 1A). Before week 44 of 2023, the median weekly HAdV positivity rate among ARTI cases nationwide was 4.29% (interquartile range (IQR) 3.92–4.53%), whereas, after week 44 of 2023, the median weekly HAdV positivity rate increased to 9.59% (IQR: 8.20–11.58%) (Appendix pp 8–16). This indicates that from October 2023 (week 44 of 2023) to August 2024, China experienced an increase in HAdV epidemic activity, with HAdV accounting for approximately 10% of pathogenic causes among patients with ARTIs nationwide. Results across different provinces show that this elevated HAdV activity was not due to a spike in cases in isolated provinces but rather a nationwide surge (Appendix pp 16–40, 46).

Association between increased circulation of HAdV and increased prevalence of species B

The tNGS data enabled us to analyze the HAdV types present in each sample. Among all 148,298 HAdV-positive cases (excluding 167 HAdV mixed-infection cases), 85,229 cases (57.47%) were from infection with HAdV species B (including 70,869 cases of HAdV-3, 914 cases of HAdV-7, 8627 cases of HAdV-21, 468 cases of HAdV-55, and 4351 cases of HAdV species B with undetermined type), and 61,617 cases (41.55%) were from infection with HAdV species C (including 38,469 cases of HAdV-1, 19,736 cases of HAdV-2, 669 cases of HAdV-5, 606 cases of other types, and 2137 cases of HAdV species C with undetermined type) (Appendix pp 12–16). Additionally, 1452 cases (0.98%) represented infection with other HAdV species.

Based on the temporal distribution of different HAdV species, China experienced a marked increase in the prevalence of HAdV species B (replacing previously dominant species C) between 2023 and 2024. From week 1 to week 44 of 2023, the dominant strain circulating in China was HAdV species C, and HAdV species B nearly disappeared, which is consistent with previous reports.[11] However, from week 45 of 2023 to week 33 of 2024, HAdV species B was dominant (Fig. 1). Pearson correlation analysis revealed a significant positive correlation between HAdV incidence and the proportion of

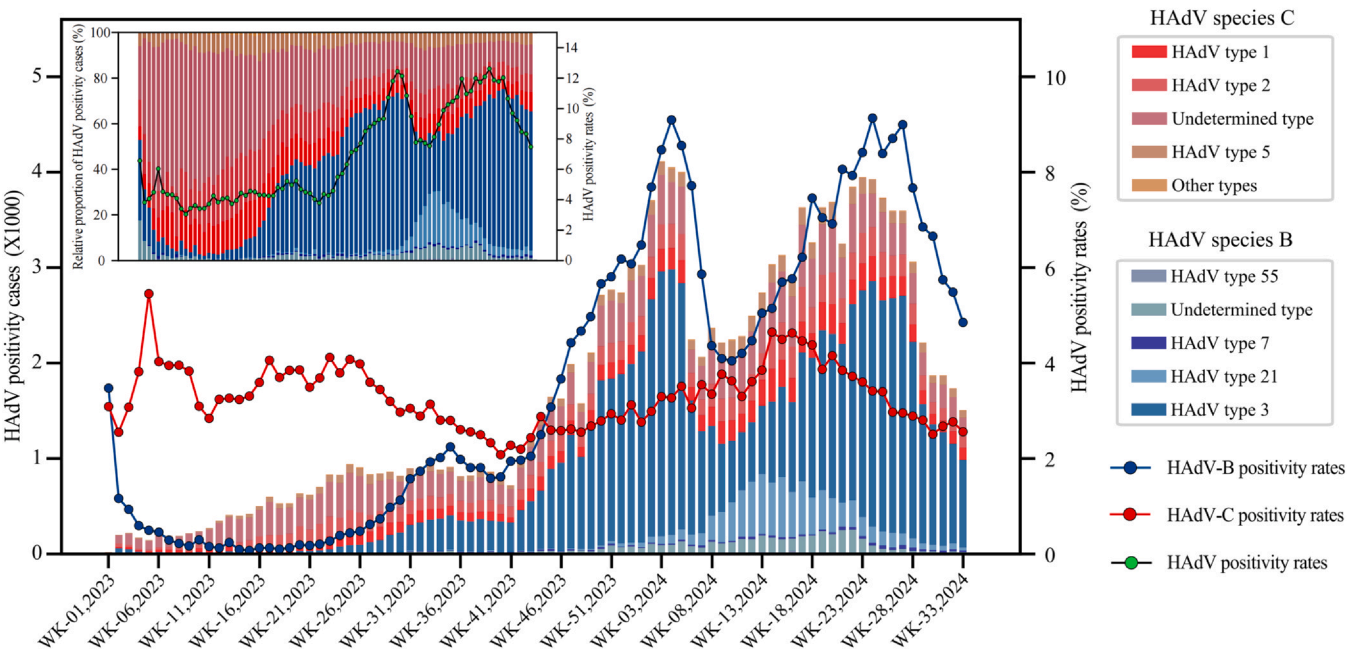

Fig. 1. Temporal trends of HAdV activity among ARTI cases in China, January 2023–August 2024. (A) Main panel: Weekly HAdV positivity rates (line chart, right y-axis) and absolute case numbers (overlaid bar chart, left y-axis) stratified by HAdV types (color-coded). The line chart plots show the nationwide positivity rates of HAdV species B (blue) and species C (red) among ARTI cases. (B) Inset: Relative weekly proportions of HAdV species B and C (stacked bars, left y-axis) and overall HAdV positivity rate (green line, right y-axis).

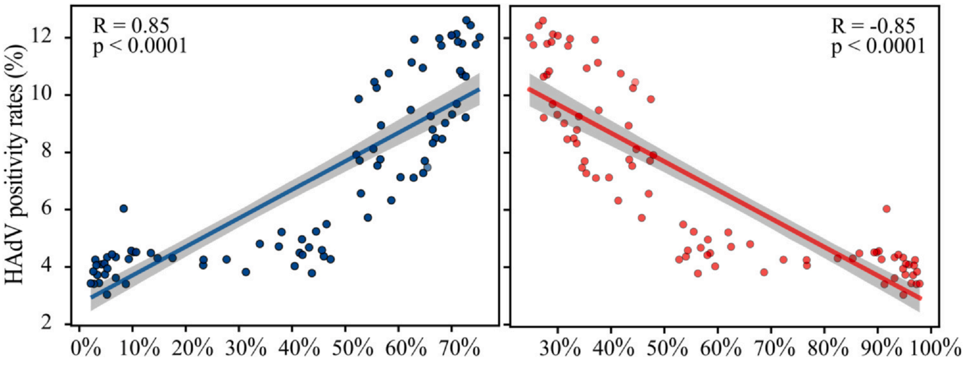

Fig. 2. Correlation analysis between HAdV positivity rates and the proportion of infections with HAdV species B or C. The left panel shows the correlation between HAdV positivity rates and the proportion of cases from infection with HAdV species B. The right panel shows the correlation between HAdV positivity rates and the proportion of cases from infection with HAdV species C. Each point represents the weekly data on the proportion of cases from infection with HAdV species B or C and the HAdV positivity rates across the country from January 1, 2023, to August 18, 2024, covering a total of 86 weeks.

cases of HAdV species B infection ($r = 0.85$, $P < 0.0001$), indicating that a higher proportion of HAdV species B cases is associated with increased HAdV incidence. Conversely, there was a significant negative correlation between the proportion of HAdV species C cases and HAdV incidence ($r = -0.85$, $P < 0.0001$) (Fig. 2).

At the provincial level, regions that experienced a significant increase in the prevalence of HAdV species B also exhibited elevated HAdV activity, with the timing of this replacing being closely associated with the period of heightened HAdV activity. For instance, in Chongqing province, HAdV species B became the predominant circulating starting in January 2024, coinciding with a significant surge in provincial HAdV infection. However, in provinces where species B did not replace circulating species C (e.g., Yunnan province), no elevated HAdV activity was observed during the study period (Appendix pp 16–40 and 46). These findings suggest that the emergence of HAdV species B as the predominant circulating strain may have contributed to the nationwide resurgence of HAdV activity observed China since October 2023.

School-associated cluster infections caused by HAdV species B contributed to the nationwide increased HAdV activity

To identify the potential drivers of the elevated HAdV activity in China, we conducted a comparative analysis of age-specific infection risks between the predominant circulating species B and species C. We observed that the highest infection rate for HAdV species C occurred in 1.84 year with risk of infection decreasing as age increased. In contrast, the HAdV species B infection rate peaked in 5.21 year (Fig. 3) (Appendix pp 41–43). Thus, compared to HAdV species C, HAdV species B was more likely to infect school-aged children (In China, children aged 3–6 years attend kindergarten, while children aged 6–12 years attend primary school), which facilitates the transmission of HAdV species B in school settings, making it more likely to cause cluster infections that could trigger the increased HAdV activity. These data strongly indicate that the increase in the prevalence of HAdV species B which primarily infects school-aged children, was a key factor in the increased circulation of HAdV in China.

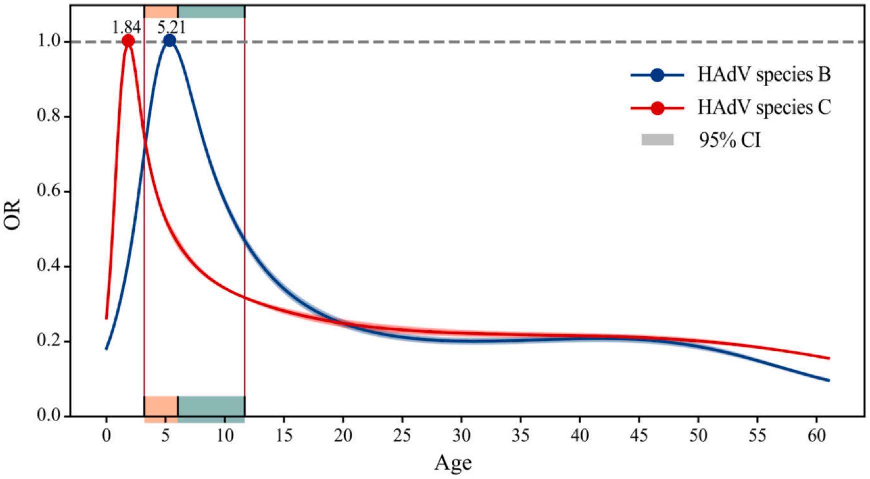

Fig. 3. HAdV positivity rates of HAdV species B and C across all age groups. The x axis represents age, with the age range 3–6 years (kindergarten) marked in blue and the age range 6–12 years (primary school) marked in purple. The y axis represents the HAdV Odds Ratio(OR) among ARTI cases, with the red line representing HAdV species C and the blue line representing HAdV species B.

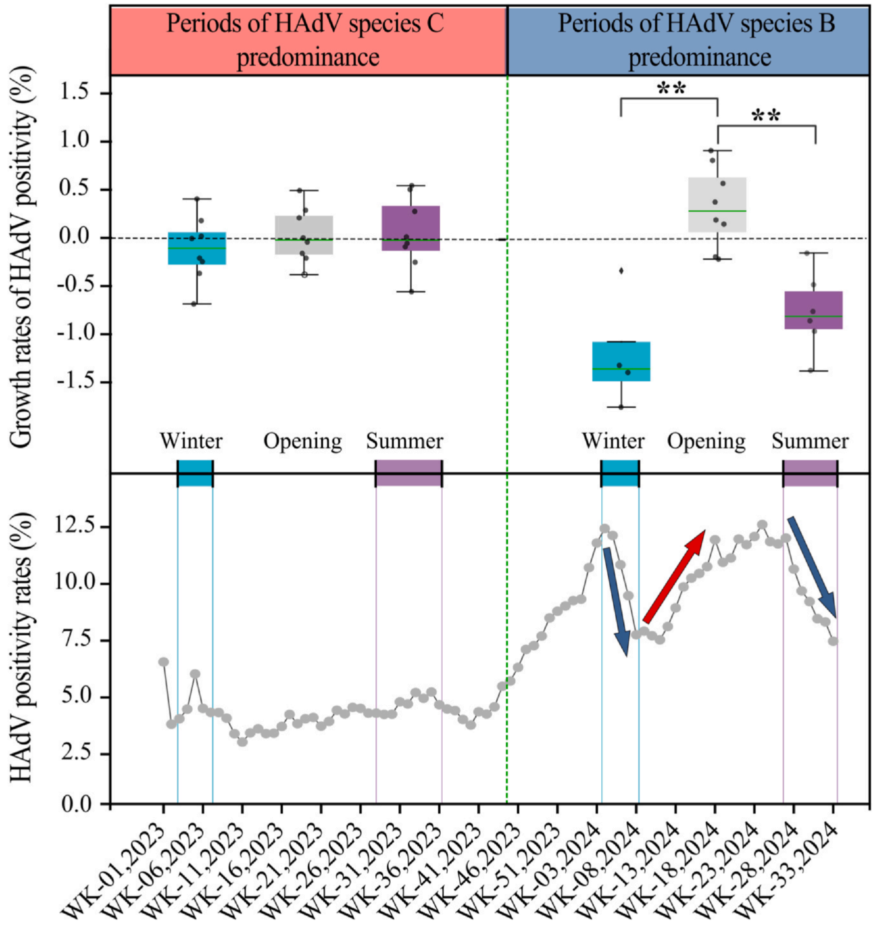

Fig. 4. Comparative analysis of HAdV positivity rates during school vacations versus school terms, split into periods dominated by HAdV species B or species C. Top: comparison of the growth rates of HAdV positivity during periods dominated by HAdV species B or species C across winter vacations, summer vacations, and school terms. Bottom: weekly HAdV positivity rates, with arrows indicating the trends in positivity rates.

To demonstrate that school-associated cluster infections of HAdV species B are the driving force behind the increased HAdV activity, we compared the trends in HAdV incidence during school terms and vacation periods for the periods dominated by either HAdV species B or HAdV species C. During the period dominated by HAdV species C (from week 1 to week 44 of 2023), no effect of school vacation periods was observed on HAdV positivity rates. However, during the period dominated by HAdV species B (from week 45 of 2023 to week 33 of 2024), HAdV positivity rates dropped rapidly during the 2024 winter vacation (from January 26, 2024, to February 25, 2024), then surged after schools reopened in spring 2024, peaking within 10 weeks before dropping rapidly again during the 2024 summer vacation (from July 7, 2024, to August 31, 2024) (Fig. 4) (Appendix pp 44–45). These findings strongly support the conclusion that school-associated cluster infections caused by HAdV species B are the drivers of the current increased circulation of HAdV that began in October 2023 in China.

Discussion

After the relaxation of NPIs following the COVID-19 pandemic, Japan, South Korea, Italy, and the United States have all reported an increase in HAdV epidemic activity. This study, for the first time, utilizes data from over one million cases to report a nationwide increased circulation of HAdV. We identified that the increased prevalence of species B was associated with the increased HAdV activity in China and, through age-specific infection risk analysis, hypothesized and confirmed that school-associated cluster infections caused by HAdV species B were the primary drivers of the increased circulation of HAdV.

This study reports on the increased HAdV activity triggered by the increased prevalence of species B. In fact, HAdV species shifts are rare, while the alternation of types within the same species occurs more frequently. Prior to the COVID-19 pandemic, the predominant HAdV in China were species B, specifically HAdV-3 and HAdV-7, which alternated in circulation.[21–25] During the increased circulation of HAdV starting in October 2023, the predominant type was HAdV-3 within species B, while the proportion of HAdV-7 was relatively low. However, HAdV-7 continued to cause a greater proportion of ICU cases compared to other types.[2] We speculate that this is a key reason why the increased HAdV activity did not result in a significant strain on China's healthcare system.

This study analyzes the age-specific infection risks for different HAdV species and reveals distinct susceptible populations for each species. We believe that these age-specific differences in infection risk are the result of a combination of the virus's biological characteristics and factors such as population immunity. Additionally, a more detailed comparison of age-specific infection risks between different types within the same species is necessary. For instance, comparing the age-specific infection risks of HAdV-3 and HAdV-7 could help us better understand the alternating circulation of these two types in China.

Notably, after the relaxation of NPIs following the COVID-19 pandemic, the alternating circulation of HAdV species has not been limited to China but has also occurred in other countries. For example,[14] in the United States from 2021 to 2023, there was also a shift in HAdV species before and after the relaxation of NPIs. During the NPIs period, countries such as China and the United States reported the disappearance of HAdV species B, but, after the relaxation of NPI regulations, these countries experienced HAdV outbreaks driven by species B.[11,17] This clearly indicates that, due to differences in susceptible populations and transmission settings, NPIs had a greater impact on species B compared to species C. It also suggests that NPIs are the main driving force behind the global shift in HAdV species.

Our findings are subject to several limitations. First, the lack of clinical outcomes data limits our ability to compare differences in clinical symptoms caused by different HAdV types. Second, the number of cases per province reflects only the included cases, not the overall severity of the outbreak, limiting our ability to compare outbreak severity across provinces. Third, this study is constrained by the relatively short surveillance period following the relaxation of NPI regulations, which limits our ability to determine whether the increased prevalence of HAdV species B represents a sustained epidemiological shift or transient post-pandemic dynamics. Lastly, the positivity rate refers to HAdV positivity among ARTI cases, not the general population, which limits our morbidity estimation.

Despite these limitations, identifying susceptible populations and transmission settings is crucial for the prevention and control of infectious diseases. In this study, we analyzed the differences in susceptible populations and transmission settings among different HAdV species, which is of great value for guiding responses to outbreaks caused by various HAdV species and for adopting more targeted prevention and control strategies.

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Author contributions

JGX, RHJ and YMS conceptualized the study. PL, and JLY contributed the original data for analysis. YMS, and TQ verified the underlying data.HL, YL, and MW performed the formal analysis, while YMS, JGX and RHJ validated the data. Data analysis was co-led by YMS and JGX. JLY and MW were responsible for data visualization. Project administration was managed by YMS and QT. The study was supervised by JGX and RHJ. YMS and TQ drafted the original manuscript, and GX and RHJ reviewed and edited it. All authors reviewed and approved the final manuscript. Each author had final responsibility for the decision to submit for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106475.

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