

Detection of Influenza A/H3N2 subclade K in Tehran, Iran, 2025

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Abstract

Introduction: The influenza A/H3N2 virus is a major cause of respiratory infections in the elderly. The emergence of new subclades of this virus may be associated with increased clinical severity and poor response to treatment. This study reports the first case of respiratory infection caused by the H3N2 subclade K in Iran.

Material and methods: A 65-year-old female patient with acute respiratory distress syndrome who was admitted to a hospital in South Tehran on first November 2025. She hospitalized in the ICU, and treatment started immediately.

Results: A throat swab sample was tested by PCR, which was positive for influenza A/H3N2. Genetic sequencing of the isolated virus identified the subtype as H3N2 subclade K. This report provides the first documented evidence of H3N2 subclade K in Iran.

Conclusion: Given the ongoing emergence of novel influenza virus subtypes, continuous molecular surveillance and genetic sequencing of positive samples are essential for timely identification of circulating subclades, especially in patients with severe clinical presentation.

Keywords: Case Report, Human Influenza, H3N2, Seasonal Influenza, Subclade K

1. Introduction

Influenza A viruses are a major public health threat worldwide and characterized by rapid evolution of the hemagglutinin gene, causing seasonal influenza epidemics. Currently, seasonal influenza viruses are A/H1N1, A/H3N2, and B/Victoria [1,2]. In the temperate northern hemisphere, influenza activity usually is between October and March, with a peak in December and January. 2025/2026 influenza season in Iran was started in late October, with a rise in November with the A/H3N2 virus as a predominant strain [3].

Reports showed that A/H3N2 subclade K accounts for 47% of the A/H3N2 virus sequences submitted to GISAID EpiFlu this year. Influenza A/H3N2 subclade K (formerly J.2.4.1) was detected at the end of the southern hemisphere 2025 influenza season and the beginning of the

northern hemisphere 2025/26 season. This was a rapid A/H3N2 virus evolution since its introduction in 1968 [4].

Initial analysis from the World Health Organization vaccine composition summit proposed low reactivity of A/H3N2 subclade K viruses with post-infection ferret antisera against the northern hemisphere vaccine strains. Influenza A/H3N2 subclade K viruses are shown by J.2.4 subclade substitutions T135K and K189R, as well as K2N, S144N, N158D, I160K, Q173R, T328A and S378N [5]. This suggests that vaccine effectiveness may be suboptimal. Given this, antiviral therapy is a crucial complementary intervention, especially for high-risk individuals. Therefore, understanding subclade K is vital for effective disease management. The rise of subclade K highlights the critical need for ongoing genomic and antigenic monitoring. Rapidly detecting these new variants is vital for improving vaccine strain updates and shaping effective prevention plans [6-7-9].

Here, we investigated to find the subclade of the A/H3N2 virus in a fatal case in Tehran, Iran.

2. Case Presentation

The 65-year-old female patient with a history of sore throat and shortness of breath started a few days ago had been referred to the hospital emergency department with symptoms of decreased consciousness. The patient had a history of chronic heart and renal disease, diabetes, and hypertension. The patient was admitted to the intensive care unit upon arrival to the hospital due to the severe shortness of breath and decreased level of consciousness. Chest X-ray showed bilateral lung infiltration, and the patient was intubated, and Oseltamivir and antibiotics were started. A respiratory sample was collected from the endotracheal tube suction and sent to Iran

National Influenza Center (NIC) laboratory. The test showed influenza A/H3N2 infection. Despite special care and timely medication, the patient died the next day.

In NIC laboratory, Real time RT-PCR was performed on the sample using the previously reported method [10]. After detection of the A/H3N2 virus, for clade determination, conventional RT-PCR was conducted for hemagglutinin gene [11]. Primer and probe sequences are summarized in Table 1. The PCR products of the HA gene were sequenced in both directions. The sequence analyzed using Nextclade tool which showed subclade K, and submitted to the GISAID. The phylogenetic tree was drawn (Figure1).

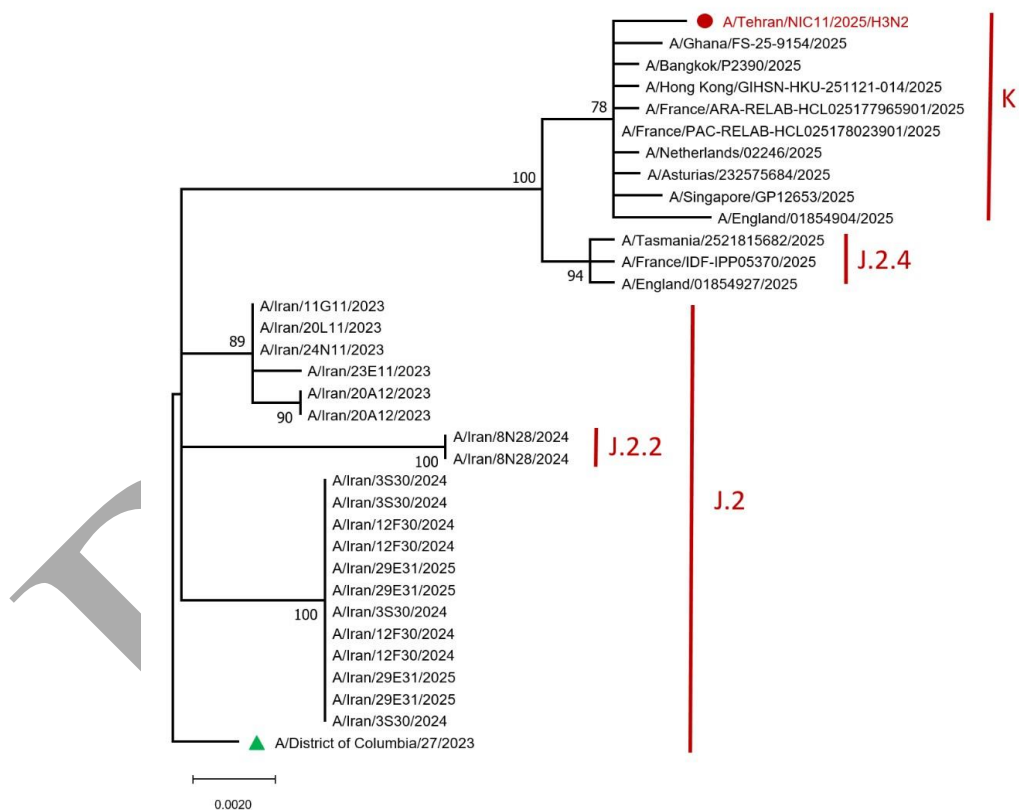


Figure 1 The phylogenetic tree shows subclade K.

Table 1. Detailed characteristics of the primers and probes, including sequences and target genes used for the detection and sequencing of Influenza A (H3N2).

Name	Gene	Sequences
Detection of Flu A (Rael-Time PCR)		
Flu A-F	M	GACCRATCCTGTCACCTCTGAC
Flu A-R	M	AGGGCATTYTGGACAAAKCGTCTA
Flu A-P	M	FAM-TGCAGTCCTCGCTCACTGGGCACG-BHQ-1
Detection of Flu A (H3N2) (Rael-Time PCR)		
H3N2-F	HA	AAGCATTCCYAATGACAAACC
H3N2-R	HA	ATTGCRCCRAATATGCCTCTAGT
H3N2-P	HA	FAM-CAGGATCACATATGGGSCCTGTCCCAG-BHQ-1
Sequencing Flu A(H3N2) (Conventional RT-PCR)		
H3N2-F1	HA	AAGCAGGGGATAATACTATTAACC
H3N2-R1	HA	AACCGTACCAACCCACCATTC
H3N2-F2	HA	CTGAACGTGACTATGCCAAACAAT
H3N2-R2	HA	AGTAGAAACAAGGGTGTITTTAAT

3. Conclusions

Despite the circulation of a drifted influenza A/H3N2 virus, there are no signs of increased mortality in healthy people. The identified patient with a fatal outcome did not have the influenza vaccine and had a history of chronic diseases. This case highlights the ongoing need for robust epidemiological surveillance to track H3N2 strains and their potential impact, especially considering the unpredictable nature of viral evolution. As routine recommendations during influenza season, all high risk groups must get the flu vaccine. All hospital staff and visitors in healthcare settings should follow infection prevention and control guidance by regular environmental cleaning, continuing strict hand sanitation, improved ventilation of closed spaces, and the suitable use of personal protective equipment to decrease transmission of influenza viruses.

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Compliance with ethical guidelines

This study protocol was performed in compliance with the ethical principles for medical research involving human subjects as outlined in the Declaration of Helsinki. In addition, it was conducted as part of the routine public health surveillance program of the National Influenza Center (NIC) of Iran. All clinical specimens were collected and analyzed in accordance with the national public health guidelines for influenza surveillance.

Conflict of interest

The authors declared no conflict of interest.

Authors' contributions

Writing the original draft: Nazanin Zahra Shafiei Jandaghi, **Methodology:** Akram Sadat Ahmadi, Saeideh Saeedeh Mahfoozi, Sevrin Zadheidar ¹, Ghazal Sadat Fateminasab ¹, Tasnim Jamalvandi. **Data acquisition:** Fatemeh Saadatmand, **Data analysis, interpretation:** Katayoun Seif-Farahi ², Zahra Hasanpoor ², Mahshid Nasehi ^{3,4}, Ghobad Moradi, **Review and editing:** Jila Yavarian.

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Data availability

The dataset presented in the study is available upon request from the corresponding author during submission or after publication. In addition, no artificial intelligence (AI) assistance was used in the preparation of this manuscript.

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