flora

RESEARCH ARTICLE/KLİNİK ÇALIŞMA

FLORA 2024;29(4):488-501 • doi: 10.5578/flora.2024041131

Influenza Surveillance Under the Shadow of COVID-19 Pandemic: Phylogenetic Analysis of Influenza A (H1N1)pdm09 Viruses in Türkiye in 2019-20 Season

COVID-19 Pandemisinin Gölgesinde İnfluenza Sürveyansı: Türkiye'de 2019-20 Sezonunda İnfluenza A (H1N1)pdm09 Virüslerinin Filogenetik Analizi

Ahmet Görkem ER[1,2*](İD), Cankut ÇUBUK[3,4*](İD), Mine DURUSU TANRIÖVER[5](İD), Ayşe Tülay BAĞCI BOSİ[6](İD), Can HOLYAVKİN[7](İD), Lale ÖZIŞIK[5](İD), Mehmet ÇAKMAK[5](İD), Oğuz Abdullah UYAROĞLU[5](İD), Ateş KARA[8](İD), Pembe Derin OYGAR[8](İD), Sibel LAÇİNEL GÜRLEVİK[8](İD), Özlem GÜZEL TUNÇCAN[9](İD), Fatma Zehra ÖZCAN[9](İD), Ergin ÇİFTÇİ[10](İD), Erdal İNCE[10](İD), Halil ÖZDEMİR[10](İD), Gül ARGA[10](İD), Fatma Şebnem ERDİNÇ[11](İD), Metin ÖZSOY[11](İD), Alpay AZAP[12](İD), Melike YILDIZ[12](İD), Serhat ÜNAL[1](İD)

[1] Department of Infectious Diseases and Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Türkiye
[2] Department of Health Informatics, Middle East Technical University, Ankara, Türkiye
[3] Centre for Experimental Medicine and Rheumatology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom
[4] Centre for Translational Bioinformatics, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom
[5] Department of Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, Türkiye
[6] Public Health Institute, Hacettepe University, Ankara, Türkiye
[7] Gen-Era Diagnostics, İstanbul, Türkiye
[8] Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Türkiye
[9] Department of Infectious Diseases and Clinical Microbiology, Gazi University Faculty of Medicine, Ankara, Türkiye
[10] Department of Pediatrics, Ankara University Faculty of Medicine, Ankara, Türkiye
[11] Department of Infectious Diseases and Clinical Microbiology, Ankara Training and Research Hospital, Ankara, Türkiye
[12] Department of Infectious Diseases and Clinical Microbiology, Ankara University Faculty of Medicine, Ankara, Türkiye

*Ahmet Görkem Er and Cankut Çubuk contributed equally to this manuscript.

Cite this article as: *Er AG, Çubuk C, Durusu Tanrıöver M, Bağcı Bosi AT, Holyavkin C, Özışık L, et al. Influenza surveillance under the shadow of COVID-19 pandemic: Phylogenetic analysis of influenza A (H1N1)pdm09 viruses in Türkiye in 2019-20 season. FLORA 2024;29(4):488-501.*

ABSTRACT

Introduction: *Influenza is a severe health problem with a massive burden of disease mainly affecting older adults, young children, and people with multimorbidity. Global Influenza Hospital Surveillance Network (GIHSN) is an active hospital surveillance network that generates solid epidemiological and medical evidence to understand influenza severity and related risk factors and support vaccine strain selection. We aimed to analyze the data from the GIHSN Project 2019-20 - Türkiye to link clinical and viral data to infer potential viral genomic risk factors in hospitalized influenza patients and to get an overview of the genetic characterization of circulating influenza viruses in this cohort.*

Materials and Methods: *This epidemiological active surveillance study followed the GIHSN core protocol and was conducted between the 51st week of 2019 and the 18th week of 2020.*

Received/Geliş Tarihi: 28/06/2024 - Accepted/Kabul Ediliş Tarihi: 14/10/2024

©Copyright 2024 by Flora. Available on-line at www.floradergisi.org.

This is an open access article under the Creative Commons AttributionNonCommercial 4.0 International (CC BY-NC 4.0) License.

Available Online Date: 26.12.2024

Results: *Two hundred seventy-three patients were enrolled in the study, and influenza positivity was detected in 84 patients (30.8%). The duration of hospitalization for patients aged five and above was 10.5 ± 1.9 days, whereas it was 7.2 ± 0.6 for patients under five (p= 0.047). There was a significant difference in the duration of hospitalization between patients with at least one comorbidity (12.2 ± 2.4) and those without any comorbidity (5.4 ± 0.6) (p= 0.013). Genetic characterization could be performed in 25 isolated strains, and 23 of them belonged to the 6B.1A.5A and 6B.1A.5a.1 subclades. The HA1 V537A mutation was associated with older age and longer hospital stays, while the H155L and N16S viral mutations were linked to severe disease.*

Conclusion: *Combining viral genomic data with hospital surveillance is essential for determining the risk factors for severe disease, developing vaccination strategies, understanding viral evolution, and establishing an early warning system to improve pandemic preparedness plans.*

Key Words: *Influenza; Surveillance; Viral evolution; Disease severity; Whole genome sequencing*

ÖZ

COVID-19 Pandemisinin Gölgesinde İnfluenza Sürveyansı: Türkiye'de 2019-20 Sezonunda İnfluenza A (H1N1)pdm09 Virüslerinin Filogenetik Analizi

Ahmet Görkem ER[1,2*], Cankut ÇUBUK[3,4*], Mine DURUSU TANRIÖVER[5], Ayşe Tülay BAĞCI BOSİ[6], Can HOLYAVKİN[7], Lale ÖZIŞIK[5], Mehmet ÇAKMAK[5], Oğuz Abdullah UYAROĞLU[5], Ateş KARA[8], Pembe Derin OYGAR[8], Sibel LAÇİNEL GÜRLEVİK[8], Özlem GÜZEL TUNÇCAN[9], Fatma Zehra ÖZCAN[9], Ergin ÇİFTÇİ[10], Erdal İNCE[10], Halil ÖZDEMİR[10], Gül ARGA[10], Fatma Şebnem ERDİNÇ[11], Metin ÖZSOY[11], Alpay AZAP[12], Melike YILDIZ[12], Serhat ÜNAL[1]

[1] Hacettepe Üniversitesi Tıp Fakültesi, İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı, Ankara, Türkiye
[2] Orta Doğu Teknik Üniversitesi, Sağlık Bilişimi Anabilim Dalı, Ankara, Türkiye
[3] William Harvey Araştırma Enstitüsü, Barts ve Londra Tıp ve Diş Hekimliği Fakültesi, Queen Mary Londra Üniversitesi, Deneysel Tıp ve Romatoloji Merkezi, Londra, Birleşik Krallık
[4] Translasyonel Biyoinformatik Merkezi, William Harvey Araştırma Enstitüsü, Barts ve Londra Tıp ve Diş Hekimliği Fakültesi, Queen Mary Üniversitesi, Londra, İngiltere
[5] Hacettepe Üniversitesi Tıp Fakültesi, İç Hastalıkları Anabilim Dalı, Ankara, Türkiye
[6] Hacettepe Üniversitesi, Halk Sağlığı Enstitüsü, Ankara, Türkiye
[7] Gen Era Diagnostik, Biyoinformatik, İstanbul, Türkiye
[8] Hacettepe Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Ankara, Türkiye
[9] Ankara Üniversitesi Tıp Fakültesi, İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı, Ankara, Türkiye
[10] Gazi Üniversitesi Tıp Fakültesi, İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı, Ankara, Türkiye
[11] Ankara Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Ankara, Türkiye
[12] Ankara Eğitim ve Araştırma Hastanesi, İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği, Ankara, Türkiye

Giriş: *İnfluenza, özellikle yaşlı yetişkinleri, küçük çocukları ve multimorbiditesi olan kişileri etkileyen büyük bir hastalık yüküne sahip ciddi bir sağlık sorunudur. Küresel İnfluenza Hastane Sürveyans Ağı (GIHSN), influenza şiddetini ve ilgili risk faktörlerini anlamak ve aşı suşu seçimini desteklemek için epidemiyolojik ve klinik kanıtlar üreten aktif bir hastane sürveyans ağıdır. Bu çalışmada hastanede yatan influenza hastalarında viral genetik risk faktörlerinin ortaya koyulması için klinik ve viral verinin ilişkilendirilmesi ve dolaşan influenza virüslerinin genetik yapıları hakkında bilgi edinilmesi amaçlandı.*

Materyal ve Metod: *Bu epidemiyolojik aktif sürveyans çalışması, GIHSN ana protokolünü takip ederek 2019'un 51. haftası ile 2020'nin 18. haftası arasında yürütülmüştür.*

Bulgular: *İki yüz yetmiş üç hasta çalışmaya dahil edilmiş ve 84 hastada (%30.8) influenza pozitifliği tespit edilmiştir. Beş yaş ve üzeri hastaların hastanede yatış süresi 10.5 ± 1.9 gün, beş yaş altı hastaların 7.2 ± 0.6 gün olarak bulunmuştur (p= 0.047). En az bir komorbiditesi olan hastalar (12.2 ± 2.4) ile herhangi bir komorbiditesi olmayan hastalar (5.4 ± 0.6) arasında hastanede yatış süresi açısından anlamlı farklılık saptanmıştır (p= 0.013). İzole edilen 25 suşun genetik karakterizasyonu yapılabilmiş ve bunların 23'ünün 6B.1A.5A ve 6B.1A.5a.1 alt kümelerine ait olduğu tespit edilmiştir. HA1 V537A mutasyonu ileri yaş ve yatış süresi ile ilişkili bulunurken, H155L ve N16S viral mutasyonları ağır hastalıkla ilişkili bulunmuştur.*

Sonuç: *Viral genomik verilerinin hastane sürveyansı ile birleştirilmesi, şiddetli hastalık için risk faktörlerinin belirlenmesi, aşılama stratejilerinin geliştirilmesi, viral evrimin anlaşılması ve pandemiye hazırlık planlarının iyileştirilmesi açısından çok önemlidir.*

Anahtar Kelimeler: *İnfluenza; Sürveyans; Viral evrim; Hastalık şiddeti; Tüm genom sekansı*

INTRODUCTION

Influenza, a viral illness, can impact individuals with specific risk factors, chronic conditions, and even those in good health. Its significance as a public health concern lies in its association with hospital admissions, workforce depletion, and increased morbidity and mortality[1-3]. Age over 65 years and under two years, immunosuppression, pregnancy and the early postpartum period, morbid obesity, chronic diseases, and being a nursing home resident are risk factors for severe disease[4].

Influenza may present with seasonal epidemics or pandemics, which result in considerable healthcare outcomes and financial burden at micro- and macro-economic levels. Surveillance systems offer valuable insights into the scope and impact of infectious diseases in real-world setting, establishing a foundation for pandemic preparedness and assessing healthcare service demands. Additionally, surveillance can help identify an impending pandemic at an early stage[5].

In the case of influenza, a vaccine-preventable communicable disease, the selection process for seasonal influenza vaccine strains is complex and relies on data from multiple countries and networks that are heavily involved in surveillance efforts. The World Health Organization (WHO) recommends vaccine strains for both hemispheres each influenza season, based on extensive viral genomic information, to make accurate projections of the impact of circulating clades and strains. During the 2019-2020 season, updates were made to the influenza A vaccine components compared to the 2018/19 season. These updates involved shifting from the clade 6B.1 strain to a clade 6B.1A1 strain with A(H1N1)pdm09 (A/Brisbane/02/2018-like), and from the clade 3C.2a1 strain to a clade 3C.3a strain with A(H3N2) (A/Kansas/14/2017-like). There were no changes to the influenza B vaccine components[6].

The COVID-19 pandemic has demonstrated how crucial it is to monitor the genetic epidemiology of a virus in real time, enabling the implementation of public health precautions and the development of effective therapeutics and vaccines. Indeed, before the emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), scientists acknowledged the implementation and importance of easily accessible bioinformatic tools processing raw data from databases that gather sequences from public repositories such as the Global Initiative on Sharing All Influenza Data (GISAID)[7-9]. The initial efforts for viruses were based mainly on influenza viruses to visualize outbreaks in as close to real-time as possible, and these foundations formed the basis of the SARS-CoV-2 surveillance[10]. Gathering the genomic data from active surveillance networks facilitates the interpretation of the viral evolution spatially and temporally while combining the genomic data with clinical data permits the interpretation of severity markers and vaccine failures.

Global Influenza Hospital Surveillance Network (GIHSN) is a unique active hospital surveillance network that generates robust epidemiological and medical evidence to understand influenza severity and related risk factors, as well as to support the vaccine strain selection process[11]. Since the 2012-13 season, Türkiye has participated in the network as a member of GIHSN, offering scientific insights into disease burden, vaccine effectiveness, and factors contributing to severe illness[12,13]. Recently, besides defining basic seasonal influenza epidemiology, the GIHSN shifted its focus to linking genetic, clinical, and epidemiological data in influenza-positive patients using a standard protocol complementary to the WHO Global Influenza Surveillance and Response System (GISRS) with the objectives of defining markers of severity and vaccine failure and supporting international capacities developed through the GISRS of laboratories to increase the availability of clinical information linked with genetic sequencing of influenza strains to expand the support of the biannual vaccine strain selection process of the WHO's formal recommendation for the composition of human influenza vaccines.

In this study, we aimed to analyze the surveillance data from the GIHSN Project 2019-20 - Türkiye to link clinical and viral genomic

data to infer potential viral genomic risk factors in hospitalized influenza patients and to get an overview of the genetic characterization of circulating influenza viruses in this cohort.

MATERIALS and METHODS

Study Design and Setting of the GIHSN Project

This multicentre, prospective, active surveillance, hospital-based epidemiological study adhered to the core protocol established by GIHSN[11]. The project was conducted in Ankara, the capital city of Türkiye, which had a population of 5.5 million people as of 2019, representing approximately 7% of the country's population between the 51st week of 2019 and the 18th week of 2020. Six hospitals in Ankara participated in the surveillance, collectively offering a total bed capacity of 3746 beds, equivalent to 20.4% of all inpatient bed capacity in the city, as per statistics from the Turkish Statistical Institution for 2017. Ethical approval was obtained from the Ethics Committee of Hacettepe University Faculty of Medicine (GO 19/1180). The study consistently followed proper clinical and laboratory protocols throughout its duration.

The study used the standardized GIHSN protocol to recruit participants. Enrolment was based on the following criteria:

- Patients with an acute illness admitted through selected wards (the emergency room, intensive care unit, infectious diseases ward, etc.) within each study hospital were screened.
- Patients who met the criteria for admission based on a predefined set of conditions potentially linked to a recent influenza infection were considered eligible for inclusion in the study.
- The investigator or designated medical staff, such as a resident or attending physician, reviewed hospital admission records, conducted chart reviews, or accessed available medical documentation to identify all eligible patients who had been hospitalized within the past 72 hours and had stayed for at least one night. Subsequently, the patient or their guardian was approached for further consent.
- Patients aged five years and above were enrolled in the study if they had been referred within seven days or less of experiencing influenza-like symptoms, such as fever, cough, headache, myalgia, and sore throat.
- Patients under five years of age were included if their symptoms had begun within seven days or less prior to hospital admission.

Patients meeting the eligibility criteria and providing consent were enlisted for additional assessment. Relevant clinical details were obtained via in-person interviews, as well as through the examination of accessible clinical records.

Following the core protocol, two swabs were collected from each patient. This included a nasopharyngeal swab for all patients and either a pharyngeal swab (for adults aged 14 years and above) or a nasal sample/nasal aspirate (for children under 14 years old), provided they met the inclusion criteria and consented to participation.

Laboratory Procedures of the GIHSN Project

EZ1 virus mini kit v2.0 (Catalog number: 955134, Qiagen, Germany) was utilized for total nucleic acid extraction. Real-time polymerase chain reaction (PCR)-based, multiplex FTD FLU/HRSV (Fast track diagnostics/catalog no. FTD-48-64) was used to detect influenza on Qiagen Rotor gene-Q (Qiagen). Influenza-positive samples were genotyped using the GISAID Protocol outlined below.

The concentration and quality-control of the RNA samples were determined spectrophotometrically (Nanodrop 2000, Thermo Scientific, USA) and fluorometrically (Qubit v3.0, Thermo Fisher, USA). The RNA samples were reverse transcribed with the primers specific to influenza A and B strains determined by Zhou et al.[14]. The primers are indicated in Table 1. For RT-PCR, 4 µl of primer-mix, 4 µl 5X PrimeScript Buffer, 1 µl Takara PrimeScript™ High Fidelity RTase, 0.5 µl RNase Inhibitor (40 U/µL), and 5 µl RNase Free dH_2O were used. PCR was performed on the Bio-Rad Thermal Cycler T100 (USA).

Table 1. The primers used for targeted reverse transcription

MBTuni-12	ACG CGT GAT CAG CAA AAG CAG
MBTuni-13	ACG CGT GAT CAG TAG AAA CAA G
B-PBs-UniF	GGG GGG AGC AGA AGC GGAG
B-PBs-UniR	CCG GGT TAT TAG TAG AAA CAC GAG
B-PA-UniF	GGG GGG AGC AGA AGC GGT G
B-PA-UniR	CCG GGT TAT TAG TAG AAA CAC GTG
B-HANA-UniF	GGG GGG AGC AGA AGC AGA G
B-HANA-UniR	CCG GGT TAT TAG TAG TAA CAA GAG
B-NP-UniF	GGG GGG AGC AGA AGC ACA G
B-NP-UniR	CCG GGT TAT TAG TAG AAA CAA CAG
B-M-Uni3F	GGG GGG AGC AGA AGC ACG CAC T
B-Mg-Uni3F	GGG GGG AGC AGA AGC AGG CAC T
B-M-Uni3R	CCG GGT TAT TAG TAG AAA CAA CGC ACT
B-NS-Uni3F	GGG GGG AGC AGA AGC AGA GGA T
B-NS-Uni3R	CCG GGT TAT TAG TAG TAA CAA GAG GAT

Whole Genome Sequencing and Data Processing

The libraries were prepared from pooled amplicons using the Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA) and sequenced on the Illumina Nextseq 500 (Illumina, USA) platform using a 2 x 150-base-pair (BP) paired-end configuration. After sequencing, the analysis's first step was removing host-derived reads. In this critical step, we used BMTagger (v3.101.5) with the human reference genome (RefSeq assembly accession: GCF_000001405.13) to remove human genomic reads from shotgun viral genome sequencing data[15]. Next, the reads were subjected to quality control. The raw data was evaluated using FastQC (v0.11.5)[16]. Low-quality bases (Phred> 30), adapter sequences, and short reads (sequence length< 30 bp) were trimmed using Trim Galore (v0.6.5)[17]. The remaining reads were first aligned to hemagglutinin (HA) sequences of the following reference strains: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), B/Malaysia/2506/2004 (influenza B), and B/Florida/4/2006 (influenza B) using the Burrows-Wheeler Alignment Tool (v0.7.1) with mem algorithm (bwa mem)[18]. Then, each sample was associated with the strain with the highest read counts on the HA gene. For this study, only H1N1 strains were retained for the subsequent steps. Once H1N1 samples were chosen, their sequences were aligned to the previously assembled sequence of the influenza A genome using the bwa mem tool. Subsequently, the Naive Variant Caller tool (v0.0.4) was employed to identify variants[19]. Variants with low quality and a low variant fraction were excluded. The remaining variants, along with the reference influenza genome, were utilized to produce consensus sequences using BCFtools (v1.9). Then, they were submitted to the GISAID database[20].

Construction of Phylogenetic Tree

The 12 global reference strains, including five vaccine viruses recommended by WHO for the northern hemisphere 2019-2020 and 2020-2021 influenza seasons and for the southern hemisphere 2020-2021 season, were used in our study. To provide global and local evolutionary background in the phylogenetic tree, 40 samples from 19 different locations outside Türkiye and 17 strains from seven different locations in Türkiye were also incorporated into the analysis. All these background samples had collection dates between January 2019 and March 2020 and had similar lengths of HA segments. Protein sequences of the hemagglutinin segments for all the samples mentioned above were retrieved from the GISAID database (gisaid.org). Supplementary Table 1

shows the details of the 94 samples analyzed in this study. Multiple sequence alignment was guided by MAFFT software (v7.48), and the alignment output was used in the phylogenetic tree construction step[21]. The construction of the tree involved utilizing an optimized substitution model (FLU+G4), selected based on the lowest Bayesian Information Criterion (BIC) score determined by the ModelFinder approach. Subsequently, an ultrafast bootstrap analysis (1000 replicates) was conducted using the UFBoot2 algorithm within the IQ-TREE software (v2.2.2.6)[22-24]. The consensus tree was annotated and visualized using the R packages ape (v5.5) and ggplot2 (v3.4.0)[25,26].

Statistical Analysis

Descriptive statistics were used to calculate the frequency and percentage distributions. Continuous variables with a normal distribution were defined using the mean and standard error. When necessary, differences between groups were assessed for statistical significance using a t-test for normally distributed continuous variables, a Mann-Whitney U-Wilcoxon test for non-normally distributed variables, and a Chi-square test for categorical variables. A Type I error rate of $\alpha = 0.05$ was defined. Statistical analyses were conducted using the IBM SPSS Statistics v22.0.

RESULTS

Characteristics and the Outcomes of the Patients in the GIHSN 2019-20- Türkiye Cohort

The project was run between the 51st week of 2019 and the 18th week of 2020. Two hundred seventy-three patients were enrolled and swabbed in the 2019-2020 season GIHSN project - Türkiye. Patients aged five years and above comprised 71.1% of the study population. The patient cohort exhibited a significant prevalence of chronic diseases; 147 (75.8%) patients greater than five had at least one chronic condition, with cardiovascular disease being the most prevalent, followed by chronic obstructive lung disease (Table 2). There were two pregnant patients in the study. Overall, the vaccination coverage rate for the 2019-20 season among patients was 9.5%.

The average duration of hospitalization for patients aged five years and above was 10.5 ± 1.9 days, while it was 7.2 ± 0.6 days for patients under five years old (p= 0.047). A notable contrast emerged in the mean hospitalization stay between patients with at least one comorbidity (12.2 ± 2.4 days) compared to those without any comorbidity (5.4 ± 0.6 days) (p= 0.013). No patients under five had been admitted to the ICU, required mechanical ventilation, or died during hospitalization. On the other hand, among the patients five years and older, 36 (18.6%) required mechanical ventilation, 37 (19.1%) were admitted to the ICU, and 18 (9.3%) died during that hospitalization episode.

Influenza Epidemiology Among the Patients in the GIHSN 2019-20- Türkiye Cohort

Influenza positivity was detected in 84 patients (30.8%), and 61 (72.6%) of these were five years and older. Influenza A was the dominant strain (n= 75, 89.3%). The weekly epidemiology of the influenza viruses among the GIHSN cohort is demonstrated in Figure 1. There was no sample between March 16 and April 13, 2020, due to the COVID-19 pandemic. After April 13, 2020, no positive cases were detected.

Genetic Characterization

Genetic characterization could be performed in 25 isolated strains. Analysis of the phylogenetic tree revealed that the viruses circulating during the 2019-2020 influenza season in Türkiye belonged to clade 6B.1A, characterized by the amino acid substitution S200P in HA1. Among these strains, 23 (92%) of the H1N1 variants fell into subclades 6B.1A.5a and 6B.1A.5a.1, which exhibit HA gene mutations resulting in amino acid substitutions HA1 T202I, N146D, and N277D. Within subclade 6B.1A.5a, there are two distinct groups distinguished by an additional HA1 amino acid substitution, V537A. Subclade 6B.1A.5a.1 viruses possess HA gene mutations encoding HA1 D204A and Q206E amino acid substitutions. Furthermore, specific HA1 amino acid substitutions, H155L and N16S, define distinct clusters of viruses within subclade 6B.1A.5a.1. Consensus tree annotation and visualization are demonstrated in Figure 2.

Table 2. Patient characteristics and comorbidities in the GIHSN 2019-20- Türkiye cohort

	Number of Patients (%)		
	Five years and older n= 194	Under five years n= 79	Total n= 273
Gender (female)	90 (46.4)	37 (46.8)	127 (46.5)
At least one chronic condition	147 (75.8)	26 (32.9)	173 (63.4)
Underlying Diseases			
Cardiovascular disease	91 (46.9)	3 (3.8)	94 (25.7)
Chronic obstructive pulmonary disease	45 (23.2)	0	45 (16.5)
Asthma	25 (12.9)	2 (2.5)	27 (9.9)
Diabetes mellitus	40 (20.6)	0	40 (14.7)
Immunodeficiency/transplant	6 (3.1)	4 (5.1)	10 (3.7)
Renal insufficiency	14 (7.2)	0	14 (5.1)
Rheumatologic/autoimmune disease	4 (2.1)	1 (1.2)	5 (1.8)
Neurological/neuromuscular disease	19 (9.8)	10 (12.6)	29 (10.6)
Cirrhosis/liver disease	4 (2.1)	0	4 (1.5)
Malignancy (active)	15 (7.7)	1 (1.2)	16 (5.9)
Obesity	3 (1.5)	0	3 (1.1)
Active tuberculosis	0	0	0
HIV infection	0	0	0
Leukemia	1 (0.5)	0	1 (0.1)
Other	15 (7.7)	8 (10.1)	23 (8.4)
Influenza Vaccine Status			
Flu vaccine 2019-20	23 (11.9) *	3 (3.8) *	26 (9.5)
Flu vaccine 2019-20, 14 days before the symptoms	17 (8.8) **	2 (2.5) **	19 (7.0)

One patient may have more than one chronic condition.
*Six patients in the five years and older group and two patients under the five years group were influenza positive.
** Five patients in the five years and older group and two patients under the five years group were influenza positive.

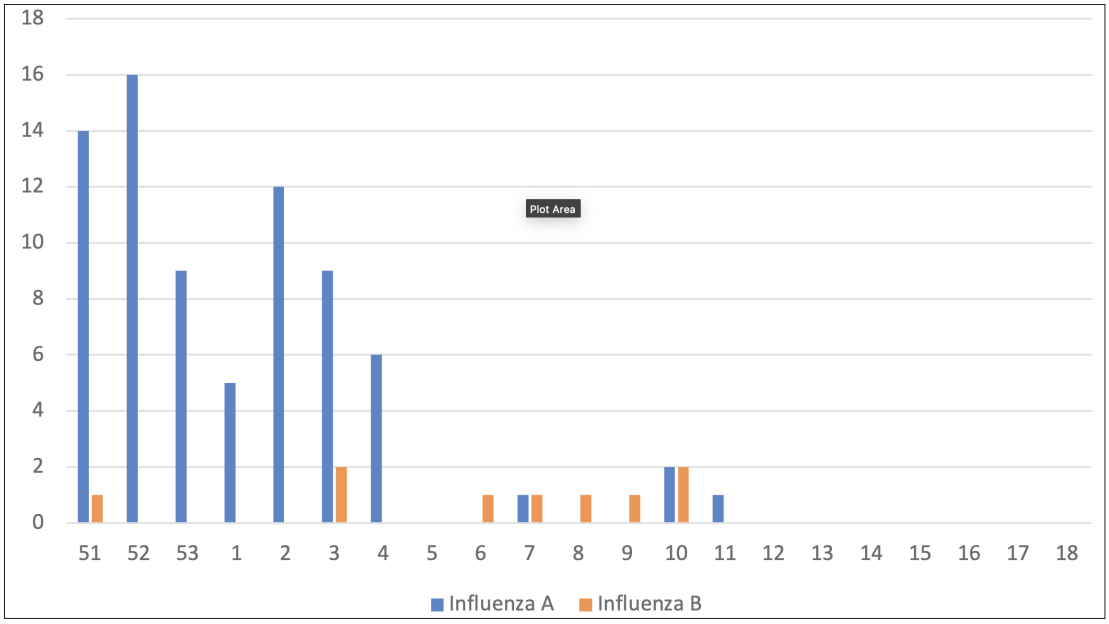

Figure 1. Weekly distribution of the number of influenza viruses during the study period.

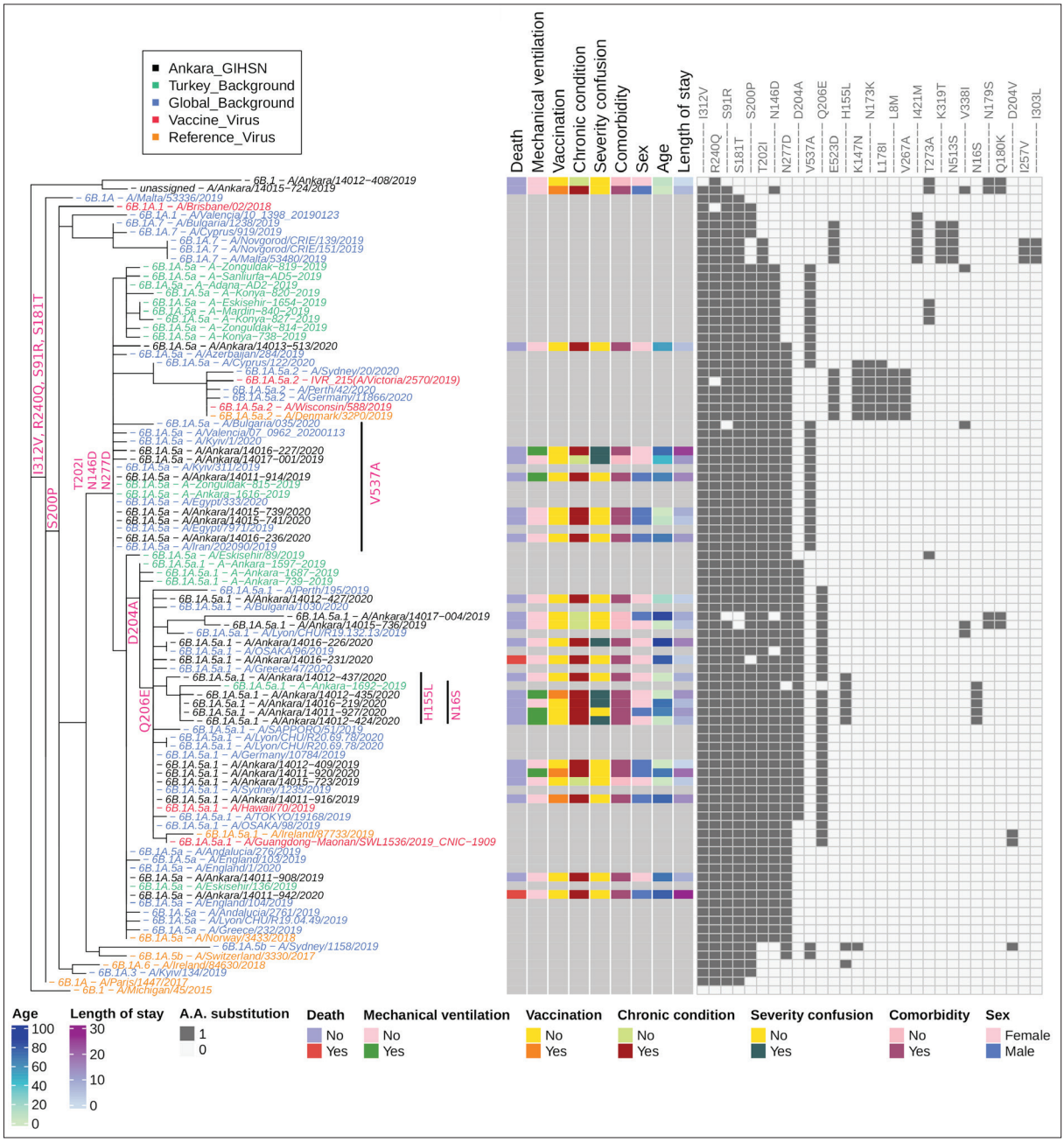

Figure 2. The left side of the figure shows the phylogenetic comparison of influenza A(H1N1)pdm09 hemagglutinin (HA) genes. The right side of the figure shows amino acid substitutions in matrix form, in which the presence of substitutions is shown in dark grey. The upper track of the matrix shows amino acid substitutions in the HA, and the side panel shows the clinical data: death, mechanical ventilation, vaccination (Vaccination 14 days before influenza-like illness symptoms), chronic condition, confusion, comorbidity, sex, age and length of stay.

Association of Influenza Subclades with Clinical Endpoints

Some of these substitutions were significantly associated with clinical endpoints. For instance, older patients tended to be infected with 6B.1A.5a, which carries V537A. This variant was also found to be significantly associated with a longer hospital stay. However, this was mainly driven by the age factor (Figure 3A).

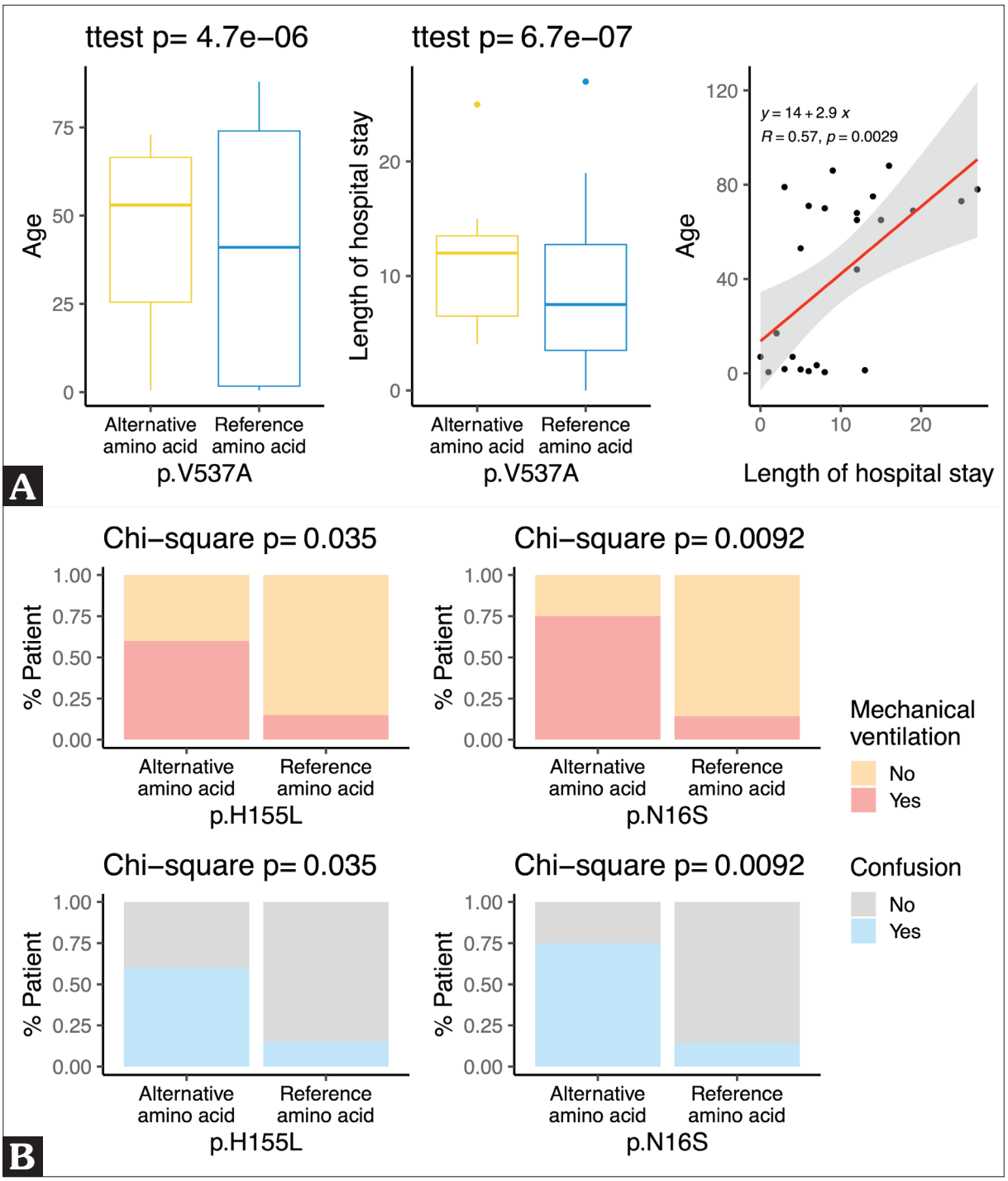

Figure 3. A. compares age and length of hospitalization in the presence of HA V537A amino acid substitution. **B.** shows the percentage of patients with mechanical ventilation and/or confusion in the presence of HA H155L and N16S amino acid substitutions.

Similarly, 6B.1A.5a.1 with H155L (n= 3, 60% vs. n= 2, 11%) and N16S (n= 3, 75% vs. n= 1, 5%) amino acid substitutions were higher in patients who underwent mechanical ventilation in the intensive care unit and/or had confusion (Figure 2, 3B).

DISCUSSION

The GIHSN study in Türkiye was conducted under the effect of the COVID-19 pandemic during the 2019-20 influenza season. We enrolled 273 hospitalized patients, among whom 84 (30.8%) demonstrated influenza positivity with molecular testing. Shortly after the WHO declared the pandemic, the first COVID-19 case in Türkiye was diagnosed on March 11, 2020, prompting hospitals to activate their pandemic plans. The organization of the hospitals and patient flow algorithms have dramatically changed, precluding the execution of the active surveillance methodology. Staff who ran the surveillance and did the swabbing were recruited for COVID-19 wards and the emergency department. The screened wards were either closed or converted to COVID-19 wards in some hospitals; in others, patients with ILI were hospitalized in the isolation wards, which were not among the wards to be screened. There was no samples between March 16th and April 13th, 2020, due to the COVID-19 pandemic. As new patients were enrolled after April 13, there were no new cases of influenza, likely due to the early end of the influenza season, attributed to COVID-19 mitigation measures and lockdowns, which reduced healthcare-seeking behavior among patients with ILI.

The 2019-20 season was the first season when the GIHSN project in Türkiye included sequencing influenza-positive samples for genetic characterization. We have commenced a methodology to establish phylogenetic trees of the hemagglutinin and neuraminidase genes of the influenza A viruses in circulation. Since pathogen genomics is reshaping the public health perception of infectious diseases, integrating clinical and genomic viral surveillance data can provide opportunities for improving the influenza surveillance[27,28]. Genomic characterization of pathogens through next-generation sequencing platform is used mainly in three domains: 1) to demonstrate pathogen evolution through space and time; 2) to characterize genetic markers such as virulence determinants and to monitor the emergence and spread of gain-of-function variants; 3) to contribute data to public databases for real-time surveillance and research[29]. Moreover, a "sequence-first" approach enables a faster and more informative sequencing and vaccine production process for influenza viruses[14]. The genomic surveillance data are reviewed during the biannual influenza vaccine strain selection consultations held by the WHO to more accurately select the clades included as vaccine components[30].

Here, we report the genomic characterization of influenza A viruses with regard to their HA proteins. The current influenza vaccines primarily provide protection by stimulating the production of neutralizing antibodies against HA[31]. Hence, vaccine efficacy primarily relies on the match of the antigenic properties of the HA of the vaccine and the circulating strains in that particular influenza season. The genomic surveillance of influenza viruses focuses on the antigenic properties of the HA protein to update the components in the vaccine[32]. On the other hand, neuraminidase (NA) has been gaining attention as a new potential vaccine component to induce better and cross-reacting immunity[33]. Research has demonstrated that higher NA inhibition titers correlate with milder illness and reduced viral shedding in individuals with influenza[34]. As a result, NA is considered a candidate for developing universal influenza vaccines, which offer better protection for matching strains and subtype-specific cross-protection[35].

Phylogenetic analysis revealed that out of 25 isolated strains, 23 belonged to 6B.1A.5A and 6B.1A.5a.1 subclades in the study population. None of the strains belonged to 6B.1A.1, the vaccine strain for influenza A (H1N1)pdm09 in the Northern Hemisphere during the study period. This pattern was observed in other locations as well. According to the European Centre for Disease Prevention and Control (ECDC) Influenza Characterization Surveillance Report published in April 2020, among the influenza A (H1N1) pdm09 samples collected from week 40/2019, clade 6B.1A.5A was observed as the dominant subclade in the WHO European Region[36]. Among specimen collections dated from week 44/2019 to 5/2020 in Canada, 245 out of 287 strains (85%) were subclade 6B.1A.5a[37].

As is known, mutations in the viral genome can affect virulence, the immune system's effectiveness against the virus, and drug resistance; therefore, they should be closely monitored[38]. For instance, the HA1 D222G mutation seen in the Norwegian population during the 2019 - 2020 Season was found to be highly prevalent in fatal and severe cases[39]. In our study, V537A was related to older age and length of stay. H155L and N16S were also found to be related to severe disease. These differences were statistically significant, suggesting a possible causal relationship between these mutations and relevant outcomes. Given that older age is associated with impaired immune response, which can lead to higher viral loads, age has the potential to influence viral evolution[40]. Conversely, elderly individuals living collectively in nursing homes may also experience an outbreak among themselves. Therefore, it is necessary to design large studies that account for social factors and further investigate the associations between age and viral mutations.

There are significant limitations in this study. First, viral genomic data could be extracted from relatively few patients. In addition, while linking viral mutations with clinical outcomes, more extensive data sets are required for more generalizable interpretations, and epidemiological relationships should be considered in detail. Moreover, since patients with mild symptoms are less likely to be admitted to the hospital, this may lead to selection bias when comparing patient groups based on disease severity. The low vaccination rate of the study cohort also prevented us from examining survival outcomes with vaccine strain mismatch. Furthermore, the emergence of the COVID-19 pandemic has led to changes in screening procedures in some healthcare facilities during the study period.

CONCLUSION

In conclusion, influenza is a severe health problem with a huge burden of disease mainly affecting older adults, young children, and people with multimorbidity. Combining viral genomic data with hospital surveillance is essential for determining the risk factors for severe disease, developing vaccination strategies, understanding the viral evolution, and establishing an early warning system to improve pandemic preparedness plans.

Acknowledgment

We extend our sincere gratitude to all the data contributors involved in this research, including the authors, for their efforts in screening and enrolling patients, as well as obtaining specimens, and to the submitting laboratories for generating the genetic sequence and metadata and for sharing this data via the GISAID Initiative. Special thanks are due to Mrs. Esra Öner for her generous hospitality and assistance during the project. This research was funded by the Foundation for Influenza Epidemiology and The Turkish Society of Internal Medicine.

ETHICS COMMITTEE APPROVAL

This study was approved by the Hacettepe University Non-Invasive Clinical Research Ethics Committee (Decision no: 2019/29-39, Date: 17.12.2019).

CONFLICT of INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: All of authors

Analysis/Interpretation: AGE, CÇ, MDT, ATBB, CH, SÜ

Data Collection or Processing: MDT, SÜ, LÖ, MÇ, OAU, AK, PDO, SLG, ÖGT, FZÖ, EÇ, Eİ, HÖ, GA, FŞE, MÖ, AA, MY

Writing: AGE, CÇ, MDT, SÜ

Review and Correction: AGE, CÇ, MDT, ATBB, CH, SÜ

Final Approval: All of authors

REFERENCES

1. *Paget J, Spreeuwenberg P, Charu V, Taylor RJ, Iuliano AD, Bresee J, et al. Global mortality associated with seasonal influenza epidemics: New burden estimates and predictors from the GLaMOR Project. J Glob Health 2019;9:020421. https://doi.org/10.7189/jogh.09.020421*
2. *Molinari NA, Ortega-Sanchez IR, Messonnier ML, Thompson WW, Wortley PM, Weintraub E, et al. The annual impact of seasonal influenza in the US: Measuring disease burden and costs. Vaccine 2007;25:5086-96. https://doi.org/10.1016/j.vaccine.2007.03.046*

3. *Lina B, Georges A, Burtseva E, Nunes MC, Andrew MK, McNeil SA, et al. Complicated hospitalization due to influenza: Results from the Global Hospital Influenza Network for the 2017-2018 season. BMC Infect Dis 2020;20:465. https://doi.org/10.1186/s12879-020-05167-4*
4. *Solomon DA, Sherman AC, Kanjilal S. Influenza in the COVID-19 era. JAMA 2020;324:1342-3. https://doi.org/10.1001/jama.2020.14661*
5. *Smith PF, Hadler JL, Stanbury M, Rolfs RT, Hopkins RS; C.S.S. Group. "Blueprint version 2.0": Updating public health surveillance for the 21st century. J Public Health Manag Pract 2013;19:231-9. https://doi.org/10.1097/PHH.0b013e318262906e*
6. *World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2021-2022 northern hemisphere influenza season. Available from: https://www.who.int/publications/i/item/recommended-composition-of-influenza-virus-vaccines-for-use-in-the-2021-2022-northern-hemisphere-influenza-season (Accessed date: 02.07.2023).*
7. *Elbe S, Buckland-Merrett G. Data, disease and diplomacy: GISAID's innovative contribution to global health. Glob Chall 2017;1:33-46. https://doi.org/10.1002/gch2.1018*
8. *Letunic I, Bork P. Interactive tree of life (iTOL) v3: An online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res 2016;44:W242-5. https://doi.org/10.1093/nar/gkw290*
9. *Argimon S, Abudahab K, Goater RJE, Fedosejev A, Bhai J, Glasner C, et al. Microreact: Visualizing and sharing data for genomic epidemiology and phylogeography. Microb Genom 2016;2:e000093. https://doi.org/10.1099/mgen.0.000093*
10. *Neher RA, Bedford T. Nextflu: Real-time tracking of seasonal influenza virus evolution in humans. Bioinformatics 2015;31:3546-8. https://doi.org/10.1093/bioinformatics/btv381*
11. *Global Influenza Hospital Surveillance Network. Protocol and questionnaires. Available from: https://gihsn.org/protocol-and-questionnaires/ (Accessed date: 06.07.2023).*
12. *Puig-Barbera J, Tormos A, Sominina A, Burtseva E, Launay O, Ciblak MA, et al. First-year results of the Global Influenza Hospital Surveillance Network: 2012-2013 Northern hemisphere influenza season. BMC Public Health 2014;14:564. https://doi.org/10.1186/1471-2458-14-564*
13. *Tanriover MD, Bagci Bosi T, Ozisik L, Bilgin E, Güzel Tunçcan Ö, Özgen Ö, et al. Poor outcomes among elderly patients hospitalized for influenza-like illness. Curr Med Res Opin 2018;34:1201-7. https://doi.org/10.1080/03007995.2017.1381078*
14. *Zhou B, Donnelly ME, Scholes DT, St George K, Hatta M, Kawaoka Y, et al. Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza a viruses. J Virol 2009;83:10309-13. https://doi.org/10.1128/JVI.01109-09*
15. *Rotmistrovsky K, Agarwala R. BMTagger: Best Match Tagger for removing human reads from metagenomics datasets. NCBI/NLM, National Institutes of Health, 2011.*
16. *Andrews S. Fast QC: A quality control tool for high throughput sequence data. 2010, Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.*
17. *Krueger F. Trim galore. A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files, 2015;516.*
18. *Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754-60. https://doi.org/10.1093/bioinformatics/btp324*
19. *Blankenberg D, Von Kuster G, Bouvier E, Baker D, Afgan E, Stoler N, et al. Dissemination of scientific software with Galaxy ToolShed. Genome Biol 2014;15:403. https://doi.org/10.1186/gb4161*
20. *Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. GigaScience 2021;10. https://doi.org/10.1093/gigascience/giab008*
21. *Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol 2013;30:772-80. https://doi.org/10.1093/molbev/mst010*
22. *Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat Methods 2017;14:587-9. https://doi.org/10.1038/nmeth.4285*
23. *Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: Improving the ultrafast bootstrap approximation. Mol Biol Evol 2018;35:518-22. https://doi.org/10.1093/molbev/msx281*
24. *Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 2020;37:1530-4. https://doi.org/10.1093/molbev/msaa015*
25. *Paradis E, Schliep K. ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 2019;35:526-8. https://doi.org/10.1093/bioinformatics/bty633*
26. *Wickham H. Data analysis, in ggplot2: Elegant graphics for data analysis. 2016, Springer International Publishing: Cham 189-201. https://doi.org/10.1007/978-3-319-24277-4_9*
27. *Armstrong GL, MacCannell DR, Taylor J, Carleton HA, Neuhaus EB, Bradbury RS, et al. Pathogen genomics in public health. N Engl J Med 2019;381:2569-80. https://doi.org/10.1056/NEJMsr1813907*
28. *Goldstein EJ, Harvey WT, Wilkie GS, Shepherd SJ, MacLean AR, Murcia PR, et al. Integrating patient and whole-genome sequencing data to provide insights into the epidemiology of seasonal influenza A(H3N2) viruses. Microb Genom 2018;4. https://doi.org/10.1099/mgen.0.000137*

29. *Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: Real-time tracking of pathogen evolution. Bioinformatics 2018;34:4121-3. https://doi.org/10.1093/bioinformatics/bty407*

30. *Hampson A, Barr I, Cox N, Donis RO, Siddhivinayak H, Jernigan D, et al. Improving the selection and development of influenza vaccine viruses - Report of a WHO informal consultation on improving influenza vaccine virus selection, Hong Kong SAR, China, 18-20 November 2015. Vaccine 2017;35:1104-9. https://doi.org/10.1016/j.vaccine.2017.01.018*

31. *Vogel OA, Manicassamy B. Broadly protective strategies against influenza viruses: Universal vaccines and therapeutics. Front Microbiol 2020;11:135. https://doi.org/10.3389/fmicb.2020.00135*

32. *European Centre for Disease Prevention and Control, Influenza virus characterisation, summary Europe, December 2015. 2016: Stockholm: ECDC.*

33. *Krammer F, Fouchier RAM, Eichelberger MC, Webby RJ, Shaw-Saliba K, Wan H, et al. NAction! How can neuraminidase-based immunity contribute to better influenza virus Vaccines? mBio 2018;9:e02332-17. https://doi.org/10.1128/mBio.02332-17*

34. *Couch RB, Atmar RL, Franco LM, Quarles JM, Wells J, Arden N, et al. Antibody correlates and predictors of immunity to naturally occurring influenza in humans and the importance of antibody to the neuraminidase. J Infect Dis 2013;207:974-81. https://doi.org/10.1093/infdis/jis935*

35. *Deng X, Wang Q, Liu M, Zheng Q, Wu F, Huang J. Tetrameric neuraminidase of influenza a virus is required to induce protective antibody responses in mice. Front Microbiol 2021;12:729914. https://doi.org/10.3389/fmicb.2021.729914*

36. *European Centre for Disease Prevention and Control, Influenza virus characterisation, summary Europe, April 2020. 2020: Stockholm: ECDC.*

37. *Skowronski DM, Zou M, Sabaiduc S, Murti M, Olsha R, Dickinson JA, et al. Interim estimates of 2019/20 vaccine effectiveness during early-season co-circulation of influenza A and B viruses, Canada, February 2020. Euro Surveill 2020;25. https://doi.org/10.2807/1560-7917.ES.2020.25.7.2000103*

38. *Shao W Li X, Goraya MU, Wang S, Chen JL. Evolution of influenza A virus by mutation and re-assortment. Int J Mol Sci 2017;18. https://doi.org/10.3390/ijms18081650*

39. *Kilander A, Rykkvin R, Dudman SG, Hungnes O. Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009-2010. Euro Surveill 2010;15. https://doi.org/10.2807/ese.15.09.19498-en*

40. *Sanz-Muñoz I, Sánchez-Martínez J, Rodríguez-Crespo C, Arroyo-Hernantes I, Domínguez-Gil M,. Rojo-Rello S, et al. Association of viral loads of influenza A (H3N2) with age and care setting on presentation-a prospective study during the 2022-2023 influenza season in Spain. Int J Infect Dis 2024;143:107034. https://doi.org/10.1016/j.ijid.2024.107034*

Address for Correspondence/Yazışma Adresi

Dr. Ahmet Görkem ER

Department of Infectious Diseases and Clinical Microbiology,
Hacettepe University Faculty of Medicine,
Ankara, Türkiye

E-posta: ahmetgorkemer@gmail.com

Supplementary_Table_GISAID

Isolate ID	Isolate name	Type	Lineage	Collection date	Originating lab	Segment accession number	Clade GISAID	City	Phylogenetic category
EPI_ISL_205499	A/Michigan/45/2015	A_/_H1N1	pdm09	2015-09-07	Michigan_Department_of_Community_Health	EPI685579	6B.1	Michigan	Reference_Virus
EPI_ISL_284660	A/Paris/1447/2017	A_/_H1N1	pdm09	2017-10-20		EPI1098411	6B.1A	Paris	Reference_Virus
EPI_ISL_338060	A/Ireland/84630/2018	A_/_H1N1	pdm09	2018-11-28	UCD_National_Virus_Reference_Laboratory	EPI1354717	6B.1A.6	Ireland	Reference_Virus
EPI_ISL_315811	A/Switzerland/3330/2017	A_/_H1N1	pdm09	2017-12-20	National_Institute_for_Medical_Research	EPI1259401	6B.1A.5b	Switzerland	Reference_Virus
EPI_ISL_391292	A/Norway/3433/2018	A_/_H1N1	pdm09	2018-10-30	National_Institute_for_Medical_Research	EPI1592753	6B.1A.5a	Norway	Reference_Virus
EPI_ISL_424676	A/Ireland/87733/2019	A_/_H1N1	pdm09	2020-01-01	National_Institute_for_Medical_Research	EPI1725283	6B.1A.5a.1	Ireland	Reference_Virus
EPI_ISL_400732	A/Denmark/3280/2019	A_/_H1N1	pdm09	2019-11-10	Statens_Serum_Institute	EPI1641015	6B.1A.5a.2	Denmark	Reference_Virus
EPI_ISL_344858	A/Brisbane/02/2018	A_/_H1N1	pdm09	2018-01-04	WHO_Collaborating_Centre_for_Reference_and_Research_on_Influenza	EPI1383389	6B.1.1	Brisbane	Vaccine_Virus
EPI_ISL_416759	A/Guangdong-Maonan/SWL1536/2019_CNIC-1909	A_/_H1N1	pdm09	2019-06-17	Guangdong_Provincial_Center_for_Disease_Control_and_Prevention	EPI1716630	6B.1A.5a.1	Guangdong	Vaccine_Virus
EPI_ISL_397028	A/Hawaii/70/2019	A_/_H1N1	pdm09	2019-10-05	State_of_Hawaii_Department_of_Health	EPI1617983	6B.1A.5a.1	Hawaii	Vaccine_Virus
EPI_ISL_404527	A/Wisconsin/588/2019	A_/_H1N1	pdm09	2019-12-19	Wisconsin_State_Laboratory_of_Hygiene	EPI1661758	6B.1A.5a.2	Wisconsin	Vaccine_Virus
EPI_ISL_459907	IVR_215(A/Victoria/2570/2019)	A_/_H1N1	pdm09	2019-01-01	CSL_Ltd	EPI1741926	6B.1A.5a.2	Victoria	Vaccine_Virus
EPI_ISL_341860	A/Greece/232/2019	A_/_H1N1	pdm09	2019-01-21	Hellenic_Pasteur_Institute	EPI1371231	6B.1A.5a	Greece	Global_Background
EPI_ISL_347665	A/Malta/53336/2019	A_/_H1N1	pdm09	2019-01-21	Mater_Dei_Hospital	EPI1393991	6B.1A	Malta	Global_Background
EPI_ISL_347669	A/Malta/53480/2019	A_/_H1N1	pdm09	2019-01-23	Mater_Dei_Hospital	EPI1393999	6B.1A.7	Malta	Global_Background
EPI_ISL_361881	A/Cyprus/919/2019	A_/_H1N1	pdm09	2019-02-13	Nicosia_General_Hospital	EPI1478269	6B.1A.7	Cyprus	Global_Background
EPI_ISL_367879	A/Bulgaria/1238/2019	A_/_H1N1	pdm09	2019-03-05	National_Centre_of_Infectious_and	EPI1506729	6B.1A.7	Bulgaria	Global_Background
EPI_ISL_367958	A/Kyiv/134/2019	A_/_H1N1	pdm09	2019-02-24	Institute_of_Epidemiology_and_Infectious_Diseases_AMS_of_Ukraine	EPI1506886	6B.1.3	Kyiv	Global_Background
EPI_ISL_403280	A/Iran/202090/2019	A_/_H1N1	pdm09	2019-11-26	Tehran_University_of_Medical_Sciences	EPI1655543	6B.1A.5a	Iran	Global_Background
EPI_ISL_407700	A/Bulgaria/035/2020	A_/_H1N1	pdm09	2020-01-05	National_Centre_of_Infectious_and	EPI1674952	6B.1A.5a	Bulgaria	Global_Background
EPI_ISL_407710	A/Greece/47/2020	A_/_H1N1	pdm09	2020-01-10	Aristotelian_University_of_Thessaloniki	EPI1674972	6B.1A.5a.1	Greece	Global_Background
EPI_ISL_409875	A/Cyprus/122/2020	A_/_H1N1	pdm09	2020-01-09	Nicosia_General_Hospital	EPI1689259	6B.1A.5a	Cyprus	Global_Background
EPI_ISL_409892	A/Kyiv/1/2020	A_/_H1N1	pdm09	2020-01-03	Institute_of_Epidemiology_and_Infectious_Diseases_AMS_of_Ukraine	EPI1689293	6B.1A.5a	Kyiv	Global_Background
EPI_ISL_409893	A/Kyiv/311/2019	A_/_H1N1	pdm09	2019-12-07	Institute_of_Epidemiology_and_Infectious_Diseases_AMS_of_Ukraine	EPI1689295	6B.1A.5a	Kyiv	Global_Background
EPI_ISL_414069	A/Germany/10784/2019	A_/_H1N1	pdm09	2019-11-02	U.S._Air_Force_School_of_Aerospace_Medicine	EPI1706309	6B.1A.5a.1	Germany	Global_Background
EPI_ISL_429886	A/Bulgaria/1030/2020	A_/_H1N1	pdm09	2020-02-19	National_Centre_of_Infectious_and	EPI1734263	6B.1A.5a.1	Bulgaria	Global_Background
EPI_ISL_508512	A/Azerbaijan/284/2019	A_/_H1N1	pdm09	2019-12-24	Republican_Anti-Plague_Station	EPI1794659	6B.1A.5a	Azerbaijan	Global_Background
EPI_ISL_351356	A/England/103/2019	A_/_H1N1	pdm09	2019-01-14	UK_Health_Security_Agency_-_Colindale	EPI1417691	6B.1A.5a	England	Global_Background
EPI_ISL_351389	A/England/104/2019	A_/_H1N1	pdm09	2019-01-16	UK_Health_Security_Agency_-_Colindale	EPI1417825	6B.1A.5a	England	Global_Background
EPI_ISL_355265	A/Andalucia/276/2019	A_/_H1N1	pdm09	2019-01-10	Instituto_de_Salud_Carlos_III	EPI1437109	6B.1A.5a	Andalucia	Global_Background
EPI_ISL_355385	A/Lyon/CHU/R19.04.49/2019	A_/_H1N1	pdm09	2019-01-05	CNR_Virus_des_Infections_Respiratoires_-_France_SUD	EPI1437348	6B.1A.5a	Lyon	Global_Background
EPI_ISL_376064	A/Valencia/10_1398_20190123	A_/_H1N1	pdm09	2019-01-23	Virology_Laboratory,_FISABIO_-_Public_Health,_Generalitat_Valenciana	EPI1536617	6B.1.1	Valencia	Global_Background
EPI_ISL_406228	A/Lyon/CHU/R19.132.13/2019	A_/_H1N1	pdm09	2019-11-23	CNR_Virus_des_Infections_Respiratoires_-_France_SUD	EPI1670630	6B.1A.5a.1	Lyon	Global_Background
EPI_ISL_410368	A/Valencia/07_0962_20200113	A_/_H1N1	pdm09	2020-01-13	Virology_Laboratory,_FISABIO_-_Public_Health,_Generalitat_Valenciana	EPI1691428	6B.1A.5a	Valencia	Global_Background
EPI_ISL_419287	A/Perth/195/2019	A_/_H1N1	pdm09	2019-11-28	Pathwest_QE_II_Medical_Centre	EPI1721596	6B.1A.5a.1	Perth	Global_Background
EPI_ISL_435189	A/Germany/11866/2020	A_/_H1N1	pdm09	2020-02-14	U.S._Air_Force_School_of_Aerospace_Medicine	EPI1735571	6B.1A.5a.2	Germany	Global_Background
EPI_ISL_480478	A/England/1/2020	A_/_H1N1	pdm09	2020-01-02	UK_Health_Security_Agency_-_Colindale	EPI1749820	6B.1A.5a	England	Global_Background
EPI_ISL_498367	A/Lyon/CHU/R20.69.78/2020	A_/_H1N1	pdm09	2020-03-09	CNR_Virus_des_Infections_Respiratoires_-_France_SUD	EPI1763726	6B.1A.5a.1	Lyon	Global_Background
EPI_ISL_498368	A/Lyon/CHU/R20.69.78/2020	A_/_H1N1	pdm09	2020-03-09	CNR_Virus_des_Infections_Respiratoires_-_France_SUD	EPI1763728	6B.1A.5a.1	Lyon	Global_Background
EPI_ISL_508510	A/Andalucia/2761/2019	A_/_H1N1	pdm09	2019-11-20	Instituto_de_Salud_Carlos_III	EPI1794655	6B.1A.5a	Andalucia	Global_Background
EPI_ISL_529122	A/Perth/42/2020	A_/_H1N1	pdm09	2020-03-07	Pathwest_QE_II_Medical_Centre	EPI1802028	6B.1A.5a.2	Perth	Global_Background
EPI_ISL_345820	A/Novgorod/CRIE/139/2019	A_/_H1N1	pdm09	2019-01-23	Center_of_Hygiene_and_Epidemiology_in_Novgorod_oblast	EPI1385050	6B.1A.7	Novgorod	Global_Background
EPI_ISL_345895	A/Novgorod/CRIE/151/2019	A_/_H1N1	pdm09	2019-01-22	Center_of_Hygiene_and_Epidemiology_in_Novgorod_oblast	EPI1385148	6B.1A.7	Novgorod	Global_Background
EPI_ISL_392486	A/TOKYO/19168/2019	A_/_H1N1	pdm09	2019-07-24	Tokyo_Metropolitan_Institute_of_Public_Health	EPI1596916	6B.1A.5a.1	TOKYO	Global_Background
EPI_ISL_398297	A/OSAKA/96/2019	A_/_H1N1	pdm09	2019-09-09	Osaka_Prefectural_Institute_of_Public_Health	EPI1627351	6B.1A.5a.1	OSAKA	Global_Background
EPI_ISL_398298	A/OSAKA/98/2019	A_/_H1N1	pdm09	2019-09-30	Osaka_Prefectural_Institute_of_Public_Health	EPI1627353	6B.1A.5a.1	OSAKA	Global_Background
EPI_ISL_400576	A/SAPPORO/51/2019	A_/_H1N1	pdm09	2019-10-07	Sapporo_City_Institute_of_Public_Health	EPI1640069	6B.1A.5a.1	SAPPORO	Global_Background
EPI_ISL_401874	A/Sydney/1235/2019	A_/_H1N1	pdm09	2019-11-14	Institute_of_Medical_and_Veterinary_Science_(IMVS)	EPI1647476	6B.1A.5a.1	Sydney	Global_Background
EPI_ISL_419292	A/Sydney/1158/2019	A_/_H1N1	pdm09	2019-07-24	Institute_of_Medical_and_Veterinary_Science_(IMVS)	EPI1721636	6B.1A.5b	Sydney	Global_Background
EPI_ISL_510039	A/Sydney/20/2020	A_/_H1N1	pdm09	2020-02-09	Clinical_Virology_Unit,_CDIM	EPI1795518	6B.1A.5a.2	Sydney	Global_Background
EPI_ISL_1519440	A/Egypt/7971/2019	A_/_H1N1	pdm09	2019-12-31	Ministry_of_Health_and_Population	EPI1857127	6B.1A.5a	Egypt	Global_Background
EPI_ISL_2932714	A/Egypt/333/2020	A_/_H1N1	pdm09	2020-01-06	Ministry_of_Health_and_Population	EPI1882565	6B.1A.5a	Egypt	Global_Background
EPI_ISL_411901	A/Ankara/14015-723/2019	A_/_H1N1	pdm09	2019-12-24	Turkish_Society_of_Internal_Medicine	EPI1695587	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_413624	A/Ankara/14017-001/2019	A_/_H1N1	pdm09	2019-12-20	Turkish_Society_of_Internal_Medicine	EPI1704284	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_413625	A/Ankara/14017-004/2019	A_/_H1N1	pdm09	2019-12-23	Turkish_Society_of_Internal_Medicine	EPI1704292	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_413626	A/Ankara/14012-409/2019	A_/_H1N1	pdm09	2019-12-25	Turkish_Society_of_Internal_Medicine	EPI1704300	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_413627	A/Ankara/14011-916/2019	A_/_H1N1	pdm09	2019-12-26	Turkish_Society_of_Internal_Medicine	EPI1704309	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_413628	A/Ankara/14011-914/2019	A_/_H1N1	pdm09	2019-12-26	Turkish_Society_of_Internal_Medicine	EPI1704317	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_413629	A/Ankara/14015-724/2019	A_/_H1N1	pdm09	2019-12-26	Turkish_Society_of_Internal_Medicine	EPI1704325	unassigned	Ankara	Ankara_GIHSN
EPI_ISL_413630	A/Ankara/14012-408/2019	A_/_H1N1	pdm09	2019-12-25	Turkish_Society_of_Internal_Medicine	EPI1704333	6B.1	Ankara	Ankara_GIHSN
EPI_ISL_413631	A/Ankara/14011-908/2019	A_/_H1N1	pdm09	2019-12-18	Turkish_Society_of_Internal_Medicine	EPI1704341	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_424765	A/Ankara/14011-942/2020	A_/_H1N1	pdm09	2020-01-16	Turkish_Society_of_Internal_Medicine	EPI1726481	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_424838	A/Ankara/14015-739/2020	A_/_H1N1	pdm09	2020-01-03	Turkish_Society_of_Internal_Medicine	EPI1726537	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_424840	A/Ankara/14015-741/2020	A_/_H1N1	pdm09	2020-01-03	Turkish_Society_of_Internal_Medicine	EPI1726544	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_424921	A/Ankara/14012-424/2020	A_/_H1N1	pdm09	2020-01-02	Turkish_Society_of_Internal_Medicine	EPI1726551	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_424991	A/Ankara/14012-427/2020	A_/_H1N1	pdm09	2020-01-13	Turkish_Society_of_Internal_Medicine	EPI1726570	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_425225	A/Ankara/14016-231/2020	A_/_H1N1	pdm09	2020-01-09	Turkish_Society_of_Internal_Medicine	EPI1727420	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_425226	A/Ankara/14013-513/2020	A_/_H1N1	pdm09	2020-02-12	Turkish_Society_of_Internal_Medicine	EPI1727428	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_425227	A/Ankara/14011-920/2020	A_/_H1N1	pdm09	2020-01-03	Turkish_Society_of_Internal_Medicine	EPI1727433	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_425230	A/Ankara/14016-226/2020	A_/_H1N1	pdm09	2020-01-07	Turkish_Society_of_Internal_Medicine	EPI1727441	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_425372	A/Ankara/14016-236/2020	A_/_H1N1	pdm09	2020-01-15	Turkish_Society_of_Internal_Medicine	EPI1727449	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_425578	A/Ankara/14011-927/2020	A_/_H1N1	pdm09	2020-01-07	Turkish_Society_of_Internal_Medicine	EPI1727457	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_428237	A/Ankara/14012-435/2020	A_/_H1N1	pdm09	2020-01-17	Turkish_Society_of_Internal_Medicine	EPI1731711	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_428246	A/Ankara/14015-736/2019	A_/_H1N1	pdm09	2019-12-31	Turkish_Society_of_Internal_Medicine	EPI1731780	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_428247	A/Ankara/14016-219/2020	A_/_H1N1	pdm09	2020-01-02	Turkish_Society_of_Internal_Medicine	EPI1731786	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_428248	A/Ankara/14016-227/2020	A_/_H1N1	pdm09	2020-01-08	Turkish_Society_of_Internal_Medicine	EPI1731794	6B.1A.5a	Ankara	Ankara_GIHSN
EPI_ISL_428249	A/Ankara/14012-437/2020	A_/_H1N1	pdm09	2020-01-17	Turkish_Society_of_Internal_Medicine	EPI1731802	6B.1A.5a.1	Ankara	Ankara_GIHSN
EPI_ISL_341344	A/Eskisehir/89/2019	A_/_H1N1	pdm09	2019-01-09	Refik_Saydam_National_Public_Health_Agency	EPI1370205	6B.1A.5a	Eskisehir	Turkey_Background
EPI_ISL_341345	A/Eskisehir/136/2019	A_/_H1N1	pdm09	2019-01-14	Refik_Saydam_National_Public_Health_Agency	EPI1370207	6B.1A.5a	Eskisehir	Turkey_Background
EPI_ISL_407643	A-Konya-738-2019	A_/_H1N1	pdm09	2019-12-08	Refik_Saydam_National_Public_Health_Agency	EPI1674855	6B.1A.5a	Konya	Turkey_Background
EPI_ISL_407644	A-Ankara-739-2019	A_/_H1N1	pdm09	2019-12-11	Refik_Saydam_National_Public_Health_Agency	EPI1674857	6B.1A.5a.1	Ankara	Turkey_Background
EPI_ISL_407654	A-Zonguldak-814-2019	A_/_H1N1	pdm09	2019-12-26	Refik_Saydam_National_Public_Health_Agency	EPI1674868	6B.1A.5a	Zonguldak	Turkey_Background
EPI_ISL_407655	A-Zonguldak-815-2019	A_/_H1N1	pdm09	2019-12-26	Refik_Saydam_National_Public_Health_Agency	EPI1674870	6B.1A.5a	Zonguldak	Turkey_Background
EPI_ISL_407656	A-Zonguldak-819-2019	A_/_H1N1	pdm09	2019-12-26	Refik_Saydam_National_Public_Health_Agency	EPI1674872	6B.1A.5a	Zonguldak	Turkey_Background
EPI_ISL_407657	A-Konya-820-2019	A_/_H1N1	pdm09	2019-12-26	Refik_Saydam_National_Public_Health_Agency	EPI1674874	6B.1A.5a	Konya	Turkey_Background
EPI_ISL_407658	A-Konya-827-2019	A_/_H1N1	pdm09	2019-12-27	Refik_Saydam_National_Public_Health_Agency	EPI1674876	6B.1A.5a	Konya	Turkey_Background
EPI_ISL_407659	A-Mardin-840-2019	A_/_H1N1	pdm09	2019-12-30	Refik_Saydam_National_Public_Health_Agency	EPI1674878	6B.1A.5a	Mardin	Turkey_Background
EPI_ISL_407660	A-Adana-AD2-2019	A_/_H1N1	pdm09	2019-12-13	Refik_Saydam_National_Public_Health_Agency	EPI1674880	6B.1A.5a	Adana	Turkey_Background
EPI_ISL_407662	A-Sanliurfa-AD5-2019	A_/_H1N1	pdm09	2019-12-26	Refik_Saydam_National_Public_Health_Agency	EPI1674883	6B.1A.5a	Sanliurfa	Turkey_Background
EPI_ISL_407664	A-Ankara-1597-2019	A_/_H1N1	pdm09	2019-11-12	Refik_Saydam_National_Public_Health_Agency	EPI1674886	6B.1A.5a.1	Ankara	Turkey_Background
EPI_ISL_407665	A-Ankara-1616-2019	A_/_H1N1	pdm09	2019-11-26	Refik_Saydam_National_Public_Health_Agency	EPI1674888	6B.1A.5a	Ankara	Turkey_Background
EPI_ISL_407668	A-Eskisehir-1654-2019	A_/_H1N1	pdm09	2019-12-09	Refik_Saydam_National_Public_Health_Agency	EPI1674892	6B.1A.5a	Eskisehir	Turkey_Background
EPI_ISL_407671	A-Ankara-1687-2019	A_/_H1N1	pdm09	2019-12-16	Refik_Saydam_National_Public_Health_Agency	EPI1674896	6B.1A.5a.1	Ankara	Turkey_Background
EPI_ISL_407672	A-Ankara-1692-2019	A_/_H1N1	pdm09	2019-12-16	Refik_Saydam_National_Public_Health_Agency	EPI1674898	6B.1A.5a.1	Ankara	Turkey_Background

Supplementary Table 1.