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Evolution of Influenza A(H3N2) Viruses in 2 Consecutive Seasons of Genomic Surveillance, 2021–2023

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Background. The circulation and the genomic evolution of influenza A(H3N2) viruses during the 2021/2022 and 2022/2023 seasons were studied and associated with infection outcomes.

Methods. Remnant influenza A–positive samples following standard-of-care testing from patients across the Johns Hopkins Health System (JHHS) were used for the study. Samples were randomly selected for whole viral genome sequencing. The sequence-based pEpitope model was used to estimate the predicted vaccine efficacy (pVE) for circulating H3N2 viruses. Clinical data were collected and associated with viral genomic data.

Results. A total of 121 683 respiratory specimens were tested for influenza at JHHS between 1 September 2021 and 31 December 2022. Among them, 6071 (4.99%) tested positive for influenza A. Of these, 805 samples were randomly selected for sequencing, with hemagglutinin (HA) segments characterized for 610 samples. Among the characterized samples, 581 were H3N2 (95.2%). Phylogenetic analysis of HA segments revealed the exclusive circulation of H3N2 viruses with HA segments of the 3C.2a1b.2a.2 clade. Analysis of a total of 445 complete H3N2 genomes revealed reassortments; 200 of 227 of the 2022/2023 season genomes (88.1%) were found to have reassorted with clade 3C.2a1b.1a. The pVE was estimated to be −42.53% for the 2021/2022 season and 30.27% for the 2022/2023 season. No differences in clinical presentations or admissions were observed between the 2 seasons.

Conclusions. The increased numbers of cases and genomic diversity of influenza A(H3N2) during the 2022/2023 season were not associated with a change in disease severity compared to the previous influenza season.

Keywords. H3N2; influenza; reassortment; vaccine.

Influenza is a respiratory illness that affects between 5% and 15% of the global population annually. Before the coronavirus disease 2019 (COVID-19) pandemic, the World Health Organization estimated that annual influenza epidemics resulted in approximately 3–5 million cases of severe disease and 290 000–650 000 deaths [1–3]. In the United States (US), mortality is strongly associated with age 65 years and older [4].

Influenza viruses belong to the Orthomyxoviridae family and have a segmented, negative-sense, single-stranded RNA genome, with each segment encoding at least 1 protein [5]. Seasonal epidemics are caused by influenza types A and B. Prior to the COVID-19 pandemic, 2 subtypes of human influenza A virus—A(H3N2) and A(H1N1) pdm09—and 2 lineages of human influenza B virus—Victoria and Yamagata—co-circulated, resulting in annual global seasonal epidemics [6].

The COVID-19 pandemic disturbed the seasonal circulation of influenza. At the Johns Hopkins Health System (JHHS), almost no influenza cases were detected during the 2020/2021 season [7]. In the 2021/2022 season, influenza activity was higher but remained relatively low overall throughout the season. This coincided with a significant increase in the prevalence of the Omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [8]. A more substantial resurgence of influenza circulation occurred during the 2022/2023 season [9].

The evolution of the A(H3N2) subclade has been remarkable since its first introduction in 1968 and evolving A(H3N2) viruses were associated with numerous severe outbreaks, poor vaccine effectiveness, and periodic seasonal vaccine strain changes [10]. In North America, influenza A(H3N2) predominated from September 2022 to January 2023 [9]. A predominance of

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clade 3C.2a1b.2a.2 was noted globally, and it subsequently evolved into multiple subclades [9]. In this study, the predominance and diversity of A(H3N2) influenza viruses were characterized across 2 consecutive influenza seasons and associated with clinical presentations and outcomes.

METHODS

Patient Consent

The design of this work has been approved by the Johns Hopkins Institutional Review Board. The research was performed under protocols IRB00306448 and IRB00221396 with a waiver of consent.

Data Availability

Genomes were made publicly available in the Global Initiative on Sharing All Influenza Data (GISAID) database.

Study Population, Samples, and Data Collection

Standard-of-care diagnostic influenza testing is conducted for both inpatients and outpatients across JHHS. Testing for influenza A/B is performed with the Cepheid Xpert Xpress SARS-CoV-2/Flu/respiratory syncytial virus (RSV) test or the ePlex RP/RP2 respiratory panels [11]. The study samples were collected between September 2021 and December 2022 (study cohort is shown in Supplementary Table 1). Clinical and demographic data were collected as previously described, involving bulk extractions that encompass all information related to encounters with positive influenza tests [12, 13]. For encounters related to influenza-like illness (ILI), the following definition was applied: patients who visited any of the 5 emergency departments (EDs) across JHHS and had either (1) a chief complaint including fever, sore throat, cough, or flu-like symptoms, or (2) an ED diagnosis of pneumonia, influenza, or "other viral agents as the cause of diseases classified elsewhere."

RNA Extraction and Multisegment Amplification by Reverse-Transcription Polymerase Chain Reaction

Viral nucleic acid extraction from influenza virus–positive samples was conducted using the Chemagic Viral RNA/DNA Kit following the manufacturer's instructions (300 µL extracted volume and 60 µL elution volume). The whole genome was amplified using a single multisegment 1-step reverse-transcription polymerase chain reaction (RT-PCR) with the MBTuni-12 and MBTuni-13 primer sets [14]. The amplification was carried out in 25-µL reactions comprising 5 µL of nuclease-free water, 12.5 µL of 2× RT-PCR buffer, 1 µL of each primer (10 µmol/L), 0.5 µL of SuperScript III One-Step RT-PCR with Platinum Taq High Fidelity (Invitrogen), and 5 µL of extracted RNA. The thermocycling conditions were as follows: 45°C for 60 minutes, 55°C for 30 minutes, and 94°C for 2 minutes, followed by 5 cycles of 94°C for 20 seconds, 40°C for 30 seconds, and 68°C for 3 minutes 30 seconds. This was followed by 40 cycles of 94°C for 20 seconds, 58°C for 30 seconds, and 68°C for 3 minutes 30 seconds, with a final extension at 68°C for 10 minutes.

Next-Generation Sequencing and Virus Genome Assembly

After the amplification, 1 µL of the PCR product was prepared for sequencing using the native barcoding genomic DNA kit (EXP-NBD196) and the NEBNext ARTIC Library Prep Kit, following the manufacturer's instructions. The samples were then sequenced using R9.4.1 flow cells on a GridION (Oxford Nanopore Technologies). The resulting fastq files were demultiplexed using the artic_guppyplex tool (Artic version 1.2.2). Nucleotide sequence assembly was performed using the FLU module of the Iterative Refinement Meta-Assembler (IRMA version 1.0.2) [12], utilizing IRMA's default settings, which include a minimum average quality score of 24 and a site depth of 100.

Real-Time RT-PCR

The cycle threshold values from the nasal swab were determined using real-time RT-PCR with primers and a probe previously described [15]. The Luna Probe One-Step RT-qPCR (New England Biolabs) was used following the manufacturer's instructions.

Phylogenetic and Reassortment Analysis

The alignment with reference sequences, retrieved from GISAID (Supplementary Table 2), was performed using Mafft (version 7.450) [16]. The phylogenetic trees for all segments were generated using the maximum likelihood method using IQ-TREE version 2.2.2.6, and the visualization was done with FigTree version 1.4.4 [17]. Complete reference genomes from GISAID were used for the phylogenetic analysis to assess probable reassortment and phylogenetic trees with connected tips were generated by R ggtree package version 3.2.1. The ModelFinder, implemented in IQ-TREE2, was used to select the best-fitted nucleotide substitution model. The robustness of the tree topology was tested with 1000 nonparametric bootstrap analyses, and bootstrap values >75% were shown on branches of the consensus trees. The nucleotide pairwise percentage identity was evaluated using Mega 11.

Nextclade was used to characterize mutations present in the hemagglutinin (HA) gene compared to vaccine strains [18]. Predicted vaccine efficacy (pVE) against circulating A(H3N2) viruses was estimated using the sequence-based model pEpitope, as previously described [19–21]. pEpitope is a mathematical model that measures the antigenic distance between the predominant circulating strains and the vaccine strains by considering the amino acid substitutions of the 5 antigenic sites. The antigenic sites with the highest $P_{epitope}$ values are considered dominant and used to estimate the pVE. $P_{epitope}$ values were calculated using MATLAB [21]. A perfect match between the circulating strain and the vaccine strain results in $P_{epitope}$ equal to zero with a pVE of 66% [19]. A negative pVE value indicates less than optimal vaccine efficacy [22].

RESULTS

Influenza Prevalence at JHHS and the Study Cohort

Between September 2021 and February 2022, influenza viruses circulated at JHHS in lower numbers than before the COVID-19 pandemic, but at greater numbers than during the 2020/2021 season (Figure 1). The start of influenza virus circulation coincided with a decrease in RSV and rhinovirus/enterovirus (Figure 1), with flattening of the influenza curve associated with the emergence of the SARS-CoV-2 Omicron variant (Figure 1). During the 2022/2023 season, influenza virus detection began in September 2022 and markedly peaked in November (Figure 1). Overall, the percentage of samples positive for influenza (A and B) between September 2021 and December 2022 was 5% (6091/121 683). Of these, 99.7% (6071/6091) were influenza A, and 0.3% (20/6091) were influenza B (Figure 2).

Among all positives detected during the study period, 13.2% (805/6091) were randomly selected (convenience sample, based on availability) for whole genome sequencing, of which 41.7% (336/805) were from the 2021/2022 season and 58.3% (469/805) were from the 2022/2023 season (Figure 2). Metadata reviews of the 805 patients showed a median age of 11, with a range from 1 month to 93 years. The age group 5–17 years was the most represented (41.4%), followed by 18–64 years (30.2%). Children aged <5 years and the elderly accounted for 23.1% and 5.3%, respectively (Table 1). There were slightly more females than males, with a male-to-female ratio of 0.91 (383 vs 421).

Clinical signs and symptoms were available for 694 patients. The most commonly reported presenting symptoms were fever (42.4% [294/694]) and cough (22% [153/694]) (Table 1). Underlying conditions were reported for 47.7% of the whole cohort of 805 patients (384/805) and were primarily lung disease (27.6% [222/805]), cancer (10.6 [85/805]), and hypertension (9.9% [80/805]). In our cohort, 5.5% (44/805) were admitted, of whom 18.2% (8/44) required an intensive care unit (ICU) level of care. A total of 50 patients (6.2%) required supplemental oxygen, most of whom were admitted. Of the 8 patients who required ICU-level care, 4 patients were positive for other pathogens at the same encounter including *Haemophilus influenzae*, yeast, *Staphylococcus aureus*, or *Corynebacterium striatum* group (Supplementary Table 1).

Phylogenetic and Sequence Analyses

Of the 805 samples for which genome sequencing was attempted, all segments were successfully generated for 462 samples (57.4%). The HA gene used for the classification of clades and subclades of influenza virus was recovered from 610 samples (Figure 2).

HA Gene Analysis

The HA sequence analysis by Nextclade showed that 95.2% (581/610) belong to H3N2 clade 3C.2a1b.2a.2, 4.4% (27/610) to H1N1pdm 6B.1A.5a.2 clade, and 0.33% (2/610) to influenza B, Victoria lineage. Among the 581 H3N2 clade 3C.2a1b.2a.2, 44.4% (258/581) were detected in the 2021/2022 season and 55.6% (323/581) in the 2022/2023 season (Figure 2). The influenza B Victoria lineage and H1N1pdm 6B.1A.5a.2 clade were reported in the 2021/2022 and 2022/2023 seasons, respectively (Supplementary Table 1).

Results from Nextclade were confirmed by phylogenetic analyses of the HA sequences. The nucleotide identity of the JHHS H3N2 genomes ranged from 97.5%–100% (Figure 3*A*). The HA gene of clade 3C.2a1b.2a.2 was reported to encode amino acid substitutions Y159N, T160I, L164Q, G186D, and D190N [9]. Our genomes showed substitutions F159N, K160I, L164Q, V186D, and D190N when reference A/Darwin/6/2021 (EPI1857216) was used for the phylogenetic analysis. Genomes of clade 3C.2a1b.2a.2 in our cohort split into multiple subclades including 3C.2a1b.2a.2a.3a.1 (2a.3a.1) (4.3%) (with G50K, D53N, N96S, I192F and H156S), 3C.2a1b.2a.2a.3 (2a.3) (9.0%) (D53N, N96S, I192F, and H156S), 3C.2a1b.2a.2a.1 (2a.1) (11.9%) (D53G, D104G, H156S, and K276R), 3C.2a1b.2a.2a.1a (2a.1a) (29.3%) (D53G, D104G, H156S, K276R, and L157I), and 3C.2a1b.2a.2b (2b) (41.3%) with G50K and F79V substitutions (Table 2 and Figure 3*B*). Subclade 2a.1a predominated in the 2021/2022 season (66%), whereas 2b was predominant in the 2022/2023 season (74%) (Table 2).

The comparison between the HA1 amino acid sequences of the 2021/2022 A(H3N2) predominant subclade 2a.1a study viruses and the 2021/2022 vaccine reference strain A/Cambodia/E0826360/2020 showed that almost all genomes had amino acid substitutions D104G, L157I, S262N, and K276R, in addition to the vaccine strain amino acid changes G53D, S156H, N159Y, I160K, Q164L, K171N, D186R, N190D, S198P, and S219F (Supplementary Table 3). On the other hand, almost all 2022/2023 HA1 predominant subclade 2b sequences differed from the season's vaccine reference strain A/Darwin/6/2021 (EPI1857216) by amino acid substitutions E50K, F79V, I140K, and S156H (Supplementary Table 3).

$P_{epitope}$ values of .33 and .09 were respectively calculated using the pEpitope model when 2 representative strains from the predominant subclades of 2021/2022 (A/Baltimore/JH-001/2021) and 2022/2023 (A/Baltimore/JHH-0336/2022) and the vaccine strains of both seasons, A/Cambodia/E0826360/2020 and A/Darwin/6/2021 (EPI1857216), were used for the analysis. The pVE during the 2021/2022 and 2022/2023 seasons was respectively estimated at −42.53% (standard deviation [SD], 18.27%) and 30.27% (SD, 8.69%).

Phylogeny of Other Segments and Reassortment Analysis

The phylogenetic analysis of the NA segment of the H3N2 viruses was carried out from 99.3% (577/581) A(H3N2) samples, 44.8% (260/581) from the 2021/2022 season and 54.6%

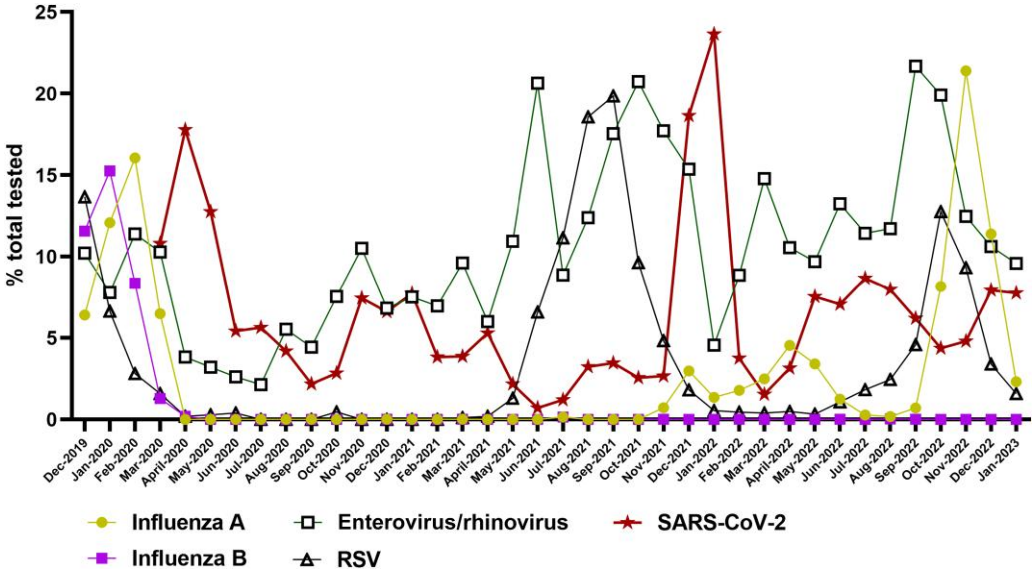

Figure 1. Circulation of influenza virus and respiratory virus positivity rates at the Johns Hopkins Health System. Abbreviations: RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

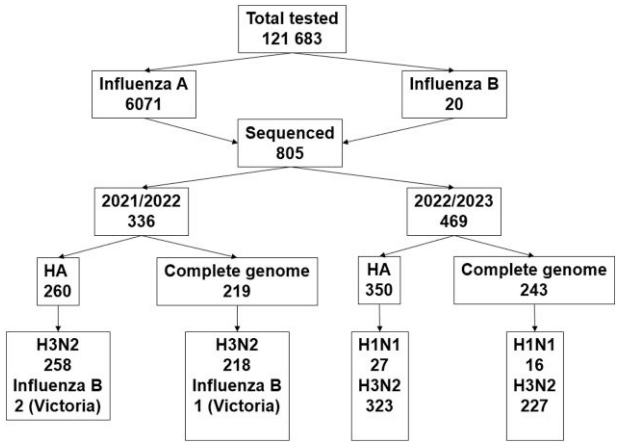

Figure 2. Flowchart of specimens used for influenza virus screening and whole genome sequencing.

(317/581) from the 2022/2023 season (Supplementary Table 1). The phylogenetic tree generated revealed that 63.7% (370/581) cluster with complete reference genomes from clade 3C.2a1b.2a.2, consistent with the HA phylogenetic analysis (Supplementary Figure 1). However, some genomes that were classified as 3C.2a1b.2a.2 clade using the HA analysis clustered with reference genomes from the 3C.2a1b.1a or 3C.2a1b.1b clades suggesting probable reassortment events. The phylogenetic trees of the other 6 gene segments (PB2, PB1, PA, NP, M, and NS; Supplementary Figure 1 and Figure 4) also suggested the interclade reassortment between 3C.2a1b.2a.2, 3C.2a1b.1a, and 3C.2a1b.1b. To further investigate the type of reassortment, we focused the analysis on samples for which we have complete genomes. A total of 445 complete H3N2 genomes were used for the analysis (Supplementary Table 4). A total of 202 genomes were probable reassortants, 200 of which were from the 2022/2023 season. Four types of reassortments were identified, namely 1:7 (77.7% [157/202]), 2:6 (16.8% [34/202]), 3:5 (4% [8/202]), and 4:4 (1.5% [3/202]) (Figure 4, Supplementary Figure 2, and Supplementary Table 4).

The comparison of clinical and demographic data, as well as disease severity between patients infected with reassortant versus nonreassortant H3N2 viruses, showed no statistically significant differences (Table 3). Notably, a higher percentage of children aged 5–17 years were infected by reassortant influenza viruses (52% vs 37.9%; $P = .003$, Fisher exact test) (Table 3).

DISCUSSION

Globally, the COVID-19 pandemic interrupted the circulation of seasonal influenza viruses. Between September 2021 and January 2022, influenza A(H1N1) pdm09, A(H3N2), and influenza B viruses circulated at very low rates, with percentage positivity of <3%, as opposed to the 17% prior to the COVID-19 pandemic [9]. In the US, influenza activity increased slightly in late 2021 but markedly in late 2022. Studying the circulation and genomic evolution of influenza is essential for understanding circulation patterns and the relationship between genomic changes and disease outcomes. As reported in several other studies around the world [23–27], A(H3N2) predominated. Interestingly, this pattern

Table 1. Clinical and Demographic Characteristics of Influenza Virus–Infected Patients With Samples Selected for Sequencing

Characteristic	2021/2022 Season	2022/2023 Season	Total
No. (%) of patients	336 (41.8)	469 (58.2)	805
Sex			
Female	173 (51.5)	248 (52.9)	421 (52.3)
Male	163 (48.5)	220 (46.7)[a]	383 (47.6)
Age group, y			
<5	78 (23.2)	108 (23)	186 (23.1)
5–17	113 (33.6)	220 (46.9)	333 (41.4)
18–64	129 (38.4)	114 (24.3)	243 (30.2)
≥65	16 (4.8)	27 (5.8)	43 (5.3)
Clinical sign			
Patients with clinical signs and symptoms at presentation	257 (76.5)	437 (93.2)	694 (86.1)
Fever	102 (39.7)	192 (43.9)	294 (42.4)
Cough	57 (22.2)	96 (21.9)	153 (22)
Headache	9 (3.5)	20 (4.6)	29 (4.2)
Breathing problem and shortness of breath	10 (3.9)	40 (9.2)	50 (7.2)
Chest pain	9 (3.5)	9 (2.1)	18 (2.6)
Sore throat	11 (4.3)	20 (4.6)	31 (4.5)
URI	9 (3.5)	18 (3.8)	27 (3.9)
Abdominal pain	7 (2.7)	15 (3.4)	22 (3.2)
Emesis	16 (6.2)	22 (5)	38 (5.5)
Flu-like symptoms	31 (12.1)	69 (15.8)	100 (14.4)
Generalized weakness or body ache	7 (2.7)	8 (1.8)	15 (2.2)
Seizures	4 (1.6)	5 (1.1)	9 (1.3)
Comorbidity (% whole cohort)			
≥1 underlying medical condition	152 (45.2)	232 (49.5)	384 (47.7)
Hypertension	34 (10.1)	46 (9.8)	80 (9.9)
Pregnancy	20 (6.0)	26 (5.5)	46 (5.7)
Lung disease	82 (24.4)	140 (29.9)	222 (27.6)
Kidney disease	16 (4.8)	22 (4.7)	38 (4.7)
Immunosuppression	28 (8.3)	40 (8.5)	68 (8.4)
Diabetes	20 (6.0)	16 (3.4)	36 (4.5)
Heart failure	11 (3.3)	13 (2.8)	24 (3.0)
Cerebrovascular disease	12 (3.6)	16 (3.4)	28 (3.5)
Cancer	40 (11.9)	45 (9.6)	85 (10.6)
Respiratory failure	15 (4.5)	27 (5.8)	42 (5.2)
Severity (% whole cohort)			
Admitted	16 (4.8)	28 (6)	44 (5.5)
ICU	4 (1.2)	4 (0.9)	8 (1)
Supplemental oxygen	17 (5)	33 (7)	50 (6.2)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ICU, intensive care unit; URI, upper respiratory tract infection.

[a]One defined as nonbinary.

was not consistent in some countries. In China, the 2021/2022 influenza epidemic was mainly caused by Victoria lineage of influenza B, whereas in Bangladesh, France, South Africa, and in some tropical countries A(H1N1)pdm09 predominated [9, 28].

In the US, the late 2021 influenza circulation declined in late December 2021 through late January 2022, which corresponded with the rapid rise of Omicron [26, 29]. This was followed by a slight increase in March and April 2022 [25–27], a trend that was not observed in several other countries including Russia [23], Italy [30], and Spain [31]. During the 2022/2023 season, influenza virus circulation began in September and peaked in November. This rapid increase in the circulation of the influenza virus can be explained by a combination of factors, including the easing of COVID-19 control measures, a decrease in community-level immunity to seasonal influenza, the return of the seasonality of influenza, and possibly the emergence of a more adapted strain [27, 32].

The phylogenetic analysis of the HA gene sequences of A(H3N2) revealed the predominant circulation of clade 3C.2a1b.2a.2 in Maryland during both seasons. Our findings align with the US data from the 2021/2022 season [25]. While the 3C.2a1b.2a.2 clade dominated during the 2021/2022 season, other clades, including 3C.2a1b.1a and 3C.2a1b.1b, were detected in Africa, Europe, and Asia [30, 33]. In the 2022/2023 season, as observed in our study, the 3C.2a1b.2a.2 clade also predominantly circulated worldwide [33].

The 3C.2a1b.2a.2 clade has evolved into multiple subclades, each typically defined by specific amino acid substitutions. Subclade 2b was predominantly detected during the 2022/2023 season, while subclade 2a.1a was predominant during the 2021/2022 season. This genetic diversity raises the possibility that several amino acid positions, such as amino acid substitutions K140I and H156S, might have been subject to selection. The amino acid change K140I affects antigenic site A [34], and the T135K change could lead to the loss of a N-linked glycosylation site [9].

In our study, all influenza A(H3N2) viruses characterized during the 2021/2022 season belonged to the 3C.2a1b.2a.2 clade, showing several mismatches with the A/Cambodia/E0826360 (3C.2a1b.2a.1) vaccine strain. Fourteen amino acid substitutions were observed in the predominant 2a.1a subclade compared to the 2021/2022 vaccine strain. This includes H156S and N159Y, which are located within major antigenic sites. Comparison of the 2022/2023 predominant 2b subclade with the vaccine reference strain A/Darwin/6/2021 (EPI1857216) revealed amino acid substitutions within antigenic sites, including C (E50K), A (I140K), and B (S156H). Notably, H156S is also situated within the receptor-binding site. Although the receptor-binding site is generally conserved, it may be subject to selective pressure, the introduction of new mutations, and evasion of antibody recognition [10]. Mutations in this domain could enable circulating strains to evade recognition of vaccine-induced antibodies. According to the pEpitope model [19–21], the pVE of vaccine strain against the A(H3N2) viruses circulating during the 2021/2022 and 2022/2023 seasons was estimated at −42.53% and 30.27%, respectively. Thus, the pVE obtained in the 2021/2022 season suggests suboptimal vaccine effectiveness; however, for 2022/2023 a better vaccine

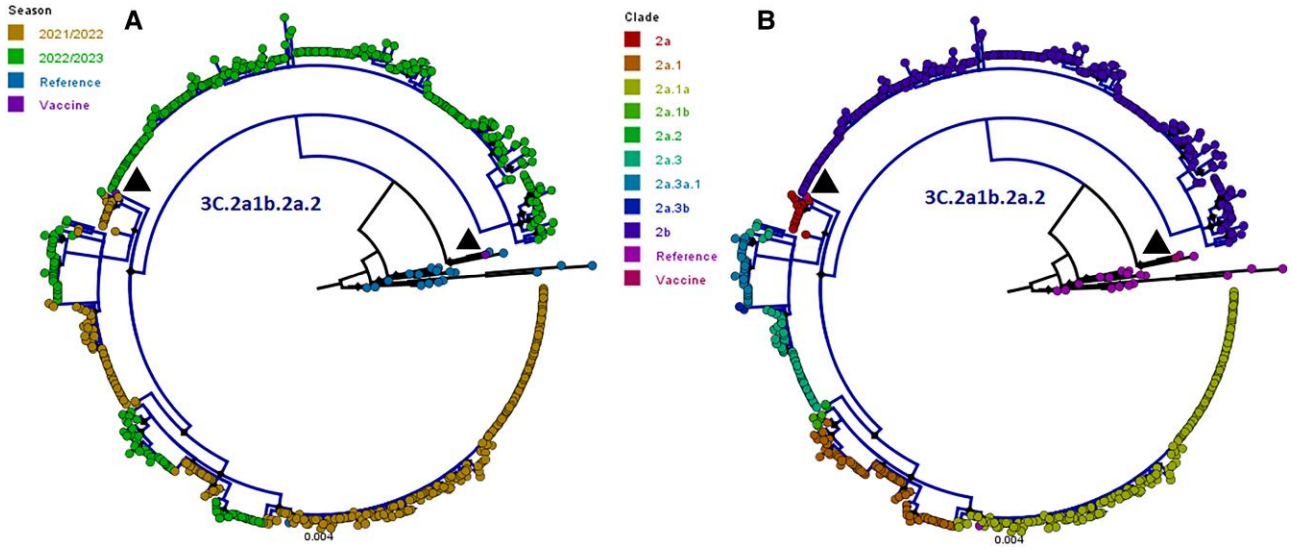

Figure 3. Phylogenetic tree of the hemagglutinin nucleotide sequences of A(H3N2) strains identified from the Johns Hopkins Health System during the period of study. The vaccine A(H3N2) strains of the northern hemisphere for 2021–2022 and 2022–2023 and reference sequences from GISAID were included in the trees. Phylogeny of the strains by season (*A*) and by subclade (*B*). Black arrowheads mark the reference vaccine strains.

Table 2. A(H3N2) Subclade Distribution by Season Based on the Phylogenetic Analysis of the Hemagglutinin Segment

Subclade	2021/2022	2022/2023	Total, No.
2a	15 (6)	1 (0)	16
2a.1	21 (8)	48 (15)	69
2a.1a	170 (66)	0 (0)	170
2a.1b	0 (0)	5 (2)	5
2a.2	2 (1)	0 (0)	2
2a.3	48 (19)	4 (1)	52
2a.3a.1	0 (0)	25 (8)	25
2a.3b	2 (1)	0 (0)	2
2b	0 (0)	240 (74)	240
Total	258	323	581

Data are presented as No. (%).

efficacy was expected. Vaccine efficacy against H3N2 influenza viruses was approximately 16% in 2021–22 [29] and rose to approximately 60% [35] based on surveillance from the US Centers for Disease Control and Prevention (CDC). Similar suboptimal vaccine efficacy during the 2021/2022 season was also reported in Italy using the same model [30]. Relatively low vaccine effectiveness was also reported against circulating A (H3N2) in 2021/2022 season in the US [29], Denmark [36], Canada (36%) [37], and Spain [31] as indicated by vaccine effectiveness observational studies. According to the CDC, during the 2022/2023 season, adults and children who were vaccinated were 44% and 42% less likely to visit an emergency department or urgent care center and 39% and 68% less likely to be hospitalized because of ILI or related complications [38]. In Canada, vaccination reduced the risk of medically attended illness by about 50% [39].

Multiple reassortments were observed during the 2022/2023 season and were primarily 1:7 involving the 3C.2a1b.2a.2 for the HA segment and the 3C.2a1b.1a for other genomic segments. Reassortant strains of the 3C.2a1b.2a.2 clade circulating in 2022/2023 season have also been reported from Canada [39] and many other countries, as indicated by the NA phylogenies available in Nextstrain [33]. Reassortment is relatively common in the evolution of influenza viruses [40, 41]. Interclade reassortments involving NA and other gene segments have been observed in recent seasons—for example, between clades 3C.2a2 and 3C.2a1a in 2017/2018 [33, 42, 43] and between clades 3C.3a1 and 3C.2a in 2018/2019 [33]. Consistent with our study, the Canadian report identified a high prevalence of reassortment between clade 3C.2a1b.2a.2 and clade 3C.2a1b.1a in 33% of their genomes (154/471) [39]. Clinically, we observed no statistically significant differences between patients infected with reassortant and nonreassortant H3N2 viruses in our study.

Our study provides the first comprehensive genomic characterization and evaluation of the correlation between genomic changes, vaccine effectiveness, clinical presentations, and outcomes within a large healthcare system in the US during the 2022/2023 season. The limitations of our study include the small sample size of admitted patients, which restricted the disease severity analyses, and the infrequently missing clinical data for some patients. Further analysis with larger sample sizes is warranted to validate our findings.

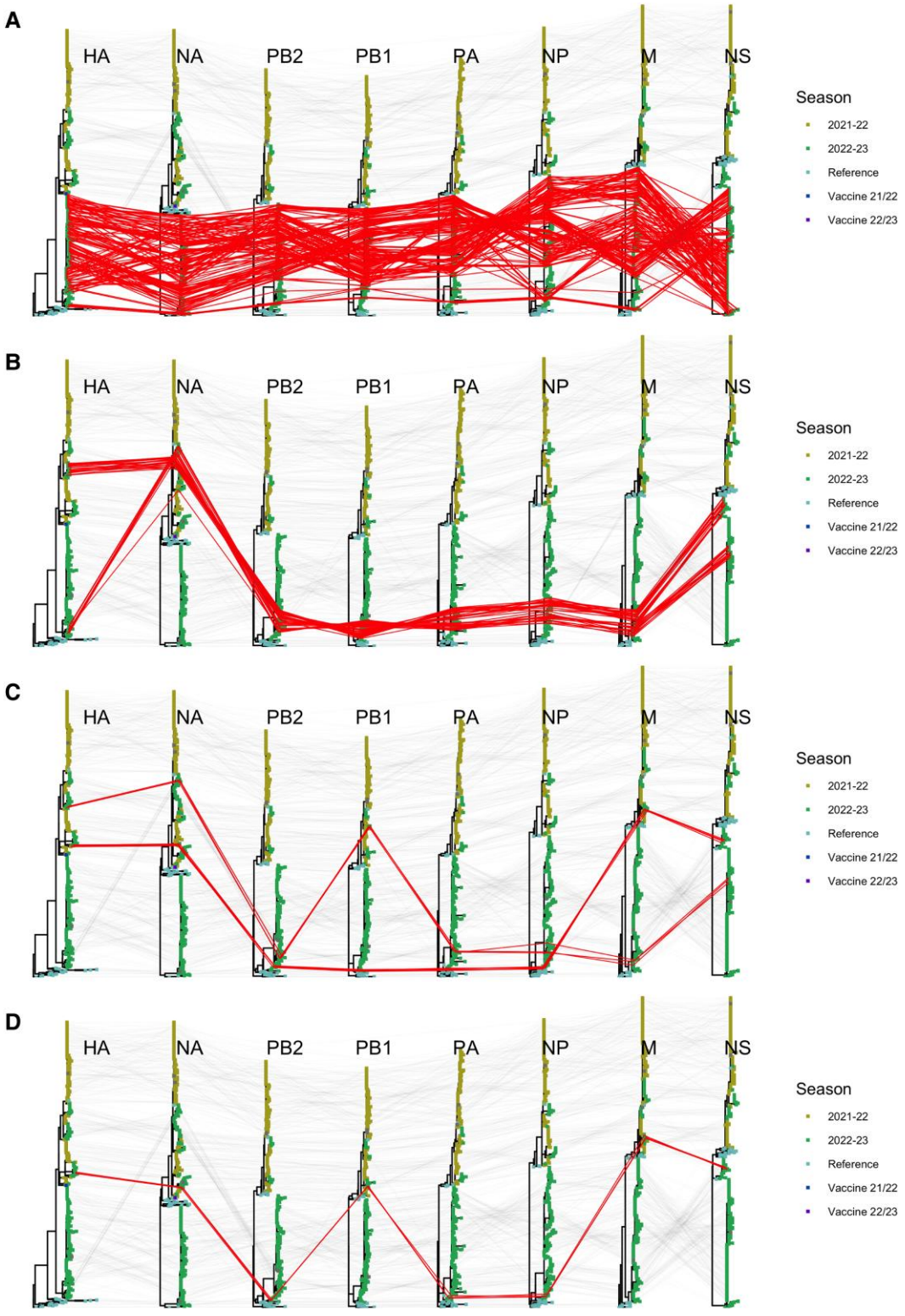

Figure 4. Phylogenetic trees of gene segments HA, NA, PB2, PB1, PA, NP, M, and NS (from left to right) of A(H3N2) strains identified from the Johns Hopkins Health System during the period of study. Reference sequences from GISAID were included in the trees. Four types of reassortments as 1:7 (*A*), 2:6 (*B*), 3:5 (*C*), and 4:4 (*D*) were identified. Lines in gray and red across segment trees connect nonreassortant and reassortant equivalent strains, respectively.

Table 3. Comparison of the Clinical and Demographic Data Between Patients Infected With Reassortant and Nonreassortant Influenza A(H3N2) Viruses

Characteristic	Nonreassortants	Reassortants
No. of patients	243	202
Sex		
Female	135 (55.6)	98 (48.5)
Male	108 (44.4)	104 (51.5)
Age group, y		
<5	56 (23)	43 (21.3)
5–17	92 (37.9)	105 (52)
18–64	84 (34.6)	47 (23.3)
≥65	11 (4.5)	7 (3.5)
Clinical sign		
Patients with clinical signs and symptoms at presentation	192 (79)	199 (98.5)
Fever	76 (39.6)	98 (49.2)
Cough	49 (25.5)	38 (19.1)
Headache	6 (3.1)	13 (6.5)
Breathing problem and shortness of breath	7 (3.6)	18 (9)
Chest pain	4 (2.1)	6 (3)
Sore throat	9 (4.7)	14 (7)
URI	6 (3.1)	5 (2.5)
Abdominal pain	3 (1.6)	6 (3)
Emesis	11 (5.7)	8 (4)
Flu-like symptoms	23 (12)	28 (14.1)
Generalized weakness or body ache	4 (2.1)	16 (8)
Seizures	6 (3.1)	1 (0.5)
Comorbidity		
≥1 underlying medical condition	111 (45.7)	102 (50.5)
Hypertension	24 (9.9)	11 (5.4)
Pregnancy	17 (7)	7 (3.5)
Lung disease	57 (23.5)	74 (36.6)
Kidney disease	8 (3.3)	8 (4)
Immunosuppression	18 (7.4)	17 (8.4)
Diabetes	14 (5.8)	4 (2)
Heart failure	8 (3.3)	4 (2)
Cerebrovascular disease	8 (3.3)	6 (3)
Cancer	33 (13.6)	15 (7.4)
Respiratory failure	12 (4.9)	11 (5.4)
Severity		
Admitted	8 (3.3)	9 (4.5)
ICU	3 (1.2)	1 (0.5)
Supplemental oxygen	10 (4.1)	15 (7.4)

Data are presented as No. (%).

Abbreviations: ICU, intensive care unit; URI, upper respiratory tract infection.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Institute of Biomedical Imaging and Bioengineering; the National Heart, Lung, and Blood Institute; the National Institutes of Health (NIH), or the US Department of Health and Human Services.

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