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MAJOR ARTICLE

Investigating Epidemiologic and Molecular Links Between Patients With Community- and Hospital-Acquired Influenza A: 2017–2018 and 2019–2020, Michigan

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Background. Hospital-acquired influenza virus infection (HAII) can cause severe morbidity and mortality. Identifying potential transmission routes can inform prevention strategies.

Methods. We identified all hospitalized patients testing positive for influenza A virus at a large, tertiary care hospital during the 2017–2018 and 2019–2020 influenza seasons. Hospital admission dates, locations of inpatient service, and clinical influenza testing information were retrieved from the electronic medical record. Time-location groups of epidemiologically linked influenza patients were defined and contained ≥1 presumed HAII case (first positive ≥48 hours after admission). Genetic relatedness within time-location groups was assessed by whole genome sequencing.

Results. During the 2017–2018 season, 230 patients tested positive for influenza A(H3N2) or unsubtyped influenza A including 26 HAIIs. There were 159 influenza A(H1N1)pdm09 or unsubtyped influenza A–positive patients identified during the 2019–2020 season including 33 HAIIs. Consensus sequences were obtained for 177 (77%) and 57 (36%) of influenza A cases in 2017–2018 and 2019–2020, respectively. Among all influenza A cases, there were 10 time-location groups identified in 2017–2018 and 13 in 2019–2020; 19 of 23 groups included ≤4 patients. In 2017–2018, 6 of 10 groups had ≥2 patients with sequence data, including ≥1 HAII case. Two of 13 groups met this criteria in 2019–2020. Two time-location groups from 2017–2018 each contained 3 genetically linked cases.

Conclusions. Our results suggest that HAIIs arise from outbreak transmission from nosocomial sources as well as single infections from unique community introductions.

Keywords. hospital-acquired; influenza; nosocomial; transmission; whole genome sequencing.

Influenza is a common cause of respiratory illness and is responsible for hundreds of thousands of hospitalizations in the United States each year [1]. Those at highest risk of severe complications of influenza include adults ≥65 years of age, children <5 years of age, persons with chronic health conditions, pregnant and postpartum women, persons living in nursing homes, and racial and ethnic minority groups [2]. These same groups are among those likely to be hospitalized for reasons other than influenza. As a result, hospital-acquired influenza virus infection (HAII) can result in longer hospital stays, more frequent admission to the intensive care unit, increased need for mechanical ventilation, and death [3–5]. HAII also leads to higher economic costs to the healthcare system due to longer hospital stays, additional laboratory tests, and healthcare worker (HCW) sick leave [6–8].

It is important to identify potential transmission routes of HAII in order to inform implementation of prevention and control strategies in hospitals to prevent morbidity, mortality, and economic burden resulting from HAII. HAII is primarily spread through large droplets and direct contact, and transmission can involve patients, HCWs, and visitors [9]. These individuals acquire influenza in the community, introduce it to the hospital, and potentially cause outbreaks within inpatient units [6, 7]. However, it is challenging to determine who infected whom and to quantify the relative contributions of patient, HCW, and visitor populations to HAII transmission.

Whole genome sequencing (WGS) has been used in a wide range of outbreak investigations to identify and link HAII cases, exclude cases from investigations, and determine sources and directionality of transmission [10–18]. However, these

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studies are often focused in response to a known HAII outbreak [10–15]. As a result, they may overestimate how often HAII occurs and the typical size of transmission clusters. In addition, outbreak-focused studies cannot determine how frequently influenza cases in the hospital may be genetically related by chance without true transmission links. This important context could be provided by also sampling community-acquired cases.

In this study, we identified all inpatients who tested positive for influenza during the 2017–2018 and 2019–2020 influenza seasons in a large tertiary care hospital. We identified HAII patients who were epidemiologically linked by time and location and applied WGS to confirm or rule out linkage between patients.

METHODS

Study Population

We identified all patients hospitalized at University of Michigan hospitals who had an influenza-positive result from clinical testing (ordered based on physician discretion) during the 2017–2018 (1 September 2017 to 4 June 2018) and 2019–2020 (26 August 2019 to 21 April 2020) influenza seasons. Residual clinical respiratory specimens were unavailable for the 2018–2019 influenza season; therefore, data from that season were not considered in this analysis. Based on predominantly circulating influenza subtypes, the 2017–2018 analysis was restricted to patients testing positive for influenza A(H3N2) or influenza A unsubtyped, and the 2019–2020 analysis was restricted to patients testing positive for influenza A(H1N1)pdm09 or influenza A unsubtyped.

Institutional infection control policies for influenza include placing individuals with symptoms of acute respiratory illness under droplet precautions pending test results, and a preference for private rooms for patients with acute respiratory illness.

Patient age and sex; admission, discharge, and transfer dates; in-hospital transfer locations; clinical influenza testing data (order and collection dates, and results); and influenza vaccination status were extracted from the electronic health record. Residual respiratory specimens collected from influenza-positive patients were retrieved from the clinical microbiology laboratory.

Patient Consent Statement

This study was reviewed and approved by the University of Michigan Medical School Institutional Review Board under a waiver of informed consent.

Hospital-Acquired Influenza and Time-Location Group Definitions

An influenza case was considered to be hospital-acquired if their first positive laboratory influenza result was from a specimen ordered >48 hours after admission (75th percentile of the influenza A incubation period [19]); sensitivity analyses considering first positive results >72 hours after admission (95th incubation period percentile) as hospital-acquired were also performed. Influenza cases identified ≤48 (or ≤72) hours after admission were considered to be community-acquired.

We defined epidemiologically linked influenza cases based on timing of influenza virus identification and residence in the same unit, defined by location and acuity level (eg, intensive care vs medical units), during the hospital admission—specifically, time-location groups contained at least 1 presumed HAII case and all other inpatients who (1) tested positive for influenza prior to the HAII case, (2) had overlapping hospital admission periods with the HAII case, and (3) resided in the same unit as the HAII case in the 4 days prior to the order date of their first positive test based on the incubation period of influenza [19]. We did not limit the window of time between identification of influenza in a potential contact and identification of HAII to allow for the possibility of transmission resulting from prolonged shedding, as is possible with immunocompromised patients. This was done to improve sensitivity of identifying potential transmission pairs linked by epidemiologic and genomic data, but this case definition would likely have low specificity in the absence of additional genetic information.

Whole Genome Sequencing Preparation

We attempted to sequence all retrieved specimens (ie, screening by viral quantitation was not performed). RNA was harvested from residual clinical respiratory samples using Applied Biosystems MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (#A48383). After RNA extraction, RNA samples were reverse-transcribed into complementary DNA and amplified using primers and probes developed by the Centers for Disease Control and Prevention Influenza Division on the SuperScript III One-Step RT-PCR system with Platinum Taq High Fidelity DNA Polymerase (ThermoFisher #12574-035). DNA cleanup was done using Ampure beads (Beckman #A63881).

Barcoded next-generation sequencing (NGS) sequencing libraries were prepared using Nextera DNA Prep Kit (Illumina #20018705). Samples were pooled (up to 96), 5 μL of each, into libraries and concentration was measured using Qubit 1× double-stranded DNA HA Assay kit (Q33231) before Illumina sequencing.

Phylogenetic Analysis

First, Cutadapt was used to remove adapter sequences [20]. Then, Fastqc was used for quality control analysis and BWA-MEM was used to map and sort the sequences using the reference sequence [21]. Sequence reads were aligned to A/Singapore/infihm-16-0019/2016(H3N2) and influenza A/Hawaii/70/2019(H1N1)pdm09 as reference sequences for specimens from the 2017–2018 and 2019–2020 seasons,

respectively. SAMtools was used to obtain sequencing coverage and consensus sequences were called using iVar with consensus bases called as the simple majority base (≥50%) at each position [22, 23]. Concatenated consensus genomes shorter than 13 000 base pairs (<99% complete) were excluded from the output data. Sequences with NGS coverage ≥20 reads at ≥80% of positions for all 8 influenza segments were defined as high quality and kept for further analysis. Finally, alignments were manually inspected and sequences with large insertions, deletions, or strings of mutations suspicious for sequencing errors were excluded. The bioinformatics pipeline workflow was written using Snakemake [24].

We attempted to sequence even those influenza A specimens that were unsubtyped as part of clinical testing. BLAST was used to confirm the subtype of those unsubtyped influenza A specimens that were successfully sequenced; those inconsistent with the analytic focus of each influenza season—A(H3N2) in 2017–2018 and A(H1N1)pdm09 in 2019–2020—were excluded.

MUSCLE was used for alignment, and maximum likelihood trees were created with IQ-TREE with 1000 ultrafast bootstrapping replicates [25, 26]. Reference genome sequences were aligned and plotted along with clinical samples. For the 2017–2018 influenza A(H3N2) phylogenetic tree, A/Texas/50/2012 was the outgroup, and A/Washington/16/2017 (3C.2a2 clade), A/Maryland/11/2018 (3C.3a clade), and A/Singapore/infihm−16−0019/2016 (3C.2a1 clade) were included as reference strains for the phylogenetic tree. For the 2019–2020 influenza A(H1N1)pdm09 phylogenetic tree, A/California/7/2009 was the outgroup, and A/Michigan/45/2015 (6B.1 clade), A/Wisconsin/588/2019 (6B.1A.5a.2 clade), and A/Hawaii/70/2019 (6B.1A.5a.1 clade) were included as reference strains. Tree files were plotted in R using the ggtree package [27].

Statistical Analysis

All statistical analyses were performed using R (R Foundation for Statistical Computing; version 4.1.1). The proportions of retrieved specimens that were successfully sequenced were compared by patient age group (<18, 18–64, and ≥65 years) and sex, influenza vaccination status (evidence of vaccine receipt >14 days prior to order date of first positive influenza test), infection source (community vs hospital), and month of first positive test using χ^2 or Fisher exact tests. The number and proportion of HAII patients who were linked by time-location and linked by time-location and genetics were determined for each influenza season. HAII patients were considered to be genetically linked if they were in the same time-location group and had ≤2 single-nucleotide variant (SNV) difference in their consensus sequence; this threshold approximately corresponds to the 0.1th percentile of the distribution of genetic distances of influenza cases without time-location links (Supplementary Figure 1). We chose this conservative SNV threshold because previous research has demonstrated very low genetic distances between influenza transmission pairs in households [28]. Patient timeline and location plots were generated using the vistime package.

RESULTS

Influenza A(H3N2) and influenza B co-circulated locally during the 2017–2018 influenza season with influenza A (H1N1) pdm09 detected at lower levels (Figure 1*A*). The 2017–2018 analysis population consisted of 230 individuals, either with influenza A(H3N2) (n = 220) or influenza A unsubtyped by clinical testing that was not resolved by sequencing (n = 10). Individuals positive for influenza A(H1N1)pdm09 (n = 30) or influenza B (n = 79) were excluded from the 2017–2018 analysis (Supplementary Figure 2). Of the 230 individuals included in the 2017–2018 analysis, 26 had influenza first identified in tests ordered >48 hours after admission and considered to be hospital-acquired; 22 of these were first identified >72 hours after admission. At least 1 residual respiratory specimen was retrieved for 220 of the 230 total influenza-positive individuals, including 25 of the 26 HAII cases.

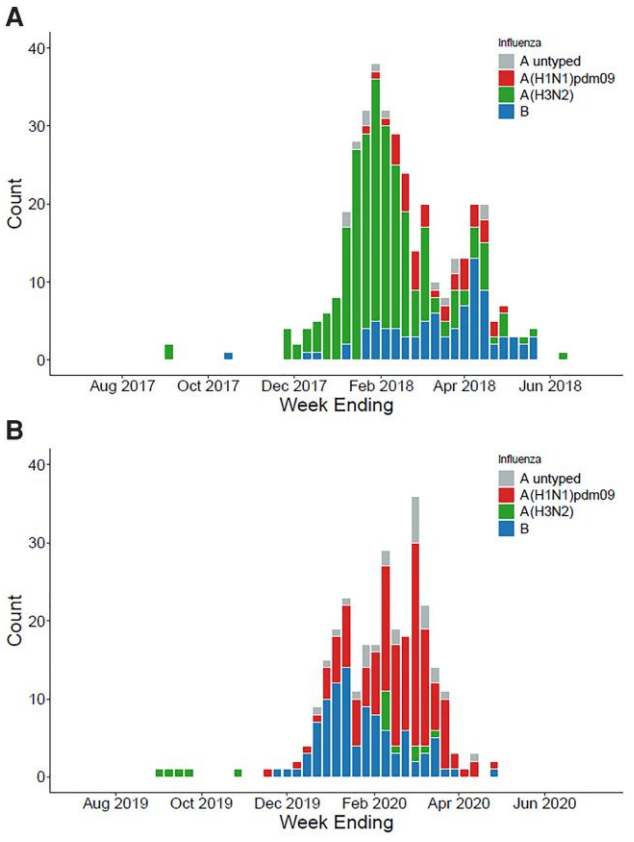

Figure 1. Number of weekly influenza-positive tests among inpatients at University of Michigan hospitals during the 2017–2018 (*A*) and 2019–2020 (*B*) influenza seasons.

In 2019–2020, influenza A(H1N1)pdm09 co-circulated with influenza B with sporadic cases of influenza A(H3N2) locally (Figure 1*B*). A total of 159 individuals were included in the 2019–2020 analysis population including 132 with influenza A(H1N1)pdm09, 1 individual with influenza A(H1N1) pdm09/B co-detection, and 26 with unsubtyped influenza A. Those positive for influenza A(H3N2) (n = 15) or B (n = 89) were excluded (Supplementary Figure 3). Among the 159 individuals included in the 2019–2020 analysis, there were 33 HAII cases identified >48 hours after admission; 22 of these were identified >72 hours after admission. At least 1 residual respiratory specimen was retrieved for 105 of the 159 influenza-positive individuals, including 22 of the 33 HAII cases. A lower proportion of specimens were retrieved in 2019–2020 compared to 2017–2018 in part because those collected after March 2020 were not retrieved due to the coronavirus disease 2019 (COVID-19) pandemic.

High-quality consensus sequences (≥20 reads at ≥80% of positions for all 8 segments) were obtained for 177 of the 220 (80%) patients with at least 1 retrieved specimen (77% of the 230 total influenza cases) including 19 (73%) of the 26 HAII cases in the 2017–2018 influenza season. Only 57 of the 105 (54%) patients with at least 1 retrieved specimen (36% of the 159 total influenza cases) resulted in high-quality consensus sequences in the 2019–2020 season, including 14 (45%) of the 33 HAII cases. Consensus sequences were more likely to be obtained for males than females in both influenza seasons (Table 1; $P < .05$). Availability of sequence data was not associated with patient age, influenza vaccination status, HAII status, or month the first positive influenza test was ordered.

Table 1. Characteristics of Influenza-Positive[a] Inpatients by Whether at Least 1 Residual Respiratory Specimen Was Retrieved and Whether Influenza Virus Consensus Sequence Was Obtained From at Least 1 Retrieved Specimen, 2017–2018 and 2019–2020 Influenza Seasons

	2017–2018					2019–2020				
			Retrieved					Retrieved		
Characteristic	Total	Not Retrieved	Sequenced[b]	No Sequence	*P* Value[c]	Total	Not Retrieved	Sequenced[b]	No Sequence	*P* Value[c]
Sex					.006					.017
Female	109 (47.4)	7 (70.0)	74 (41.8)	28 (65.1)		89 (56.0)	30 (55.6)	26 (45.6)	33 (68.8)	
Male	121 (52.6)	3 (30.0)	103 (58.2)	15 (34.9)		70 (44.0)	24 (44.4)	31 (54.4)	15 (31.2)	
Age group, y					.3					.8
<18	36 (15.7)	0 (0.0)	32 (18.1)	4 (9.3)		36 (22.6)	13 (24.1)	12 (21.1)	11 (22.9)	
18–64	91 (39.6)	3 (30.0)	68 (38.4)	20 (46.5)		81 (50.9)	24 (44.4)	30 (52.6)	27 (56.2)	
≥65	103 (44.8)	7 (70.0)	77 (43.5)	19 (44.2)		42 (26.4)	17 (31.5)	15 (26.3)	10 (20.8)	
Vaccination status					.7					.7
Vaccinated	128 (55.7)	5 (50.0)	100 (56.5)	23 (53.5)		69 (43.4)	25 (46.3)	25 (43.9)	19 (39.6)	
Unvaccinated	102 (44.3)	5 (50.0)	77 (43.5)	20 (46.5)		90 (56.6)	29 (53.7)	32 (56.1)	29 (60.4)	
Source[d]					.6					.3
Community	204 (88.7)	9 (90.0)	158 (89.3)	37 (86.0)		126 (79.2)	43 (79.6)	43 (75.4)	40 (83.3)	
Hospital	26 (11.3)	1 (10.0)	19 (10.7)	6 (14.0)		33 (20.8)	11 (20.4)	14 (24.6)	8 (16.7)	
Month[e]					.2					.8
September	2 (0.9)	0 (0.0)	2 (1.1)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
October	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
November	6 (2.6)	0 (0.0)	6 (3.4)	0 (0.0)		1 (0.6)	0 (0.0)	0 (0.0)	1 (2.1)	
December	22 (9.6)	2 (20.0)	18 (10.2)	2 (4.7)		14 (8.8)	4 (7.4)	6 (10.5)	4 (8.3)	
January	111 (48.3)	3 (30.0)	88 (49.7)	20 (46.5)		33 (20.8)	2 (3.7)	16 (28.1)	15 (31.2)	
February	59 (25.7)	2 (20.0)	44 (24.9)	13 (30.2)		75 (47.2)	12 (22.2)	35 (61.4)	28 (58.3)	
March	16 (7.0)	1 (10.0)	8 (4.5)	7 (16.3)		34 (21.4)	34 (63.0)	0 (0.0)	0 (0.0)	
April	13 (5.7)	2 (20.0)	10 (5.6)	1 (2.3)		2 (1.3)	2 (3.7)	0 (0.0)	0 (0.0)	
May	1 (0.4)	0 (0.0)	1 (0.6)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

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

[a]Inpatients positive for influenza A(H3N2) or unsubtyped influenza A were included in the analysis population for 2017–2018, and inpatients positive for influenza A(H1N1)pdm09 or unsubtyped influenza A were included for 2019–2020.

[b]High-quality consensus sequence of influenza virus (≥20 reads at ≥80% of positions for all 8 segments) obtained from at least 1 residual respiratory specimen.

[c]Pearson χ^2 test; Fisher exact test.

[d]Hospital-acquired cases were defined as those with influenza first identified in a test ordered >48 hours after hospital admission. Those with influenza first identified in a test ordered ≤48 hours after hospital admission were considered community-acquired.

[e]Month the first influenza positive test was ordered.

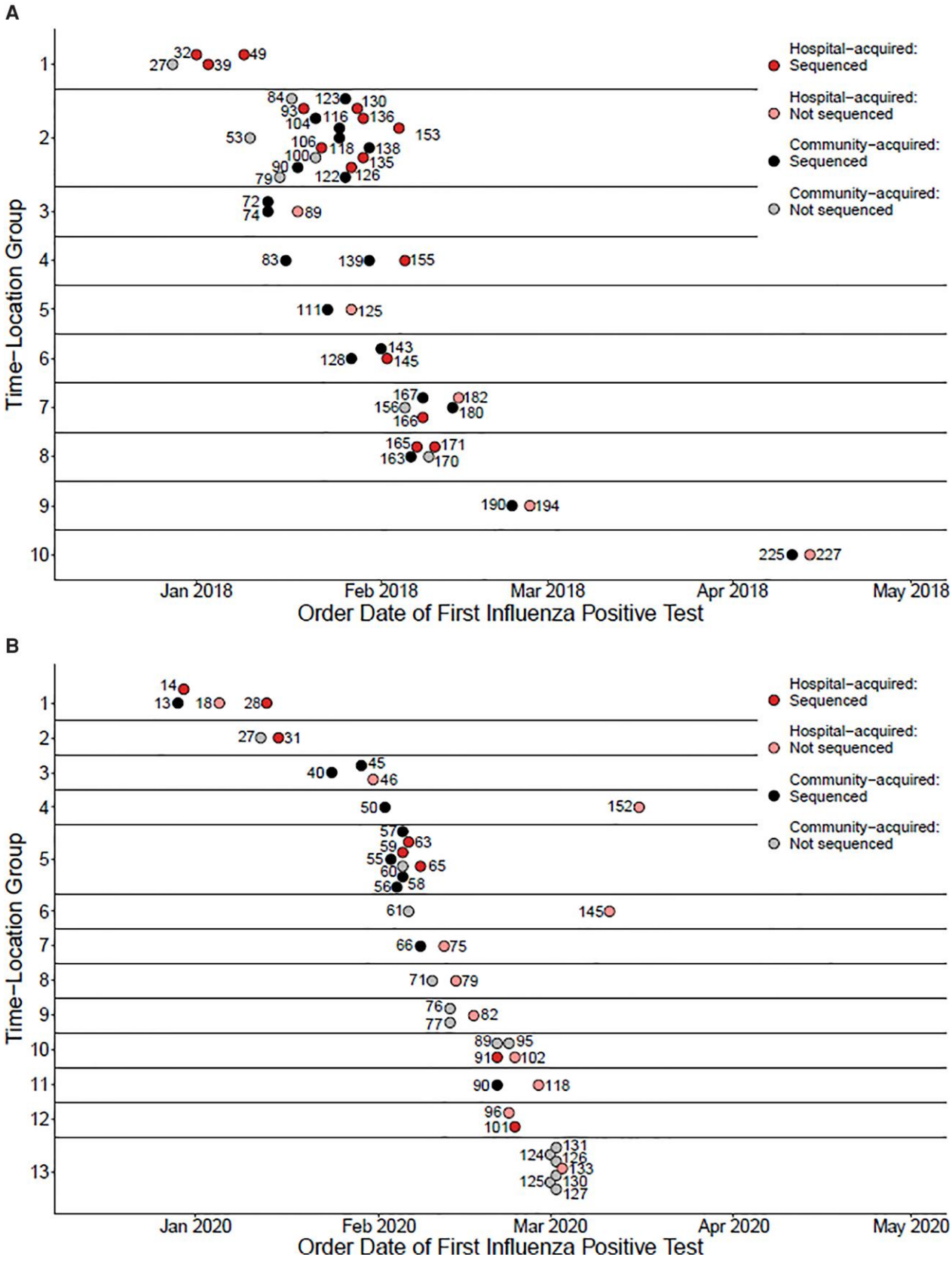

Figure 2. Groups of influenza-positive inpatients with epidemiologic links to hospital-acquired influenza cases defined by time of influenza identification and hospital unit locations, 2017–2018 (*A*) and 2019–2020 (*B*). Row numbers indicate time-location groups, dots are anchored to the date the first influenza-positive test was ordered and are jittered vertically within groups to avoid overlap, and labels are study identification numbers.

Table 2. Summary of Epidemiologic and Phylogenetic Linkages Between Hospital-Acquired Influenza Cases and Other Inpatients With Influenza During the 2017–2018 and 2019–2020 Influenza Seasons

	2017–2018[a]		2019–2020[a]	
Hospital-Acquired Influenza Case Disposition	Hospital-Acquired[b] (48 h) (n = 26)	Hospital-Acquired[b] (72 h) (n = 22)	Hospital-Acquired[b] (48 h) (n = 33)	Hospital-Acquired[b] (72 h) (n = 22)
Sequenced[c]	19 (73.1)	16 (72.7)	14 (42.4)	10 (45.5)
Phylogenetic link with ≥1 other case[d]	9 (34.6)	8 (36.4)	3 (9.1)	3 (13.6)
In time-location group[e]	20 (76.9)	17 (77.3)	19 (57.6)	11 (50.0)
Sequenced and in time-location group with ≥1 other sequenced case	15 (57.7)	13 (59.1)	5 (15.2)	2 (9.1)
In time-location group with phylogenetic link to ≥1 other case	6 (23.1)	5 (22.7)	0 (0.0)	0 (0.0)

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

[a]Inpatients positive for influenza A(H3N2) or unsubtyped influenza A were included in the analysis population for 2017–2018, and inpatients positive for influenza A(H1N1)pdm09 or unsubtyped influenza A were included for 2019–2020.

[b]Hospital-acquired cases were defined as those with influenza first identified in a test ordered >48 hours after hospital admission. A secondary definition included those with influenza first identified in a test ordered >72 hours after hospital admission.

[c]High-quality consensus sequence of influenza virus (≥20 reads at ≥80% of positions for all 8 segments) obtained from at least 1 residual respiratory specimen.

[d]Difference of ≤2 single-nucleotide variants (SNVs) in consensus sequence and closely related based in the phylogenetic tree. A conservative SNV threshold was chosen because previous research has demonstrated low genetic distances between influenza transmission pairs in households [28].

[e]Potential transmission linkage to another influenza case based on overlapping time spent in the same hospital unit in the 4 days prior to the date the hospital-acquired case's first influenza-positive test was ordered.

We defined groups of patients with epidemiologic links to HAII cases through overlapping hospitalization periods, influenza identification prior to the HAII cases, and being roomed in the same unit as the HAII case in the 4 days prior to the HAII identification (Figure 2). We identified 10 groups of time-location–linked patients in 2017–2018, and 13 groups in 2019–2020. Time-location groups ranged in size from only 1 HAII case and 1 community-acquired case to 18 individuals including 7 HAII cases (2017–2018 group 2; Figure 2*A*). However, most were small, with 19 of 23 groups containing ≤4 total cases, and only 4 groups contained ≥3 HAII cases.

In 2017–2018, 20 of the 26 HAII cases were epidemiologically linked to ≥1 other influenza A case within 10 time-location groups (Table 2). Overall, 6 of the 10 time-location groups had ≥2 individuals with consensus sequence data including ≥1 HAII case. Sequence availability was more limited in 2019–2020. Of 33 total 2019–2020 HAII cases, 19 were linked to ≥1 other influenza A case in 13 time-locations groups. However, only 2 of the 13 time-location groups in 2019–2020 had ≥2 individuals with consensus sequence data including ≥1 HAII case. Among the time-location groups with ≥3 HAII cases (2017–2018: groups 1 and 2; 2019–2020: groups 1 and 5), all had high sequence coverage.

After examining the 2017–2018 phylogeny, 2 possible transmission clusters were identified (Figure 3) among the 6 time-location groups with sufficient sequence availability. There were no influenza cases that were closely related in the 2 time-location groups with sufficient sequence availability in 2019–2020 (Figure 4). Patients 58 and 59 in 2019–2020 time-location group 5 appeared close on the phylogenetic tree, but their consensus sequences differed by 7 SNVs. Across both seasons, we identified a total of 33 specimen pairs that had ≤2 SNV differences in their consensus sequences, but only 5 (15%) of these pairs had epidemiologic evidence suggesting a true transmission link.

In the 2017–2018 time-location group 1, 3 HAII cases (patients 32, 39, 49) had nearly identical consensus sequences (≤1 SNV difference); the fourth inpatient included in group 1, a community-acquired case, did not have sequence data available. All 4 influenza cases in group 1 had overlapping time of being located on the same adult inpatient unit (Figure 5). Another 3 HAII cases (patients 130, 135, and 136) from the 2017–2018 time-location group 2 had ≤2 SNV differences in their consensus sequences (Figure 3). Of the 18 total individuals included in group 2, 14 had sequence data available for at least 1 specimen, but none of the other 11 influenza cases were closely related to the 3 previously mentioned HAII cases. A community-acquired case from 2017–2018 group 7 (patient 180) did have an identical consensus sequence, as did 1 of the HAII cases (patient 136) in the group 2 cluster. However, this community-acquired case's specimen collection date was approximately 2 weeks after that of the HAII case.

The inpatient transfer histories of those included in group 2 were more complex than those of group 1, and not every patient had obvious links to all other patients (Figure 5). For example, patients 53 and 122 had time-location links only to patient 136 while patient 136 had links to several other patients. Among the 3 genetically linked patients, patients 135 and 136 had overlapping time in the emergency department and patients 130 and 136 had overlapping time in an adult inpatient unit <4 days prior to the order of their first positive test.

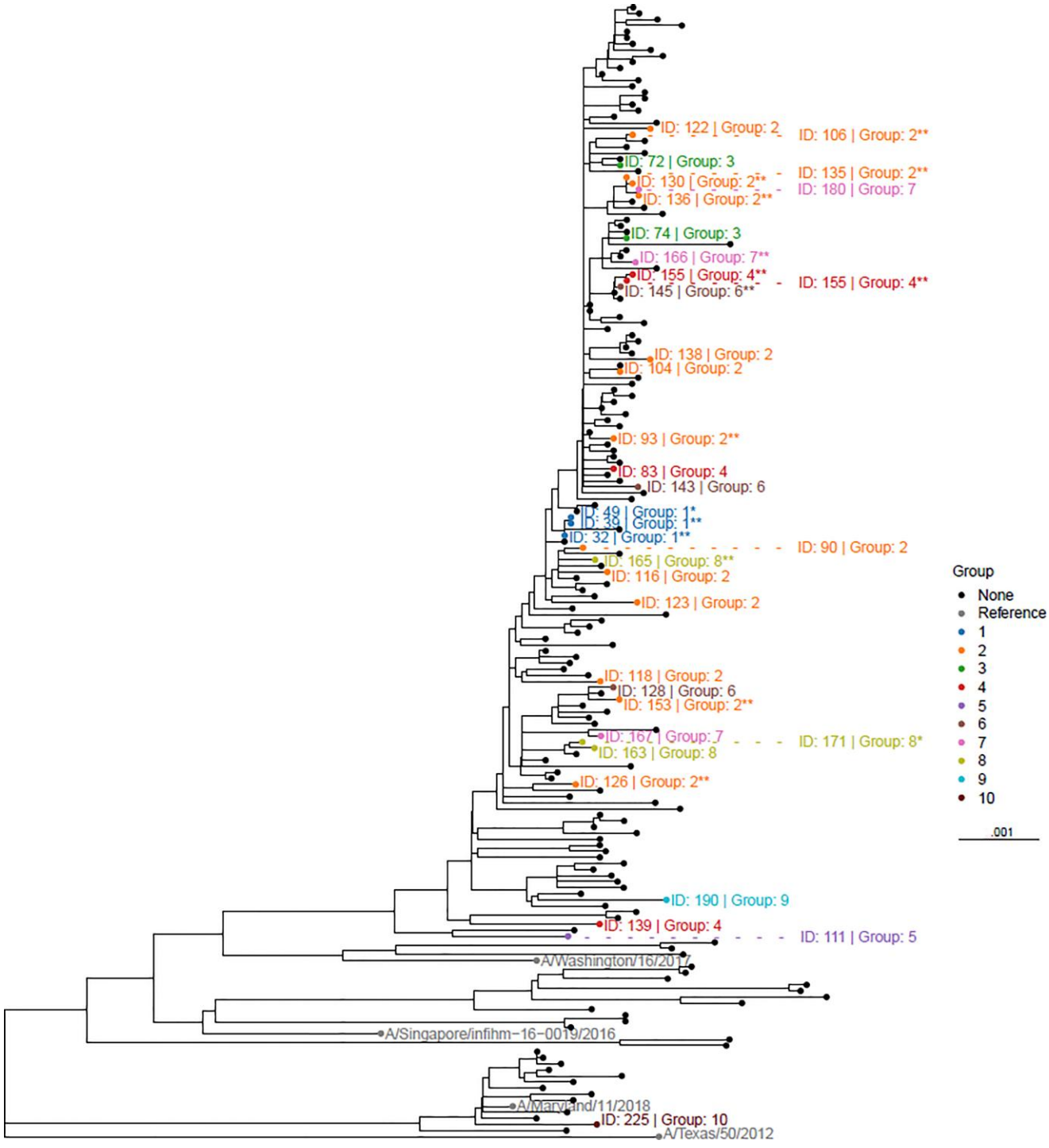

Figure 3. Maximum likelihood phylogenetic tree for 2017–2018 inpatient influenza cases. Labeled tips are those belonging to time-location groups with epidemiologic links to a hospital-acquired influenza case, colored by group. Tip labels are study identification numbers followed by time-location group number; tip labels are extended by dashed lines when needed to avoid overlapping text. *Hospital-acquired case with first influenza-positive test ordered >48 hours after admission. **Hospital-acquired case with first influenza-positive test ordered >72 hours after admission.

DISCUSSION

There was little evidence of sustained in-hospital transmission of influenza A during the 2 years included in the study period. Most epidemiologically linked time-location groups were small: 19 of 23 had ≤4 total patients, and only 4 groups contained ≥3 HAII cases. Only 8 time-location groups had sufficient sequence

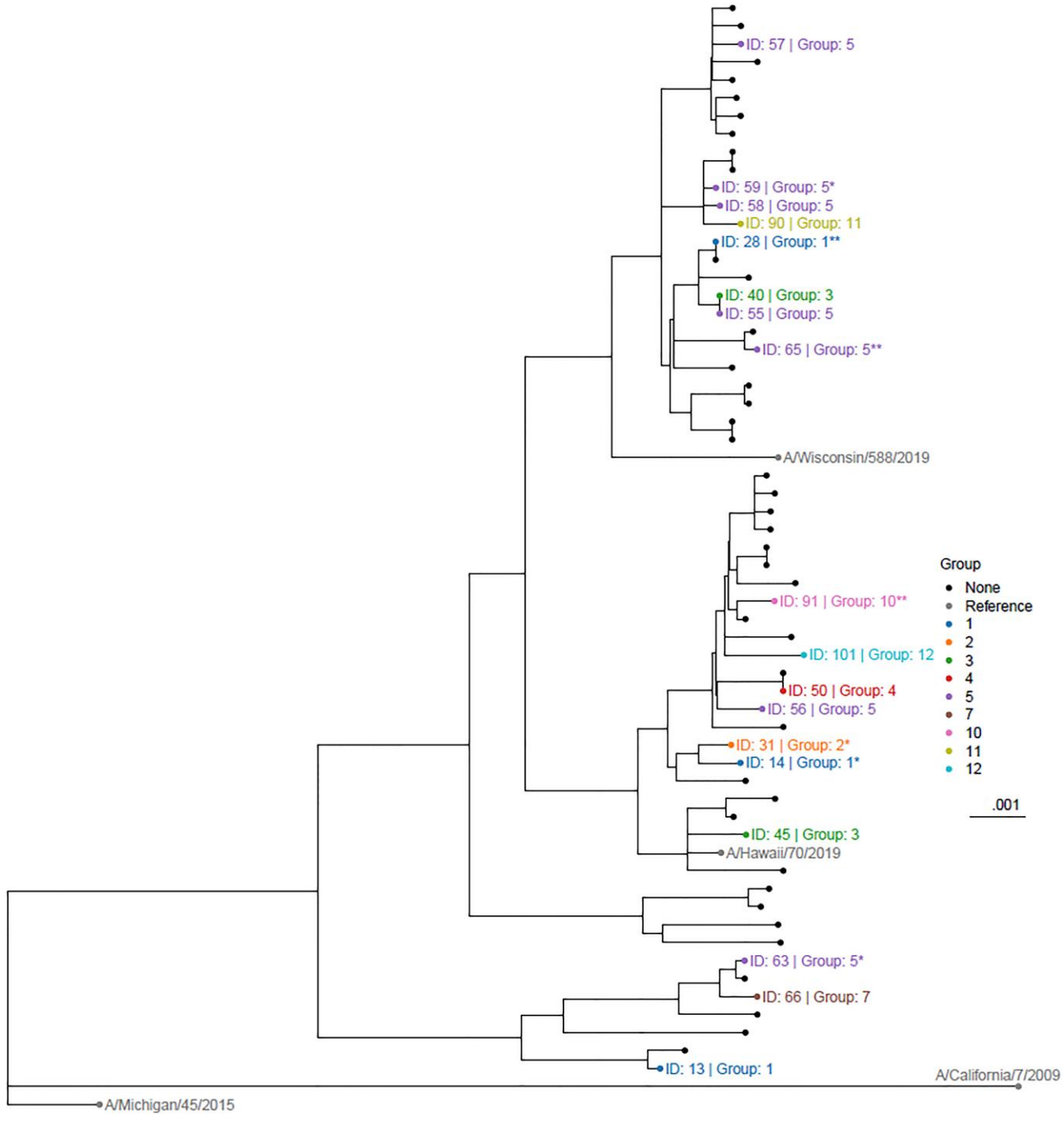

Figure 4. Maximum likelihood phylogenetic tree for 2019–2020 inpatient influenza cases. Labeled tips are those belonging to time-location groups with epidemiologic links to a hospital-acquired influenza case, colored by group. Tip labels are study identification numbers followed by time-location group number. *Hospital-acquired case with first influenza-positive test ordered >48 hours after admission. **Hospital-acquired case with first influenza-positive test ordered >72 hours after admission.

coverage (≥2 patients with sequence data, including at least 1 HAII case), including all 4 with ≥3 HAII cases. Of these 8 groups, only 2 contained genetically linked infections. In each of these 2 time-location groups, there were 3 HAII cases that were genetically linked; 1 of these groups contained 4 additional HAII cases that were not genetically linked. Taken together, these results suggest that HAIIs arise from outbreak transmission from nosocomial sources (eg, patient to patient transmission or multiple transmissions from a single HCW) as well as single infections from unique community introductions.

In addition to standard infection control practices to prevent in-hospital transmission (eg, droplet precautions, private

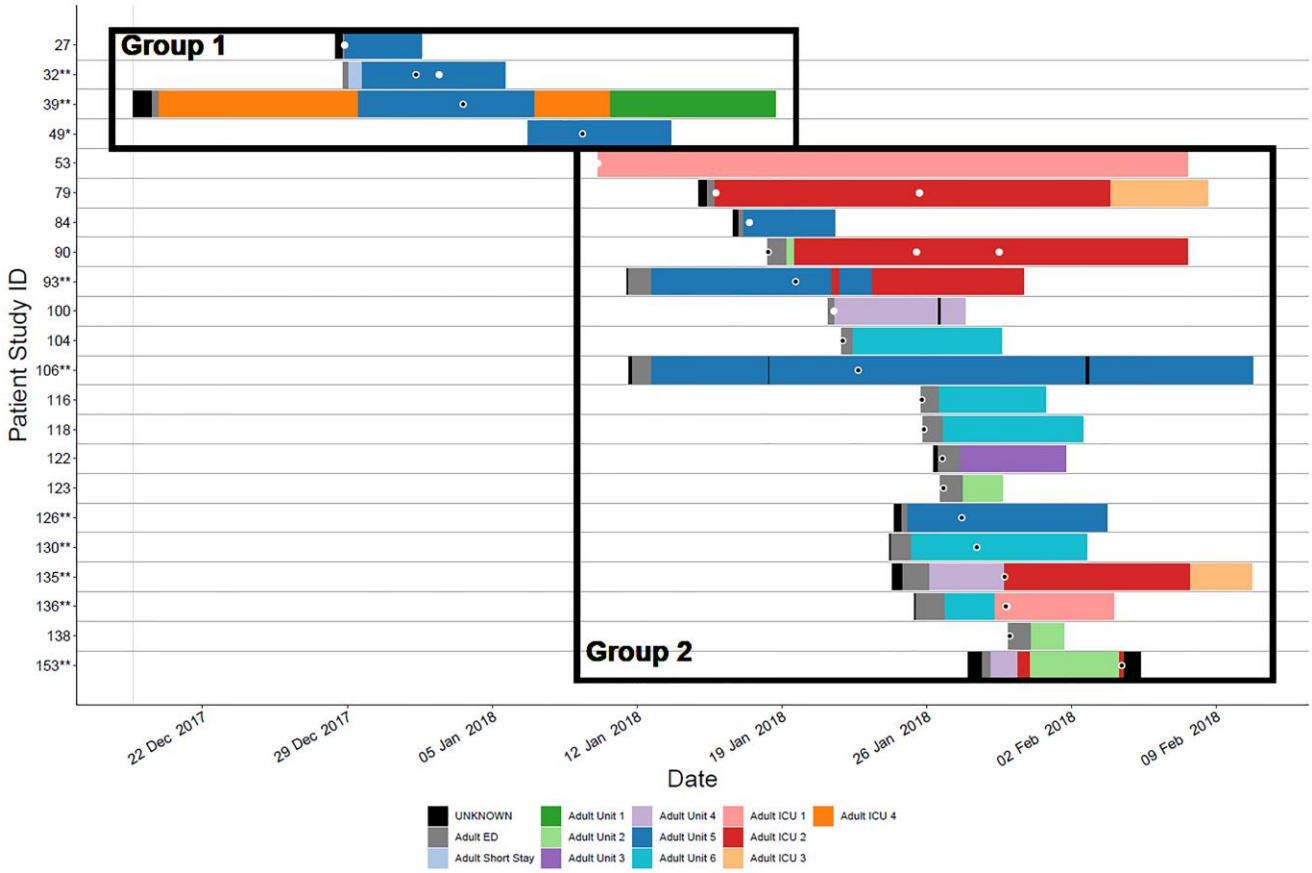

Figure 5. Hospital unit transfer history for 2017–2018 time-location groups of patients with epidemiologic links to hospital-acquired influenza that contained phylogenetically linked influenza cases. Rows represent individual patients; single and double asterisks identify hospital-acquired cases with first influenza-positive test ordered >48 and >72 hours after admission, respectively. Dots represent collection of an influenza-positive specimen; those with a black center were successfully sequenced, and those completely white were not. Patients 32, 39, and 49 in group 1 were phylogenetically linked; patients 130, 135, and 136 in group 2 were phylogenetically linked. Abbreviations: ED, emergency department; ICU, intensive care unit.

rooms), our results highlight the importance of measures that prevent influenza introduction in the first place. For HCWs, influenza vaccination is a cornerstone of HAII prevention and has been demonstrated to be effective in improving patient safety [29]. Influenza vaccination coverage was 98% among HCW in our system in the 2 study seasons [30], but there is room for improvement in the United States overall with vaccination coverage declining to 81% during the 2020–2021 season [31]. HCW presenteeism, or working when ill, has been a challenging issue in infection control that has persisted during the COVID-19 pandemic [32, 33]. Supporting HCWs in staying home when ill and identifying barriers to doing so is a priority for further reducing HAII. Source control via masking and proper hand hygiene compliance are also important interventions for the prevention of HAII. Enhanced visitor restrictions and universal masking policies, such as those implemented during the COVID-19 pandemic, could also be effective in reducing HAII, but further research is needed to find the optimal balance of risks and benefits of these policies [34, 35].

Previous studies attempting to link HAII by WGS have often focused on known outbreaks [10–15]. In the current study, we attempted to perform WGS for all hospitalized patients with laboratory-confirmed influenza, including both community-acquired and HAII cases. By sampling community-acquired cases, it is possible to assess how frequently hospitalized patients with influenza might have similar sequences by chance without a transmission link. Overall, we identified 33 specimen pairs that had ≤2 SNV differences in their consensus sequences across the 2 influenza seasons, but only 15% of these pairs had epidemiologic evidence suggesting a true transmission link. The lack of agreement between epidemiologic and genetic definitions of HAII transmission is consistent with previous studies that have sequenced both community-acquired and HAII cases across an influenza season [16–18]. This suggests that the combination of data sources is more powerful than either alone.

Although we were able to retrieve residual respiratory specimens from a high proportion of patients with positive

influenza test results, high-quality WGS data were only obtained for a portion of specimens (80% in 2017–2018 and 54% in 2019–2020). Therefore, it is possible we were unable to identify all instances of within-hospital transmission. In addition, males were more likely to have specimens successfully sequenced than females in both years, potentially biasing our results. We may have also missed intermediate cases because we did not collect specimens from HCWs or visitors, and asymptomatic patients were unlikely to be tested clinically. Better characterization of infection within these populations is necessary to confirm our findings. Finally, this study was carried out over 2 seasons in a single hospital and focused on influenza A; patterns of transmission may not be generalizable to other settings or viruses.

Our time-location group definitions were based on unit-level locations. It is possible that patients could have come into contact with each other during procedures or other short-term in-hospital transfer events. Similarly, we were additionally unable to account for possible transmission from HCWs who may work across units such as physicians and support staff. The time-based definitions we used to define HAII are also subject to misclassification. For example, community-acquired cases with delayed testing might be considered hospital-acquired, but their influenza viruses would not necessarily be expected to be genetically related to others in the hospital.

In summary, we found a relatively small number of genetically linked hospital clusters over 2 influenza seasons and suspect most HAII cases were from independent introductions from the community. Because most HAII occurrences were likely independent of one another, additional preventive measures could focus on reducing introductions by HCWs and visitors while maintaining current infection control practices to prevent hospital outbreaks.

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

Acknowledgments. Consensus genomes generated in this study are available at the Global Initiative on Sharing Avian Influenza Data (GISAID). Genome accessions and analysis code are available at https://github.com/jgpetrie/HAII_transmission.

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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