Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Arons MM, Hatfield KM, Reddy SC, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. N Engl J Med. DOI: 10.1056/NEJMoa2008457

Supplementary Appendix

Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility

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Detailed Methods and Results

Cohort Description

Details of our cohort from Facility A are presented in Figure 1. There were 89 residents living in Facility A on March 3, the day the first positive resident was identified, and by March 13, there were 82 residents. There were no admissions to Facility A since March 3. Of the 82 residents living in Facility A on March 13; two were hospitalized earlier in the day, prior to the first point prevalence survey, one was not present (receiving hemodialysis offsite), three declined testing, and 76 completed the symptom screening and testing. By March 26, 57 residents were positive for SARS-CoV-2; 48 of these had participated in our point prevalence surveys, 8 residents were unavailable for point prevalence surveys but tested positive during hospitalizations, and 1 resident who declined to participate in the point prevalence survey tested positive post-mortem.

Symptom Screening Form

At the time of the first point prevalence study, the nursing staff from Facility A provided information on a symptom screening form for each resident including date of birth, gender, unit and room number. The form required the nursing staff to complete a medical chart review for documentation of any pre-existing conditions, including history of: chronic lung disease, diabetes mellitus, cardiovascular disease (e.g., congestive heart failure), cerebrovascular accident/stroke, renal disease, cognitive impairment, obesity, and current treatment with hemodialysis.

Symptoms were assessed by nursing staff on all participating residents at both point prevalence surveys and for those asymptomatic with a positive test, one week following the last survey. The data collection tool assessed if the resident had any of the following in the preceding 14 days: typical symptoms such as fever, cough, shortness of breath, or atypical symptoms such as chills, myalgia (body aches), malaise (feeling generally unwell), sore throat, runny nose or congestion, confusion or sleepiness, dizziness, headache, diarrhea, and nausea and/or vomiting. For each symptom selected (except for fever or chills), the form requested information on whether that symptom was chronic (i.e., ongoing for more than 14 days) for that resident. For all symptoms selected, the form then asked if the symptom was new or worsened, and to provide the date of onset. The form then asked if the resident was still experiencing this symptom on the day of the survey, and if not, to list the date the symptom resolved.

At the time of the first point prevalence survey, clinicians interviewed residents to confirm symptoms reported by nursing staff on the screening tool. The outbreak investigation team used the electronic medical record to identify pre-existing conditions if not listed on the symptom screening form. At the conclusion of both point prevalence surveys, the outbreak investigation team reviewed the electronic medical record to corroborate symptoms onset dates for all patients with positive test results.

The most commonly reported symptom among residents with a positive SARS-CoV-2 test was cough (33%). Residents also reported malaise (13%), and fever (10%), although residents with negative SARS-CoV-2 tests also reported fever (14%), cough (14%), and malaise (11%) most frequently (Table S1). Characteristics, including age and pre-existing conditions, were also similar when positive residents were stratified by symptom status at time of test (Table S2). Among the 24 residents who were asymptomatic at the time of testing and developed symptoms in the subsequent week, the most commonly reported symptoms were fever, cough, and malaise (Table S3).

Cognitive impairment was common among residents with both positive (58%) and negative tests (46%). Of those with a positive test, similar proportions of residents who reported symptoms at the time of testing had cognitive impairment (62%), compared to those who did not report symptoms (56%).

Specimen Testing

For the initial point prevalence survey, nasopharyngeal (NP) and oropharyngeal (OP) swabs (if collected) were placed in the same viral transport media. For the follow-up survey, on March 19-20, 62 residents were tested and NP and OP swabs were placed in separate tubes and analyzed separately. Specimen collection and diagnostic testing were conducted in accordance with CDC guidelines for respiratory specimens [1]. The Washington State Public Health Laboratory performed one step real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) assay on all NP/OP combined samples from March 13 and all NP samples from March 19-20. In addition, 33 OP samples from March 19-20 were tested (15 OP samples sent to the lab were not tested). The limit of detection for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (RNA copies /ul) was determined to be 100.5 and 100 using the QIAGEN EZ1 Advanced XL or DSP Viral RNA Mini Kit, respectively; at these concentrations, the CDC rRT-PCR panel identified N1 and N2 positive targets at a rate of 100% [2]. Additionally, the percent agreement of the CDC rRT-PCR diagnostic panel with a composite comparator was 100% (n=13, 95% CI: 77.2% - 100%) for positive specimens and 100% (n=104, 95% CI; 96.4% - 100%) for negative specimens [2].

Of the 76 tested residents on March 13, 23 tests were positive for SARS-CoV-2, and 53 were negative, including one resident with a negative test on March 13 who had a preceding positive test on March 8 and reported sore throat and fever. This resident was considered positive with typical symptoms for our symptom analyses.

On March 19-20, 62 residents were retested for SARS-CoV-2, including 49 who were negative on March 13. From this second point prevalence survey, 36 residents were positive, including 24 newly identified positives among the 49 who were negative as of March 13. Of the 33 residents from the second point prevalence study who had both NP and OP results available, six residents had discordant NP and OP tests; all six had a positive OP test and negative NP test. These residents were considered positive for the second point prevalence survey.

Cycle threshold (Ct) values were reported for two genetic markers: N1, N2 [2] for 47 residents who tested positive during the point prevalence studies (i.e., not including the resident with a positive test result only from March 8). For residents with both NP and OP Ct values reported for the second point prevalence survey, we selected the swab with the lower Ct value.

The median Ct values for each of the symptom status groups were similar (asymptomatic resident's median Ct value: 25.5 (Q1 to Q3: 25.3 to 31.1), presymptomatic: 23.1 (17.1 to 30.8), atypical symptoms: 25.3 (19.8 to 29.7), typical symptoms: 21.5 (19.8 to 25.7)). Similar to findings shown in Figure 2 in the manuscript, Ct values did not appear to be correlated with the days from symptom (typical or atypical) onset to testing (Figure S1). Among twelve residents with two consecutive positive tests for SARS-CoV-2, symptom onset date did not appear to be associated with increases or decreases in serial Ct values (Figure S2).

Culture and Sequencing

Specimens were shipped to CDC's laboratory for viral culture, where the rRT-PCR-positive specimens were used to inoculate Vero-CCL-81 cells (one culture not assessed). Cells showing cytopathic effect were used for SARS-CoV-2 rRT-PCR to confirm isolation and viral growth in culture. The mean Ct values for genetic marker N1 for samples positive by rRT-PCR with positive culture growth was lower than for those without (22.4 vs 30.6).

Nucleic acid was extracted from rRT-PCR positive specimens and amplified for subsequent Oxford Nanopore MinION sequencing [3]. Consensus sequences by nanopore sequencing were generated using minimap 2.17 and samtools 1.9. Phylogenetic trees were inferred with the Neighbor-Joining method

using the Hasegawa-Kishino-Yano nucleotide substitution model with gamma-distributed rate variation among sites (HKY + G) with 500 bootstrap replicates using Geneious Prime (Biomatters, INC., San Diego, CA) and MEGA X [4].

Sequencing results for 39 positive primary specimens from 34 residents and resulted in nine closely-related sequences with 1 to 4 nucleotide (NT) difference compared to each other (Supplementary Table S5). Sequences were submitted to Genbank and GISAID (Table S6). For 5 residents with two specimens sequenced, the first and specimen sequence were identical. Of the 34 residents, 27 fell into two clusters (sequence B and sequence H,) with 1 NT difference (Supplementary Table S3). All sequences are identical or highly similar to previously reported USA-WA sequences branching form USA-WA1/sequences from China (Supplementary Figure S3).

Facility A Transmission

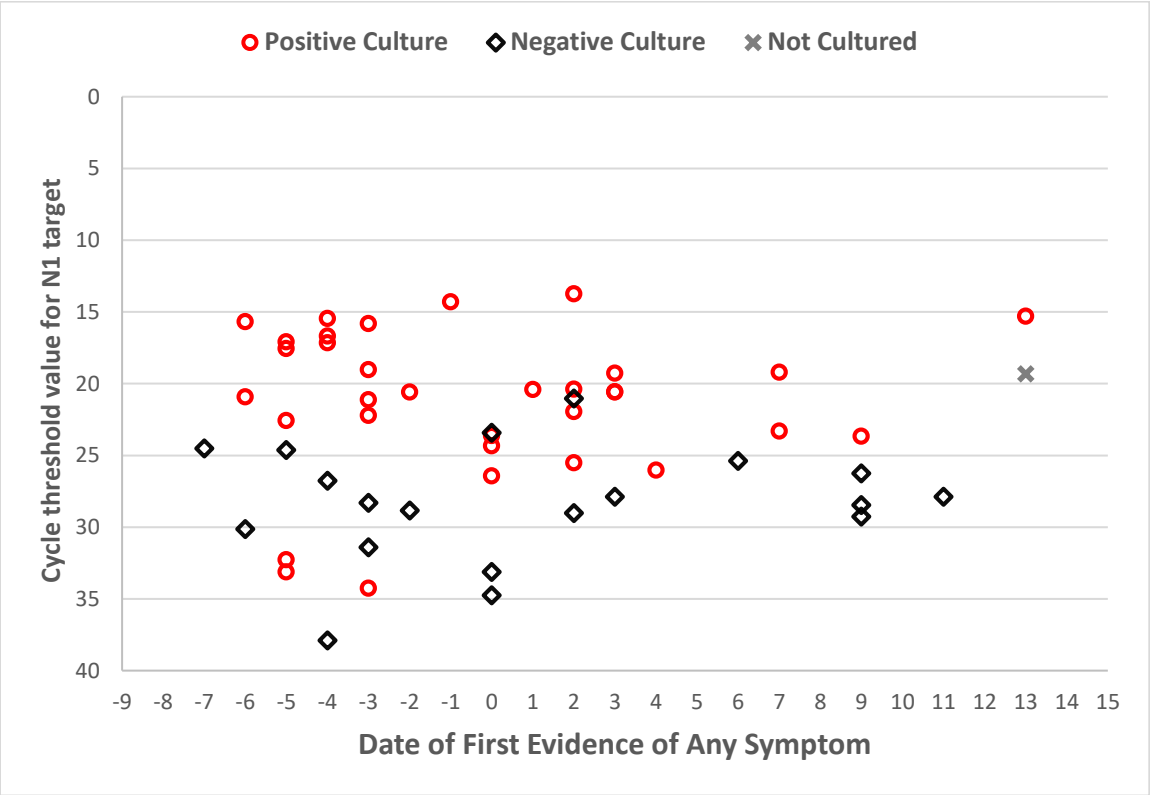
To evaluate facility transmission dynamics, residents with positive test results for SARS-CoV-2 were identified from March 3 to March 26, including those tested in acute care settings and post-mortem. No admissions to Facility A occurred during this period. The daily growth rate was estimated using least-squares regression of the log-transformed daily cumulative counts of residents positive for SARS-CoV-2, from March 3 through our second point prevalence survey, March 20. Doubling time was estimated as the natural log(2) divided by the daily growth rate. The daily growth rate was estimated as 0.204 (95% CI: 0.131, 0.278) and the doubling time among residents in this facility was estimated to be 3.4 days (95% CI: 2.5 to 5.3, Table S4). The doubling time for all residents of King County with positive tests for SARS-CoV-2 was estimated using the same method and time period using counts reported through PHSKC COVID-19 data dashboard as 5.5 days (95%CI: 4.8, 6.7) [5].

Residents were mapped according to their room assignment and their infection onset date. Infection date is symptom onset date or test date for those asymptomatic at time of testing. Unit of residence appears to be associated with infection date, with the majority of unit one infections occurring first. Sequencing results identified most sequenced isolates from unit 1 and 2 were from one cluster (sequence H), while isolates from unit 4 were from the second cluster (sequence B, Figure S4).

Tables and Figures

Figure S1. Cycle threshold values relative to date of first evidence of any symptoms (typical or atypical).

Shown are N1 target cycle threshold values and viral culture results for each resident's positive tests for SARS-CoV-2 shown by day since first evidence of fever, cough, or shortness of breath (N=53*).



*Includes 42 residents with known onset dates for first reported symptoms; 11 residents have two specimens positive for SARS-CoV-2 included. One resident reported having experienced atypical symptoms in the 14 days preceding testing, but no onset date was listed.

Figure S2. Serial cycle threshold values for the N1 target for 12 residents with two positive tests**.**

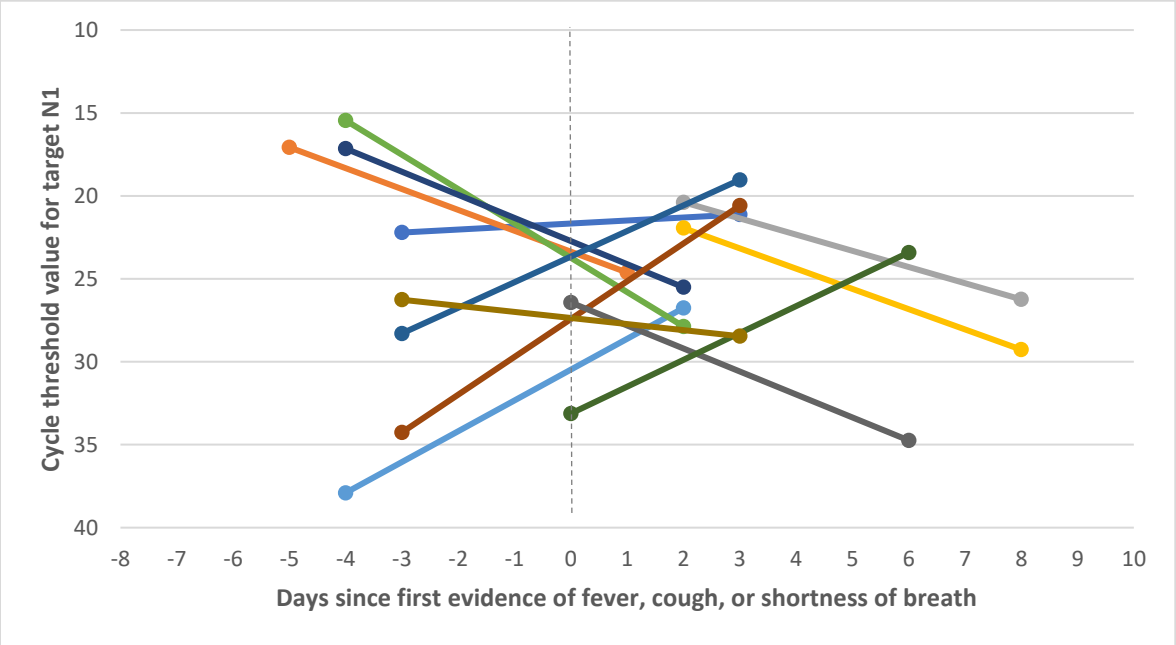


Figure S3. Phylogenetic tree of selected SARS-CoV2 genomes including 39 genomes from this study.

Select full genome sequences available as of March 24, 2020 were included in this Neighbor-Joining tree. A) Polar tree layout of sequences analyzed. Blue branches indicate sequences from USA-WA and red branches indicate sequences from this study (USA-WA, nursing home). B) Branch view of USA-WA nursing home cases (red). The 39 genomes were from specimens of 34 residents. All 5 residents with 2 specimens had identical sequences from the first and second specimens.

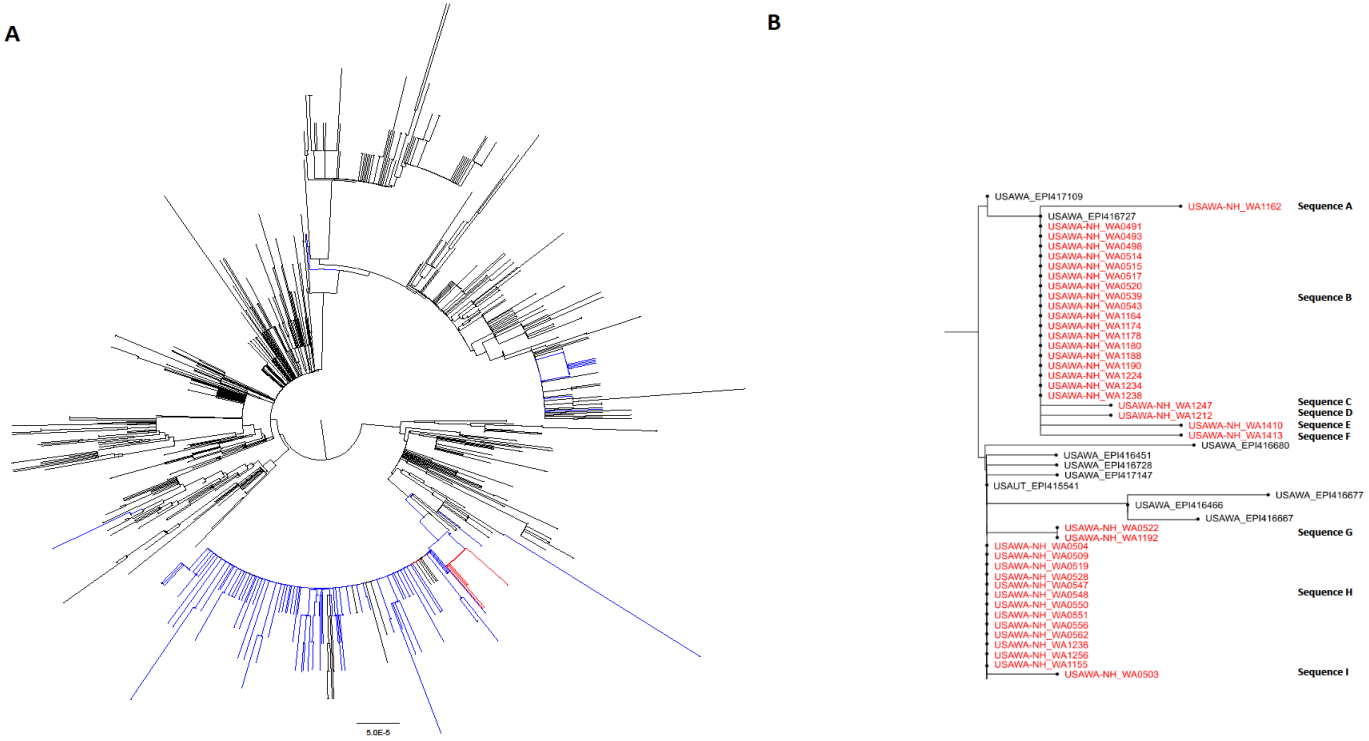
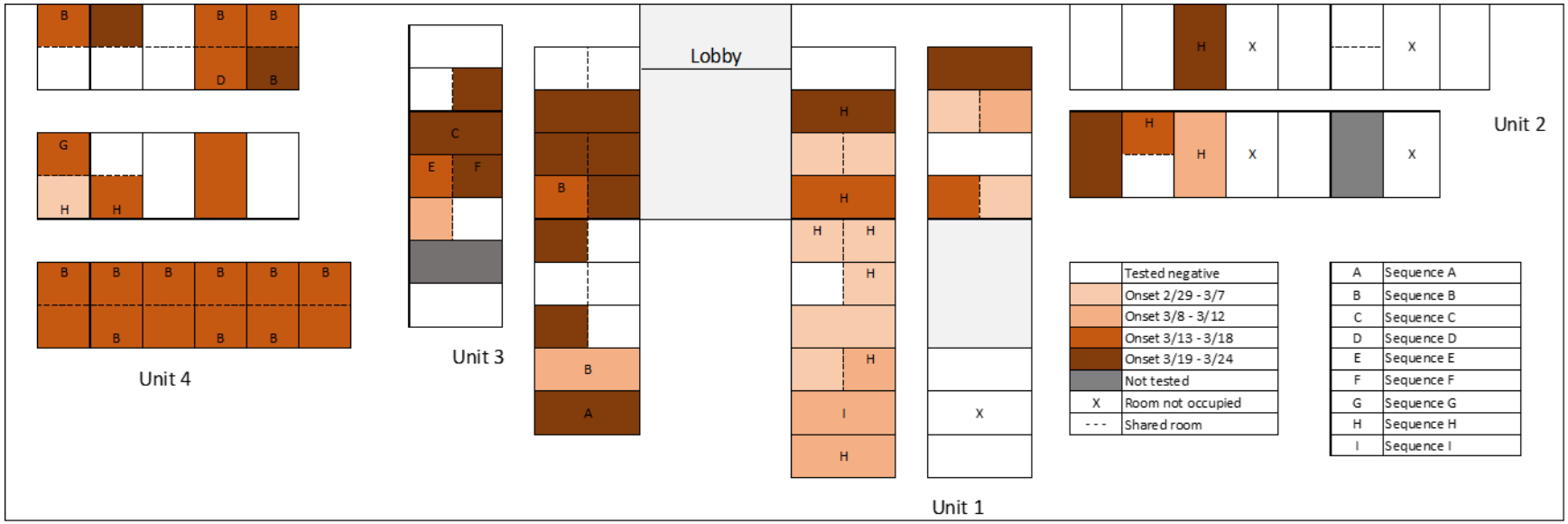


Figure S4. Map of residents of Facility A with positive tests for SARS-CoV-2.
Shown here is a map of facility A denoting residents positive for SARS-CoV-2. Coloring of room indicates date of symptom onset*, and letter in room denotes the genetic sequence of isolates** from that room, double occupancy rooms are denoted by a dashed line



*For asymptomatic or presymptomatic residents, onset is the date of the first positive test.

**Sequences A-I represent nine closely-related sequences from 34 residents with up to 4 nucleotide (NT) difference (see Figure S3 and Table S5)

Table S1. Specific symptoms for residents of Facility A at time of testing, by SARS-CoV-2 test results — King County, Washington, March 2020

Characteristics	SARS-CoV-2 Test Results	
	Positive, No. (%)	**Negative, No. (%)**
Overall	48 (63%)	28 (37%)
Specific signs and symptoms reported as new/worse in preceding 14 days		
Typical symptoms		
Fever	5 (10%)	4 (14%)
Cough	16 (33%)	4 (14%)
Shortness of breath	4 (8%)	0 (0%)
Atypical symptoms		
Sore throat	3 (6%)	2 (7%)
Chills	0 (0%)	0 (0%)
Confusion	2 (4%)	1 (4%)
Rhinorrhea or congestion	1 (2%)	1 (4%)
Myalgia	0 (0%)	0 (0%)
Dizziness	2 (4%)	0 (0%)
Malaise	6 (13%)	3 (11%)
Headache	1 (2%)	0 (0%)
Nausea	3 (6%)	1 (4%)
Diarrhea	2 (4%)	2 (7%)
Symptoms reported as chronic and stable for preceding 14 days		
Cough	12 (25%)	6 (21%)
Shortness of breath	1 (2%)	3 (11%)
Confusion	12 (25%)	3 (11%)
Rhinorrhea or congestion	3 (6%)	2 (7%)
Myalgia	1 (2%)	1 (4%)
Dizziness	1 (2%)	3 (11%)
Headache	1 (2%)	2 (7%)
Nausea	2 (4%)	1 (4%)

*Results include all residents present in facility March 13 and assented to screening. Facility-wide cohort symptom screens and testing performed on March 13 and March 19-20, 2020. Positive residents include those with at least one positive test from facility-wide cohort screening on March 13 and March 19-20 and one resident who was negative on March 13 but tested positive in unit-wide cohort screening on March 8.

Table S2. Demographic characteristics and comorbidities stratified by symptom grouping at time of SARS-CoV-2 test

Characteristics	SARS-CoV-2 Test Results		
	Positive, Symptoms	Positive, Asymptomatic	Negative
Overall	21 (28%)	27 (36%)	28 (37%)
Female gender	16 (76%)	14 (52%)	18 (64%)
Age, years, Mean (SD)	82.0 (7.4)	75.9 (10.2)	73.8 (11.5)
SNF length of stay <90 days prior to test	10 (48%)	13 (48%)	14 (50%)
Reported any chronic stable symptoms	10 (48%)	15 (56%)	11 (39%)
Presence of pre-existing conditions and comorbidities	20 (95%)	27 (100%)	28 (100%)
Chronic Lung disease	10 (48%)	8 (29%)	8 (29%)
Diabetes	8 (38%)	10 (37%)	11 (39%)
Cardiovascular disease	16 (76%)	23 (85%)	17 (61%)
Cerebrovascular accident	8 (38%)	11 (41%)	8 (29%)
Renal Disease	8 (38%)	10 (37%)	9 (32%)
Received Hemodialysis	1 (5%)	2(7%)	1 (4%)
Cognitive Impairment	13 (62%)	15 (56%)	13 (46%)
Obesity	6 (29%)	5 (19%)	6 (21%)

Table S3. Symptoms reported at 7-day follow-up among residents with a positive SARS-CoV-2 test who were asymptomatic at time of testing (N=27).

Asymptomatic at time of testing (n= 27)	No. (%)
Remained Asymptomatic	3 (11%)
Presymptomatic (developed new symptoms)	24 (89%)
Time to symptom onset, days, Median (Q1 to Q3)	4 (3 to 5)
Fever	17
Cough	13
Malaise	10
Shortness of breath	7
Confusion	4
Rhinorrhea/congestion	3
Diarrhea	3
Sore throat	2
Dizziness	1
Headache	1

Table S4. Number of residents of Facility A with a positive test for SARS-CoV-2 by date used to calculate doubling time.

Date	Number of Positive Tests	Cumulative Number of Positive Tests	Ln (Cumulative Number of Positive Tests)
3/3/2020	1	1	0
3/5/2020	1	2	0.693147
3/6/2020	5	7	1.94591
3/8/2020	5	12	2.484907
3/11/2020	1	13	2.564949
3/13/2020	18	31	3.433987
3/18/2020	1	32	3.465736
3/19/2020	20	52	3.951244
3/20/2020	4	56	4.025352
Growth Rate (slope)	0.204	95% CI: 0.131, 0.278	
Doubling Time	3.4	95% CI: 2.5, 5.3	

Table S5 Nucleotide differences between sequences among 39 genomes from this study.

39 genomes were sequenced from 34 residents; 5 residents had two specimens sequenced (which produced identical sequences)

Sequence name (no. positive cases)	Sequence A	Sequence B	Sequence C	Sequence D	Sequence E	Sequence F	Sequence G	Sequence H	Sequence I
Sequence A (1)		2	1	1	2	2	2	1	2
Sequence B (18)			1	1	2	2	2	1	2
Sequence C (1)				2	3	3	3	2	3
Sequence D (1)					3	3	3	2	3
Sequence E (1)						4	4	3	4
Sequence F (1)							4	3	4
Sequence G (2)								3	4
Sequence H (13)									1
Sequence I (1)									

Table S6. Genbank accession numbers and GISAID identification numbers for the 39 genomes sequenced in this study

Study Identification	GenBank Accession number	GISAID ID
2019-nCoV/USA/WA-NH2/2020	MT262896	418771
2019-nCoV/USA/WA-NH3/2020	MT262897	418772
2019-nCoV/USA/WA-NH4/2020	MT262898	418773
2019-nCoV/USA/WA-NH5/2020	MT262899	418774
2019-nCoV/USA/WA-NH6/2020	MT262900	418775
2019-nCoV/USA/WA-NH7/2020	MT262901	418776
2019-nCoV/USA/WA-NH8/2020	MT262902	418777
2019-nCoV/USA/WA-NH9/2020	MT262903	418778
2019-nCoV/USA/WA-NH10/2020	MT262904	418779
2019-nCoV/USA/WA-NH11/2020	MT262905	418780
2019-nCoV/USA/WA-NH12/2020	MT262906	418781
2019-nCoV/USA/WA-NH13/2020	MT262907	418782
2019-nCoV/USA/WA-NH14/2020	MT262908	418783
2019-nCoV/USA/WA-NH17/2020	MT262909	418784
2019-nCoV/USA/WA-NH18/2020	MT262910	418785
2019-nCoV/USA/WA-NH19/2020	MT262911	418786
2019-nCoV/USA/WA-NH20/2020	MT262912	418787
2019-nCoV/USA/WA-NH21/2020	MT262913	418788
2019-nCoV/USA/WA-NH22/2020	MT262914	418789
2019-nCoV/USA/WA-NH23/2020	MT262915	418790
2019-nCoV/USA/WA-NH24/2020	MT262916	418791
2019-nCoV/USA-WA_0428/2020	MT350264	426439
2019-nCoV/USA-WA_0434/2020	MT350266	426442
2019-nCoV/USA-WA_0442/2020	MT350267	426445
2019-nCoV/USA-WA_0480/2020	MT350263	426450
2019-nCoV/USA-WA_0418/2020	MT350268	426436
2019-nCoV/USA-WA_0427/2020	MT350265	426438
2019-nCoV/USA-WA_0429/2020	MT350276	426440
2019-nCoV/USA-WA_0440/2020	MT350278	426443
2019-nCoV/USA-WA_0448/2020	MT350272	426447
2019-nCoV/USA-WA_0478/2020	MT350273	426449

2019-nCoV/USA-WA_0484/2020	MT350275	426451
2019-nCoV/USA-WA_0441/2020	MT350277	426444
2019-nCoV/USA-WA_0711/2020	MT350279	426452
2019-nCoV/USA-WA_0432/2020	MT350270	426441
2019-nCoV/USA-WA_0422/2020	MT350274	426437
2019-nCoV/USA-WA_0447/2020	MT350280	426446
2019-nCoV/USA-WA_0797/2020	MT350269	426453
2019-nCoV/USA-WA_0457/2020	MT350271	426448

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