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MAJOR ARTICLE

Surveillance of Severe Acute Respiratory Syndrome Coronavirus 2 and Variants Using Digital Droplet Polymerase Chain Reaction at a Large University and Healthcare System in California

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Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with different infectivity, transmission potential, and morbidity change the characteristics of local epidemics and affect vaccine effectiveness. As part of the University of Southern California COVID-19 Pandemic Research Center's efforts to understand, control, and inform local community on coronavirus disease 2019 (COVID-19), we implemented a SARS-CoV-2 surveillance program among students, employees, and USC Keck Medical Center patients. We present the epidemiology and distribution of SARS-CoV-2 and its variants among the population.

Methods. We used digital droplet reverse-transcriptase polymerase chain reaction (PCR) to analyze in real-time remnant SARS-CoV-2 PCR-positive saliva specimens stored at the USC Keck Medicine laboratory between September 2020 and April 2022. Samples were tested for the original strain (A20) and 9 SARS-CoV-2 variants: α(B.1.1.7, Q.1–Q.8), β(B.1.351, B.1.351.2, B.1.351.3), γ(P.1, P.1.1, P.1.2), δ(B.1.617.2), δ+(or δ417N), ϵ(B.1.427 and B.1.429), η(B.1.525), λ(C.37) and o(B.1.1.529, BA.1, BA.2). We reviewed deidentified health information from positive cases including demographics, history of COVID-19 (eg, symptoms, hospitalizations, and repeat infections), and COVID-19 vaccination status.

Results. We reviewed 1169 cases and determined the variant type of 482 specimens: 77 specimens were original strain, 119 "Delta", 165 "Omicron". The original strain was detected during the third and fourth quarters of 2020. The Delta variant appeared during the second quarter of 2021, whereas Omicron appeared in the fourth quarter of 2021.

Conclusions. Prospectively tracking SARS-CoV-2 variants in a university population and a hospital system, utilizing a low-cost, high-throughput PCR assay, was feasible. Local variant monitoring remains important to inform prevention and control efforts among university and clinical settings.

Keywords. COVID-19; SARS-CoV-2; surveillance; university; variants.

Since the introduction of severe acute respiratory syndrome coronavirus 2 ([SARS-CoV-2] December 2019), the virus that causes coronavirus disease 2019 (COVID-19), many SARS-CoV-2 variants have emerged worldwide. Due to accumulated genetic mutations, these variants demonstrate differences in their capacity to cause disease, clinical manifestations, transmissibility, and their capacity to evade the immune system [1–3]. Those characteristics help the virus cause new outbreaks and maintain endemicity. To date, the Centers for Disease Control and Prevention (CDC) has characterized the Delta (PANGO B.1.617.2 and AY lineages) and Omicron (B.1.1.529 and BA lineages) SARS-CoV-2 variants as variants of concern (VOC), while actively monitoring an additional 10 variants (Table 1). Strategic, continuous monitoring of the local epidemiology of SARS-CoV-2 variants is important to inform local disease control policies.

Academic institutions, such as universities and colleges, are communities with characteristics that could lead to outbreaks;

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Table 1. Demographics and Clinical Characteristics of Cases Tested Positive for SARS-CoV-2: University of Southern California Variant Study 2020–2022

Characteristics	Students, Employees (n = 70)	Patients (n = 1099)	Total (N = 1169)
Mean age, years (standard deviation)	27.4 (±12.0)	48.0 (±19.4)	46.7 (±19.7)
Male	24 (34.3%)	535 (48.7%)	559 (47.8%)
Heritage	…	…	…
Non-Hispanic White	36 (51.4%)	339 (30.9%)	375 (32.1%)
Hispanic or Latino	4 (5.7%)	358 (32.6%)	362 (31%)
Black or African American	5 (7.1%)	47 (4.3%)	52 (4%)
Asian and Middle Eastern	11 (15.7%)	81 (7.4%)	92 (7.8%)
Native Hawaiian/Pacific Islander and Native American/Alaska Native	0	1 (0.1%)	1 (0.1%)
Multiracial	2 (2.9%)	37 (3.4%)	39 (3.3%)
Other or Unknown	12 (17.1%)	236 (21.5%)	247 (21.1%)
Pregnant	0	6 (0.6%)	6 (0.5%)
Symptomatic Upon Testing	…	…	…
Yes	51 (72.9%)	646 (58.8%)	697 (59.6%)
No	17 (24.3%)	252 (22.9%)	269 (23%)
Clinical Evaluation Not Occurred	2 (2.9%)	201 (18.3%)	202 (17.3%)
Reported Comorbidities	…	…	…
Yes	7 (10.0%)	810 (73.7%)	817 (69.8%)
No	44 (62.9%)	249 (22.7%)	293 (25%)
Clinical Evaluation Not Occurred	19 (27.1%)	40 (3.6%)	58 (5%)
Required Hospitalization	…	…	…
Yes	0	91 (8.3%)	91 (7.8%)
No	68 (97.1%)	932 (84.8%)	1000 (85.5%)
Clinical Evaluation Not Occurred	2 (2.9%)	76 (6.9%)	78 (6.7%)
Repeat Infections	6 (8.6%)	27 (2.5%)	33 (2.8%)
Vaccination Status at Time of Infection	…	…	…
Up-to-date vaccinations[a]	11 (15.7%)	241 (21.9%)	252 (21.6%)
Fully vaccinated[b]	38 (54.3%)	399 (36.3%)	437 (37.4%)
Partially vaccinated[c]	0	0	0
Unvaccinated	21 (30.0%)	459 (41.8%)	480 (41.1%)

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Up-to-date, fully vaccinated and receipt of an additional dose of Pfizer-BioNTech or Moderna COVID-19 vaccine.

[b]Fully vaccinated, receipt of 2 vaccine doses for persons who received Pfizer-BioNTech, Moderna, or unspecified US-authorized or approved mRNA COVID-19 vaccine, or receipt of 1 dose for persons who received Janssen.

[c]Partially vaccinated, receipt of the first vaccine dose of Pfizer-BioNTech, Moderna mRNA COVID-19 vaccine.

that is, a high number of individuals residing in proximity, engaging in frequent travel, and participating in large number of indoor events (eg, parties, musical shows). Modeling studies showed that universities have an increased risk of outbreaks during their return to class season [4–6]. As expected, outbreaks occurred among students [7–9] and in response, universities developed operational plans, testing strategies, surveillance systems, and risk mitigation strategies [10, 11]. Since the onset of the pandemic, hospitals and medical care systems have been routinely testing newly admitted patients presenting for healthcare and treatment [12]. Regular tracking of test positivity rates and COVID-19 morbidity rates, such as hospitalizations, morbidity, and mortality, has been crucial in the management of the COVID-19 pandemic. As the COVID-19 pandemic switches from control to mitigation, it is important to maintain efficient and low-cost surveillance systems that could readily monitor new variants.

As part of the University of Southern California (USC) COVID-19 Pandemic Research Center's efforts to understand, control, and inform the local community on COVID-19, we developed a surveillance system for SARS-CoV-2 and its variants among students, employees, and Keck Medical Center of USC patients. Using a novel, low-cost, easily adaptable, high-throughput polymerase chain reaction (PCR) and combining analysis of stored remnant-positive specimens from routine weekly USC campus SARS-CoV-2 testing with continually collected metadata, we attempted to monitor and describe the real-time epidemiology of SARS-CoV-2 and its variants.

METHODS

We reviewed the medical records and analyzed remnant specimens of cases that tested positive for SARS-CoV-2 infection. The study population consists of (1) USC students and staff and (2) patients of the Keck Medical Center who tested for COVID-19 either as part of the university-wide COVID-19 screening program or as part of their clinical care.

We acquired patient clinical information directly from their medical records. A waiver of informed consent was acquired for the patients of Keck Medical Center. Students and staff were invited to participate via weekly, university-wide email invitations. Potential participants would visit the study enrollment page, where they received information about the study, and they were asked to provide informed consent and sign a Health Insurance Portability and Accountability Act of 1996 (HIPAA) release form. We included available specimens and data that were collected between September 2020 and April 2022.

Patient Consent Statement

Participants provided their written consent was obtained and permission to access data was granted by the University regulations. The study has been approved by the ethical committee of the University of Southern California Institutional Review Board under IRB# UP-21-00393 and HS-21-00366.

Specimen Collection and Processing

Saliva samples were collected using a saliva-chew method; individuals chewed on a plastic straw until approximately 2 mL saliva was collected in a VACUETTE tube without additives. Samples were transferred to USC Clinical Laboratories,

Molecular Pathology within 2 hours of collection. Specimens were transported at room temperature upon collection and after processing they were stored at −80°C. Upon receipt, samples were heat inactivated at 65°C before SARS-CoV-2 testing with reverse-transcriptase PCR (RT-PCR). Remnant solutions were biobanked at −80°C before further processing. Only samples with confirmed SARS-CoV-2 detection and a cycle threshold less than or equal to 30 were included in the study.

Development and Validation of the Assay

For variant determination, the specimens were analyzed using RT-digital droplet PCR (ddPCR) [13, 14]. Our team developed molecular probes and validated the assay for remnant saliva specimens, as described elsewhere [15]. In brief, we designed 7 primer/probe sets targeting hallmark mutations in the SARS-CoV-2 spike protein gene that would enable characterization of VOC. Primers were made to amplify wild-type or mutated sequences at S-gene amino acid positions 69/70, 222, 241-3, K417 and K417T, L452R, N501Y, and P681R. In addition, an internal control set of primers targeting the human RNase P/Protein Coding Gene 30 (RPP30) was included, using the sequences previously published by the US CDC as an internal control for SARS-CoV-2 (CDC number 2019-nCoV EUA-01). The Magbind Viral DNA/RNA 96 Kit (Omega Bio-Tek) was used to extract total nucleic acids from patient samples following the manufacturers protocol. Extraction was done on either the KingFisher Apex Purification System (Irwindale, CA) or KingFisher Duo Prime Purification System. The One-Step RT ddPCR Advanced Kit for Probes (Irvine, CA) was combined with primers. Samples were loaded onto a Bio-Rad CFX96 Deep Well Real-Time thermocycler. Droplet fluorescence was measured using a QX200 Droplet Reader (Bio-Rad) paired with QuantaSoft software (Bio-Rad). Highly concentrated samples were diluted before ddPCR to avoid saturation of the assay.

Heat-inactivated viruses of the Washington Isolate USA-WA1/2020 (NR-52286), Alpha (NR-55245), Beta (NR-55350), Delta (NR-56128), and Omicron (NR-56495) variants were obtained from the National Institutes of Health's Biodefense and Emerging Infections Research Resources Repository (BEI Resources). In addition, remnant saliva samples from patients infected with Gamma, Delta, Delta K714N, and Omicron variants that were heat-inactivated, sequenced, and stored at –80°C in Zymo DNA/RNA Shield (Zymo) were provided by Curative Inc. (San Dimas, CA).

Finally, we confirmed the ddPCR identification in a subset of 124 samples by whole-genome sequencing (WGS), including 120 samples clearly identified by ddPCR and the 4 samples with an unexpected pattern. The subset of clearly identified samples included 13 samples with the original strain lineage, 9 Alpha, 72 Delta, 1 Mu, and 25 Omicron samples. The WGS data confirmed 100% of the ddPCR results.

The assay was adapted as new variants emerged allowing the continual identification and monitoring of new variants. Samples were tested for the original strain (A20) and 9 SARS-CoV-2 variants: Alpha (B.1.1.7, Q.1–Q.8), Beta (B.1.351, B.1.351.2, B.1.351.3), Gamma (P.1, P.1.1, P.1.2), Delta (B.1.617.2), Delta + (or δ417N), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Lambda (C.37), and Omicron (B.1.1.529, BA.1, BA.2).

Data Extraction and Analysis

Data extracted from the medical record were entered into REDCap. Cases with repeated SARS-CoV-2 positive test results more than 30 days after initial detection were considered a new case of infection ("reinfection"). We followed the CDC's definitions on reporting vaccination status at the time of infection [16] (see Table 2). Continuous variables are expressed as mean (standard deviation), and categorical variables are expressed as percentages and absolute frequencies. We performed the data cleaning and analyses utilizing SAS Software Version 9.4 (Cary, NC). Data visualizations were created in Tableau (Seattle, WA).

Human Subjects Considerations

The study was reviewed and approved by the ethical committee of the University of Southern California Institutional Review Board (IRB) under IRB Number UP-21-00393 and HS-21-00366.

RESULTS

During the project period, 6280 samples were tested at USC Clinical Laboratories, Keck Medicine by RT-quantitative PCR and 3698 were included in this study because they had a cycle threshold value less than 30. We extracted the data from 1099 Keck patient records and analyzed 412 available samples. In addition, 3074 USC students and staff members consented to data-use and variant testing: 239 tested positive for SARS-CoV-2, and 70 had remnant samples suitable for molecular variant analysis. Reasons for samples not being available included the following: (1) samples tested in outside laboratories, eg, Los Angeles County Public Health laboratory; (2) "self-reported" test results; or (3) remnant specimen not stored after testing. In total, we extracted data from 1169 case records and analyzed 482 available remnant specimens from both populations.

Demographic and clinical characteristics of the cases are shown on Table 1. Among the 482 samples analyzed, 116 samples yielded no result due to insufficient quantity of the sample. Of the remaining 366 samples, we identified the original SARS-CoV-2 strain (20A) as well as variants of concern ("Delta", "Omicron") and variants of interest ("Alpha", "Gamma") (Table 3). The original strain (20A) was detected

Table 2. Demographic and Clinical Characteristics of Cases by Variant Type: University of Southern California Variant Study 2020–2022

Characteristics	Original (20A) (n = 77)	Delta (n = 119)	Omicron (n = 165)	*P* Value
Age Group				
0–17 years old	3 (3.9%)	12 (10.1%)	13 (7.9%)	.62
18–44 years old	34 (44.2%)	45 (37.8%)	65 (39.4%)	…
45–64 years old	22 (28.6%)	38 (31.9%)	58 (35.2%)	…
65+ years old	18 (23.4%)	24 (20.2%)	29 (17.6%)	…
Gender				
Male	39 (50.7%)	66 (55.5%)	77 (46.7%)	.34
Female	38 (49.4%)	53 (44.5%)	88 (53.3%)	…
Heritage				
Non-Hispanic White	47 (61.0%)	46 (38.7%)	43 (26.1%)	<.01
Hispanic or Latino	11 (14.3%)	29 (24.4%)	43 (26.1%)	…
Black or African American	2 (2.6%)	8 (6.7%)	11 (6.7%)	…
Asian/Middle Eastern	3 (3.9%)	7 (5.9%)	18 (10.9%)	…
Other/ Unknown	10 (13.0%)	26 (21.9%)	44 (26.8%)	…
Multiracial	4 (5.2%)	3 (2.5%)	6 (3.6%)	…
Vaccination Status at Time of Infection				
Up-to-date vaccination[a]	0	4 (3.4%)	50 (30.3%)	<.01
Fully vaccinated[b]	0	65 (54.6%)	75 (45.5%)	…
Partially Vaccinated[c]	0	0	0	…
Unvaccinated	77 (100%)	50 (42%)	40 (24.2%)	…
Time Between Completion of Vaccination and Infection[d]				
…	N = 0	N = 69	N = 125	<.01
0–3 months	0	8 (11.6%)	6 (4.8%)	…
4–6 months	0	25 (36.2%)	10 (8.0%)	…
6–9 months	0	30 (43.5%)	44 (35.2%)	…
9–12 months	0	6 (8.7%)	64 (51.2%)	…
≥12 months	0	0	1 (0.8%)	…

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Up-to-date, fully vaccinated and receipt of an additional dose of Pfizer-BioNTech or Moderna COVID-19 vaccine.

[b]Fully vaccinated, receipt of 2 vaccine doses for persons who received Pfizer-BioNTech, Moderna, or unspecified US-authorized or approved mRNA COVID-19 vaccine, or receipt of 1 dose for persons who received Janssen.

[c]Partially vaccinated, receipt of the first vaccine dose of Pfizer-BioNTech, Moderna mRNA COVID-19 vaccine.

[d]Unvaccinated individuals not included.

Table 3. SARS-CoV-2 Variant Distributions Among Analyzed Specimens: University of Southern California Variant Study 2020–2022

Specimen Results	Students, Staff (n = 12)	Patients (n = 354)	Total (N = 366)
Original (20A)	6 (50%)	71 (20%)	77 (21%)
Alpha (B.1.1.7)	0	3 (0.8%)	3 (0.8%)
Gamma (P.1)	0	1 (0.2%)	1 (0.3%)
Delta and Delta Plus (B.1.617.2)	4 (33%)	115 (32.5%)	119 (32.5%)
Omicron (B.1.1.529)	2 (16%)	163 (46%)	165 (45.1%)
Omicron BA2	0	1 (0.2%)	1 (0.3%)

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

during the third and fourth quarter of 2020. Cases infected with the "Delta variant" started appearing during the second quarter of 2021, whereas cases with the Omicron variant started appearing on the fourth quarter of 2021 (Figure 1).

We explored differences between cases infected with the original, the Delta, and the Omicron variant (Table 2). We did not identify any differences in age and sex between the 3 groups. A higher proportion of individuals identifying as non-Hispanic White were infected with the original strain (61.0%) compared with Delta (38.7%) and Omicron (26.1%), whereas a higher proportion of individuals identifying as Hispanic or Latino were infected with Omicron (28.4%) compared with the other variants ($P < .01$). The proportion of individuals who were unvaccinated at the time of infection declined (98.7% for original strain, 41.2% for the Delta strain, and 22.4% for the Omicron; $P < .01$).

DISCUSSION

We implemented a surveillance program monitoring SARS-CoV-2 variants in a large university and medical center in Los Angeles, California. The program combined a low-cost, high-throughput RT-ddPCR assay, with health information data review. Digital droplet PCR has been validated and utilized for detection of other infectious diseases [17], but to our knowledge this is one of the few programs utilizing it for SARS-CoV-2 variant determination. We examined remnant samples from patients with SARS-CoV-2 infection and detected a similar pattern of variant distribution as that identified within the community of Los Angeles County determined by convential sequencing methods, as shown in Figure 1.

The SARS-CoV-2 variant surveillance program we described in this manuscript could be used complementary to WGS in settings, where genome sequencing may not be widely available or too expensive to use broadly [18]. The core of the program relies on a widely available PCR methodology, so this assay can be scaled to different settings from large centers processing hundreds of samples to smaller laboratories. A surveillance program using RT-ddPCR could be used to test a large volume of samples, which would allow real-time monitoring of the variants among those tested. Sequencing would be limited to a small number of positive samples selected randomly or based on epidemiological or clinical criteria to detect emerging variants. An additional benefit would be that this RT-ddPCR assay can be easily adapted to track new and emerging variants for ongoing surveillance. However, further research would be

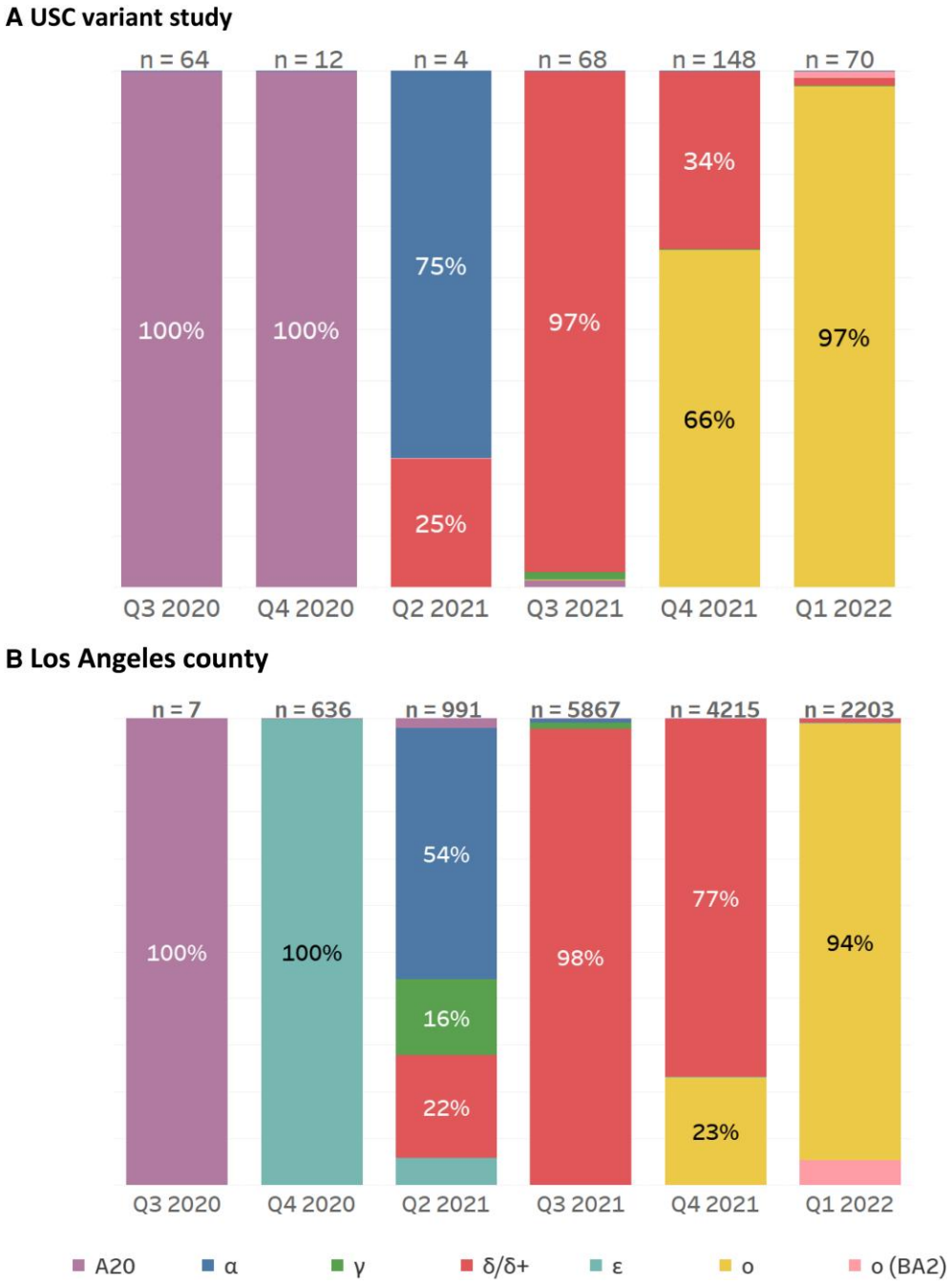

Figure 1. University of Southern California SARS-CoV-2 Variant Study—Distribution of SARS-CoV-2 variants detected among the study cases and comparison with cases detected in Los Angeles County, September 2021 to April 2022. Los Angeles county Data source: GISAID (https://www.gisaid.org/hcov19-variants/). Proportions <5% are not shown in the figure.

needed to fully evaluate the efficacy and cost-effectiveness of our RT-ddPCR-based testing algorithm.

The ddPCR surveillance program could also provide information to assist clinical practice. The type of circulating SARS-CoV-2 variants impacts the selection of therapeutics [19], such as neutralizing antibodies, so the local epidemiology of variants is clinically important. The ddPCR surveillance program we describe can provide rapid and low-cost variant information that if integrated as a reflex to clinical SARS-CoV-2 testing could provide timely and actionable clinical information. The ddPCR is not cleared by the US Food and Drug Administration and is not currently approved for clinical management. As a laboratory-developed test, it could be verified and used in a clinic setting in accordance with the Clinical Laboratory Improvement Amendments (CLIA). Our study did not use the assay in that way; however, this is a potential next step for future research.

We extracted the data and analyzed the available samples of patients. However, we collected a limited number of data elements and specimens from USC faculty or staff, which limited our capacity to study the variant distribution in this population. Access to databases and medical records of USC students and employees was restricted due to University policies that were in place to require mandated testing of students and the workforce. Our team had to acquire additional consent and HIPAA release from individuals due to University policies. Limited access and use of samples only after obtaining the additional consent led to slow study enrollment and delays in acquiring the samples. That resulted in delayed sample acquisition and variant reporting. Restrictive data and testing policies are important to protect individuals' privacy and confidentiality, but they can serve an adverse role in disease monitoring and limit public health responsiveness. As described by Mahraj et al [20], close collaboration by institutions, researchers, and public health specialists is paramount to adapt policies that ensure easier access to data while maintaining privacy and confidentiality.

The distribution of variants detected among the study population had a similar pattern to the variant distribution to samples from Los Angeles County, analyzed by the Los Angeles County Public Health Laboratory. Our team did not identify any patients infected with the Gamma variant during the fourth quarter of 2020 and the second quarter of 2021. During that period, the USC Clinical Laboratories, Keck Medicine were sending whole specimens to the Public Health laboratory for genetic sequencing, thus limiting the availability of remnant samples for inclusion in this study. With the increasing prevalence of SARS-CoV-2 among communities and changing clinical presentations (eg, fewer symptoms and lower disease severity), it may be efficient to establish monitoring of diverse populations at risk for outbreaks. Hospital systems that serve large areas and institutions of higher education could be 2 such candidate populations.

Throughout the project, we invested in the University's student workforce. We hired and trained a large team of Public Health and Health Science students to assist in sample preparation and data collection. Student researchers completed the basic human subjects' research and HIPAA trainings, as well a training on electronic medical record review and standardized data collection. Training was fast and simple, because the project took advantage of university applications and software such as REDCap, with which students were familiar from their coursework. Surveillance projects are great opportunities for future members of the public health workforce to acquire actual experience.

There are certain limitations that should be taken into consideration. Data were extracted from the available health information records of students, staff, and patients. It is possible that these individuals received testing or care from providers outside the USC medical care network, so we may be missing information, such as cases of reinfection. We enrolled only a small number of USC faculty or staff, which limits the representativeness of our findings to the USC population.

CONCLUSIONS

The COVID-19 pandemic is now entering its fourth year. Mass vaccination programs, easy access to testing, and effective treatment have changed the face of the epidemic. New variants of potential public health importance, such as the recent BA.4/5 and BA.2.75, are constantly emerging. Ongoing monitoring of the distribution of variants among incident cases is important. Public health organizations need to leverage widely available, accessible, and low-cost tools and work towards adapting policies that will permit greater collaborative efforts between researchers and public health scientists to best exploit all available resources.

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Potential conflicts of interest. All authors: No reported conflicts of interest.

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