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# Investigation of SARS-CoV-2 Epsilon Variant and Hospitalization Status by Genomic Surveillance in a Single Large Health System During the 2020-2021 Winter Surge in Southern California

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## ABSTRACT

**Objectives:** This study aimed to assess whether the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Epsilon variant (B.1.429/427) is more virulent, leading to more hospitalization and more severe disease requiring intensive care unit (ICU) admission.

**Methods:** SARS-CoV-2 genomic surveillance was performed on respiratory samples from 231 unique patients, collected at a single large health system in Southern California between November 2020 and March 2021 during the winter surge.

**Results:** The frequencies of the Epsilon variant among outpatients, hospitalized patients, and ICU patients were indifferent.

**Conclusions:** Our study suggests that the Epsilon variant is not associated with increased hospitalization and ICU admission.

## INTRODUCTION

Between November 2020 and March 2021, California experienced a winter surge of coronavirus disease 2019 (COVID-19), with peak daily new cases surpassing 44,000 (https://covid19.ca.gov/). During this surge, the prevalence of two emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineages, B.1.429 and B.1.427 (Epsilon variant), increased steadily and became dominant in California.[1] Many questions were raised, including whether this variant is more contagious, is more virulent, or can negatively affect efficacies of vaccines and/or therapeutic neutralizing antibodies.[2] Studies have shown the Epsilon variant is indeed more transmissible and has reduced susceptibility to bamlanivimab, which prompted the Centers for Disease Control and Prevention (CDC) to consider it a variant of concern (VOC) until it became less frequent in June 2021.[3,4] However, few studies have existed to suggest whether the Epsilon variant is more virulent, leading to more hospitalization, and more severe disease requiring intensive

## KEY POINTS

- The SARS-CoV-2 Epsilon variant dominated the 2020 to 2021 COVID-19 winter surge in Southern California.
- Genomic surveillance showed that the frequencies of the Epsilon variant among outpatients, hospitalized patients, and ICU patients were indifferent.
- The Epsilon variant was found to be not associated with increased hospitalizations and ICU admissions.

## KEY WORDS

SARS-CoV-2; Epsilon variant; B.1.429; B.1.427; Hospitalization; ICU; California; Genomic surveillance; COVID-19

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care unit (ICU) admission. This genomic surveillance study was aimed to provide an assessment to address this concern.

## MATERIALS AND METHODS

From November 20, 2020, to March 10, 2021, a total of 231 upper respiratory tract samples from 231 unique patients (210 randomly selected and 21 from deceased ICU patients) with a polymerase chain reaction cycle threshold (Ct) less than 25 from our institution were included in the study. The randomly selected samples were submitted weekly to the Los Angeles County Public Health (LACDPH) laboratory for sequencing. The 21 samples from deceased ICU patients were specifically chosen to assess the possibility of any dominant variant that caused most severe disease and death during the surge. The patient's demographic information and hospitalization status were required by the LACDPH when the samples were submitted. Three months after the last sample was collected (March 10, 2021), the hospitalization status of all cases was checked again, and the analysis in this study was based on the hospitalization status updated at least 3 months after the samples were collected. If a patient had ever been admitted to either the hospital or the ICU due to COVID-19, the case was counted as an inpatient or an ICU patient, respectively. Most samples were collected in January (n = 98) and February (n = 77) 2021. Samples were sequenced on MiSeq (Illumina) by an amplicon-based protocol using the ARTIC primers version 3 (https://artic.network/resources/ncov/ncov-amplicon-v3.pdf) or a shotgun metagenomics approach using the NEBNext Ultra II RNA protocol (NEB) (Supplemental Methods; all supplemental materials can be found at *American Journal of Clinical Pathology* online). Quality criteria for sequences include (1) percent genome coverage of more than 90%, (2) mean sequencing depth greater than 1,000×, (3) mean base quality (average Phred score) more than 30, and (4) mean mapping quality higher than 30. Statistical analysis was performed using JMP Pro v14 (SAS). To obtain the lineage identification, each consensus sequence was uploaded and analyzed with the open-source platform, Pangolin COVID-19 Lineage Assigner (https://pangolin.cog-uk.io), using the version most updated at the time of analysis. The Pearson $\chi^2$ test was used to identify any association between viral lineages and basic hospitalization status (outpatient, non-ICU inpatient, ICU). $P < .05$ was considered statistically significant. Viral lineage prevalence in LA County and California state was calculated based on metadata (November 2020 to March 2021) downloaded from GISAID.[5,6] All the sequencing results were deposited to both GISAID and the NCBI database once they became available.

## RESULTS

Among the 231 patients, the age distribution ranged from less than 1 year to 96 years, with 7.4% aged 12 years or younger, 45.9% aged 13 to 49 years, 24.7% aged 50 to 64 years, and 22.1% aged 65 years or older. Females and males accounted for 45.0% and 55.0%, respectively (Supplemental Table S1). Most (73.6%, n = 170) were outpatients; 61 (26.4%) were hospitalized, with 37 (16.0%) admitted to the ICU.

The top five lineages identified in this study were B.1.429 (44.6%), B.1.2 (16.9%), B.1.427 (8.7%), B.1 (7.8%), and B.1.1.7 (4.3%) TABLE 1. The frequency of the Epsilon variant (B.1.429 and B.1.427) was 53.2%, which was slightly higher than the prevalence of the Epsilon variant in LA County (50.0%) and the state of California (45.7%) during the same period of this study. Notably, B.1.1.7, another VOC, was found only in outpatients (n = 10, 4.3%). No other VOC, including the Delta variant (B.1.617.2), was found in this study. Of note, the frequency of lineage B.1.2, one of the major non-Epsilon variants circulating in California during the winter surge, was 16.9%, which was nearly identical to the prevalence of B.1.2 in LA County (16.7%) and California (17.1%). This has served as a control and demonstrated that the cases included in our study had a good representation of the general COVID-19 patient population in LA County.

The percentage distribution of the top four major lineages (B.1.429, B.1.2, B.1.427, and B.1) among the three different patient populations

**TABLE 1** Frequencies and Percentage Distribution of Major SARS-CoV-2 Lineages Among Patients With Different Hospitalization Status

| Characteristic | ICU, No. (%) | Hospitalized (Including ICU), No. (%) | Outpatient, No. (%) | Total No. | Frequency (This Study), % | Prevalence (LA County),[a] % | Prevalence (California State),[a] % |
|---|---|---|---|---|---|---|---|
| Pangolin lineage | | | | | | | |
| B.1.429 | 16 (43.2) | 29 (47.5) | 74 (43.5) | 103 | 44.6 | 43.3 | 29.7 |
| B.1.2 | 6 (16.2) | 8 (13.1) | 31 (18.2) | 39 | 16.9 | 16.7 | 17.1 |
| B.1.427 | 3 (8.1) | 5 (8.2) | 15 (8.8) | 20 | 8.7 | 6.8 | 16.1 |
| B.1 | 3 (8.1) | 6 (9.8) | 12 (7.1) | 18 | 7.8 | 4.6 | 4.0 |
| B.1.1.7 | 0 (0.0) | 0 (0.0) | 10 (5.9) | 10 | 4.3 | 1.9 | 2.7 |
| Others | 9 (24.3) | 13 (21.3) | 28 (16.5) | 41 | 17.7 | 26.8 | 30.4 |
| Total | 37 (100) | 61 (100) | 170 (100) | 231 | 100.0 | 100.0 | 100.0 |
| Epsilon variant (B.1.429/427) | 19 (51.4) | 34 (55.7) | 89 (52.4) | 123 | 53.2 | 50.0 | 45.7 |
| Non-Epsilon variant | 18 (48.6) | 27 (44.3) | 81 (47.6) | 108 | 46.8 | 50.0 | 54.3 |

ICU, intensive care unit.
[a]LA County and California state prevalence was based on data (November 20, 2020, to March 10, 2021) from GISAID.


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**TABLE 2** Lack of Correlation Between the Frequency of the Epsilon Variant and Hospitalization Status (Age and Sex Stratified)

| Characteristic | Epsilon Variant, No. (%) | Non-Epsilon Variant, No. (%) | Total No. | $\chi^2$ | *P* Value |
|---|---|---|---|---|---|
| Age ≥65 y | | | | | |
| ICU | 6 (32) | 13 (68) | 19 | 3.622 | .1635 |
| Hospitalized (including ICU) | 13 (42) | 18 (58) | 31 | | |
| Outpatient | 13 (65) | 7 (35) | 20 | | |
| Age ≥50 y | | | | | |
| ICU | 13 (43) | 17 (57) | 30 | 0.943 | .6241 |
| Hospitalized (including ICU) | 25 (49) | 26 (51) | 51 | | |
| Outpatient | 29 (51) | 28 (49) | 57 | | |
| Male | | | | | |
| ICU | 14 (54) | 12 (45) | 26 | 1.101 | .5767 |
| Hospitalized (including ICU) | 25 (60) | 17 (40) | 42 | | |
| Outpatient | 47 (55) | 38 (45) | 85 | | |
| Female | | | | | |
| ICU | 5 (45) | 6 (55) | 11 | 0.064 | .9684 |
| Hospitalized (including ICU) | 9 (47) | 10 (53) | 19 | | |
| Outpatient | 42 (49) | 43 (51) | 85 | | |

ICU, intensive care unit.

(ICU, hospitalized including ICU, outpatient) was similar (maximum difference = 5.1%) TABLE 1. No significant correlation was found between the frequencies of these lineages and the hospitalization status ($\chi^2 = 3.019$, $P = .9331$). For the Epsilon variant, the percentage distribution among the three different patient populations was 51.4%, 55.7%, and 52.4%, respectively. No significant correlation was found between the frequency of the Epsilon variant and hospitalization status ($\chi^2 = 0.933$, $P = .6271$), even after stratification by sex and age TABLE 2.

## DISCUSSION

Our study suggested that the Epsilon variant is not associated with increased hospitalization and ICU admission. This is consistent with the CDC's decision to downgrade it from a VOC to a variant of interest on June 29, 2021, as the prevalence of the Epsilon variant has decreased dramatically since April 2021 in both California and the United States. More recently (October 2021), CDC downgraded the Epsilon variant again to a variant being monitored due to the dominancy of the Delta variant with more than 99% frequency in the United States. Notably, one study showed current vaccines are still effective against the Epsilon variant.[7] Interestingly, another genomic surveillance study from January to March 2021 in Colorado showed that among 211 patients infected with the Epsilon variant, 193 (91%) were symptomatic and 46 (14%) were hospitalized, and these rates were slightly higher than national average (85% symptomatic illness and 5% hospitalization rates), suggesting the Epsilon variant might be more virulent.[8] Both studies (this study and the Colorado study) had a relatively small sample size (n = 231 and 211), which may partially explain the contradictory findings. In addition, the methods for determining virulence were also different, with our study comparing the Epsilon variant frequencies among the three patient populations vs the Colorado study comparing the percentage of hospitalizations among the patients infected with the Epsilon variant with the national average percentage of hospitalizations among COVID-19 cases.

Limitations of this study include a relatively small case number, especially for the ICU patients, and how these cases were chosen. Although the samples were mainly chosen to be sequenced in a random and unbiased manner, due to the limited ICU case numbers randomly chosen, 21 additional samples from deceased ICU patients (representing the most severe disease) were added to this study. In addition, setting the criterion of a Ct less than 25 for sequencing could potentially select the cases with higher viral loads and lead to a certain level of bias, which may confound the conclusion of this study. Last, this study did not incorporate essential clinical information such as comorbidities into the analysis and therefore could not rule out the possibility that the Epsilon variant could still be more virulent among certain specific patient populations.

Although the Epsilon variant has faded away in both California and the United States since summer 2021, our study adds valuable data to help understand the behavior of one of the past dominant variants from a historic perspective and shed light on the unpredictability and ever-changing nature of this highly dynamic pandemic.

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