Changing SARS-CoV-2 variants in Karachi, Pakistan from alpha to delta through COVID-19 waves three and four

Zahra Hasan (zahra.hasan@aku.edu)
The Aga Khan University, Karachi, Pakistan https://orcid.org/0000-0001-7580-372X
Uzma Bashir Aamir
World Health Organization, Islamabad, Pakistan
Asghar Nasir
The Aga Khan University, Karachi, Pakistan
Akbar Kanji
The Aga Khan University, Karachi, Pakistan
Azra Samreen
The Aga Khan University, Karachi, Pakistan
Ali Raza Bukhari
The Aga Khan University, Karachi, Pakistan
M Asif Syed
Department of Health, Sindh, Pakistan
Mansoor Wassan
Department of Health, Sindh, Pakistan
Syed Faisal Mahmood
The Aga Khan University, Karachi, Pakistan
Rumina Hasan
The Aga Khan University, Karachi, Pakistan

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Abstract

We investigated the presence of SARS-CoV-2 variants of concern (VoC) in Karachi, Pakistan between April and July 2021 in specimens received at the Aga Khan University Clinical Laboratories. VoC were identified using a PCR based approach targeting lineage specific mutation. Of the 710 clinical isolates tested, 63% were VoC comprising; 36% alpha, 37% beta, 7% gamma and 21% delta variants. Alpha variants remained the majority whilst, delta strains increased to 43% of cases in July. Thirty-six per cent of all cases were admitted COVID-19 in-patient samples. Of the in-patient cases, 41% were alpha, 28% were beta, 8% were gamma and 24% were delta variants. Overall, we report an increase of delta variants in Karachi over the past two months which is concordant with the currently observed exacerbation in COVID-19 morbidity and mortality.

Background

SARS-CoV-2 strains have evolved from the novel coronavirus (2019-nCoV) identified in Wuhan in December 2019 [1–2]. To date, 199 million infections have been reported with a 4.24 million death toll [1]. Variations observed in Orf1ab, Orf3a, N and S genes are associated with evolutionary changes and particular single nucleotide variations (SNVs) divide strains into lineages as defined by GISAID [2]. Further, Centers for Disease Control and Prevention identify four variants of concern (VOC) circulating globally characterized by unique signature mutations; alpha (B.1.1.7), beta (B.1.351), gamma (P.1) and delta (B.1.617.2) [3]. Alpha (known as 20I/501Y.V1 or VOC 202012/01), was first identified in the UK in December 2020 with a deletion in the S-gene (69–70 deletion). Beta (known as 20H/501Y.V2) identified in the Republic of South Africa and gamma (known as 20J/501Y.V3) identified in Brazil were both reported in December 2020. The delta variant first identified in India in early 2021, is growing as the globally dominant variant. It is more transmissible than previously known VoC and neutralizing antibodies are less efficacious against it [4].

The first wave of COVID-19 in Pakistan occurred between March and July 2021 [1] and the second from October 2020 until January 2021. The third wave occurred from March to May 2021 [5] and soon after in July a fourth wave started [6]. An estimated 1,039,695 cases have been reported in the country with death toll of 23,462 (Data available till 1st August 2021) [6]. The Southern province of Sindh has reported most cases (36%) and 21% of these are from Karachi. To date, the case fatality rate (CFR) due to COVID-19 in Pakistan has been 2 % with some regional variations [7]. Morbidity has been relatively lower as compared with many other countries [8] [9]. However, with slow vaccination rates and an increase in SARS-CoV-2 VoC, it is expected the number of cases will continue to rise.

Genomic epidemiology of SARS-CoV-2 has allowed interrogation of viral diversity and the understanding of disease transmission in different countries [10]. Global genomic sequencing efforts have led to the input of data of 2,503,415 strains into public databases such as, GISAID and Nextstrain, NCBI SARS-CoV-

2 Resources. However, genomic sequencing requires complex technical instrumentation and expertise and is costly. Therefore, it is challenging for resource-poor regions to use it for routine rapid surveillance of SARS-CoV-2 variants. Alternately, based on the detection of lineage-specific target mutations, it is possible to identify VoC using a PCR-based approach.

World Health Organization, Pakistan supported the implementation of genomic surveillance for SARS-CoV2 at major diagnostic laboratories including Aga Khan University Hospital (AKUH) that are part of a national consortium notified by the National COVID Operation and Command (NCOC). The objectives were to allow for early detection of circulating SARS-CoV2 variants and monitor their spread in different regions. Here, we have identified alpha, beta, gamma and delta variants in Karachi, Sindh province through testing SARS-CoV-2 positive specimens received at AKUH Clinical Laboratories, Karachi.

Methods

This study received approval by the Ethical Review Committee, The Aga Khan University (AKU). Samples identified as positive for SARS-CoV-2 Cobas 6800 Roche assay were retrospectively included in the study. Inclusion criteria: samples from AKUH inpatient between April 1 and July 18, 2021, with a CT value of < 30 and all random selection of 5–10 outpatient samples each day from Karachi. We excluded samples with CT > 31, samples and those from outside of Karachi.

RNA was extracted using the QiaAmp RNA MiniKit, Qiagen, USA. Discrimination for alpha, beta and gamma variants was based on N501Y and A570D mutations. RNA was set up for RT-PCR using the Novatype II assay, Eurofins, GmBH (supplementary file 1). Samples which showed no amplification or were negative on the Novatype II assay were further tested on the Promocure L452R, delta variant PCR assay.

Results of cases where SARS-CoV-2 variants were reported directly to the Health Department, Sindh, Government of Pakistan, National Institutes of Health (Pakistan) and to WHO, Pakistan.

Results

In total, 710 respiratory samples met our criteria for variant testing. Four hundred and forty-eight of samples were found to have one of four VoC. Whilst, 262 specimens did not have VoC of which 67 were wild-type sequences and 195 were undetermined.

Of the COVID-19 cases identified to have with VoC, 264 were males and 184 were females. Cases were aged between 1 and 85 years. The month-wise distribution of the isolates is shown in Fig. 1. Alpha variants comprised 36%, beta variants were in 37%, gamma comprised 7% and delta comprised 21% of cases. Alpha variants were predominant at 86%, 48% and 41% of cases in April, May and July. However, in May we observed an increase in beta variants (40%) which peaked in June (59%) and reduced to 1% by July. Gamma variants remained steady at 10% and 11% of cases in May and June, respectively. Delta

variants first identified in May, increased to comprise 11% of all variants in June and 43% of cases by July.

Overall, the SARS-CoV-2 variants identified, 36% (n = 160) comprised in-patients and 64% (n = 288) were out-patient specimens (Fig. 2). The median age of in-patient cases was 43 years and of out-patients was 37 years.

Studying each variant type separately, in-patients comprised 40%, 27%, 39% and 42% for alpha, beta, gamma and delta positive SARS-CoV-2 specimens (Fig. 2). When only in-patient cases were considered; 41% were alpha, 28% were beta, 8% were gamma and 24% were delta.

Discussion And Conclusions

Our data provides critical insights into the transmission patterns of SARS-CoV-2 in Pakistan. We highlight continued transmission of VoC with an increase in delta variants to 43% of all cases in July 2021. This typifies the highly transmissible nature of delta, supporting global observations that this rapidly spreading VoC is establishing itself as the dominant variant in countries such as, the USA [11] and UK [12].

Genomic sequencing of SARS-CoV-2 strains and can support infection control, epidemiological investigations and viral responses to vaccines and treatments [13]. However, this is restricted by on the technical capacity financial resources of countries. We have demonstrated that a PCR-based targeted approach can be used to monitor VoC in a low-resource setting. One limitation is that this approach cannot identify new variants of interest (VOI) such as, epsilon (B.1.427/ B.1.429) identified California, USA, eta (B.1.525) in UK/Nigeria, iota (B.1.526) in New York, USA and kappa (B.1.617.1) identified in India [3].

Identification of VoC must be coupled with a robust response at the field level to trace contacts and to implement quarantine and control measures. COVID-19 variant cases need to monitored both in hospital in-patients and in cluster outbreaks. Variant trends are likely to be impacted by the lockdown measures implemented, vaccination status of affected individuals. Further, it will be important to extend this work to cities outside of urban Karachi to predict spread to smaller urban and rural parts of Sindh province. Finally, identification of these 4 VoC in the 20 million strong population of Karachi city a harbinger of potential new SARS-CoV-2 VoIs emerging in coming weeks and months with as yet unclear implications.

Declarations

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Declarations

The authors have no conflicts of interest to declare.

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Figures

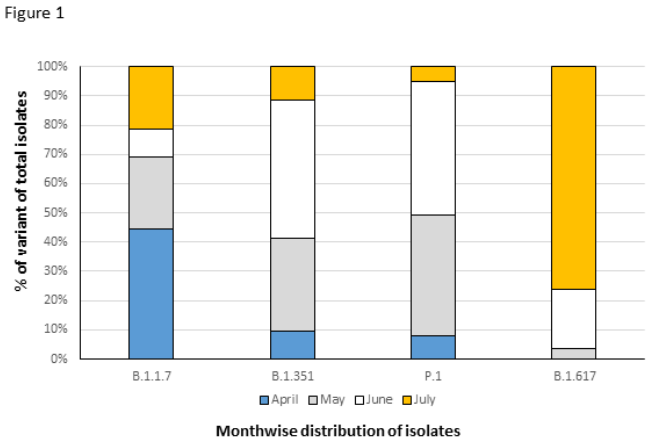

Figure 1

SARS-CoV-2 variants in Karachi. Data presented is of 448 SARS-CoV-2 samples in which alpha, beta, gamma or delta variants were identified. A month-wise distribution of the SARS-CoV-2 variants is presented as a percentage of the total number for each month April (n=42), May (n=48), June (n=201) and July (n=157).

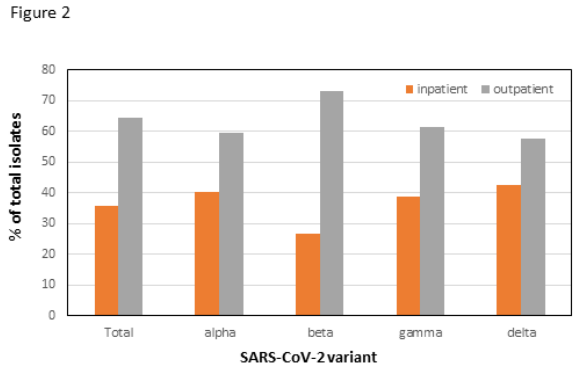

Figure 2

Distribution of SARS-CoV-2 variants within in-patient and out-patient samples. Data presented is of 488 cases where variants were identified. These comprised 160 hospital in-patient and 288 laboratory out-patient specimens. The graph depicts the percentage of total alpha (n=65), beta (n=44), gamma (n=12) and delta (n=39) variants as in-patient or out-patient cases for each.

Supplementary Files

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- AN1SupplementaryFile.docx