INSACOG BULLETIN

(18 June 2021)

The **Indian SARS-CoV-2 Genomics Consortium (**INSACOG) is a consortium of 10 regional genome sequencing laboratories (RGSLs) established on 18 January 2021. Its overall aim is to sequence SARS-CoV-2 RNA from Covid-19 infections in India to monitor the emergence and community circulation of viral variants and variants of concern (VOC).

SARS CoV2 viral sequencing in India in 2020

India started sequencing SARS CoV2 viral genomes in 2020. The first reports were from NIV, ICMR from initial cases in Kerala (January 2020). Samples were sequenced from international passengers who arrived in India from UK, Brazil or South Africa or transited through these countries and states reporting sudden surge in cases were sequenced on priority. This was further expanded through efforts of Council of Scientific and Industrial Research, Department of Biotechnology and National Centre for Disease Control, as well as individual Institutions.

7970 genomes were sequenced between Jan 2020 and December 2020. Of these 6922 are of a quality suitable for analysis, shown at https://nextstrain.org/community/banijolly/Phylovis/COVID-India?c=pangolin_lineage&dmax=2020-12-26. B.1 lineage was the most common with B.1.1 and B.1.36 seen frequently. B.1 has not been formally referred to as a VOC

During this period, India identified the B.6 variant (Nextstrain A3i in the graph) associated with a spike of cases around the country. This variant did not have any concerning mutations and the surge was a super-spreading event, rather than increased transmissibility. B.6 was progressively replaced by B.1 variants with D614G mutation that led to increased transmissibility. By June 2020, during the first wave B.1 lineages were the default strain across the nation. This was a likely contributor to the surge in India in June 2020. B.1 is the parent strain of all current VOC.

SARS CoV2 viral sequencing in India in 2021

The initial focus of India was on restricting spread of global variants of concern – Alpha (B.1.1.7), Beta (B.1.351) and Gamma (P.1), which had high transmission and varying degree of immune escape. The entry of these variants was carefully tracked by INSACOG and of these, Alpha was found to be spreading in North India, especially Punjab while Beta was seen at low levels in Bengal. Gamma was restricted to travelers.

INSACOG investigations of outbreaks in Maharashtra led to identification of a novel variant characterized by mutations L452R, E484Q and P681R. This was variously referred to as double or triple mutant until it received a lineage B.1.617. To show phylogenetic evidence, a new lineage must meet all of the following criteria: (a) it exhibits one or more shared nucleotide differences from the ancestral lineage; (b) it comprises at least five genomes with >95% of the genome sequenced; (c) genomes within the lineage exhibit at least one shared nucleotide change among them; and (d) a bootstrap value >70% for the lineage-defining node Detailed descriptions of the B.1.617 lineage and experimental work to characterize its immune properties has been published by the National Institute of Virology

A sub-lineage B.1.617.2 (Delta variant) was associated with the outbreak in Delhi. Part of this work was carried out in collaboration with colleagues in UK, where also Delta was rising rapidly. This represents an important effective international collaboration of INSACOG to find answers to questions of global relevance.

1. In terms of fraction of positive samples, Delta replaced Alpha in Delhi in about one month. Thus, it had a much higher net transmissibility with Delta infected patients infecting more people than Alpha infected people (Rt of Delta > Rt of Alpha). The difference in Rt was about 0.5, which is as much more as Alpha was over its parent strain.

2. Net transmissibility can be because of two factors, either greater transmissibility in uninfected people, or because of ability to escape cross-neutralization by previous infections or vaccine and thereby greater transmissibility in otherwise immune people (immune escape). This is understood in two ways.

   First, by vaccine escape data and reinfection data. We noted that in double vaccinated subjects who got infected, Delta was the most common cause while Alpha was not seen. In separate work covering multiple hospitals, it was noted that breakthroughs with Delta were likely to form larger clusters. We also noted that in a cohort of CSIR employees, 9 of 76 subjects showed serological signs of reinfection during the second wave

   Second, by computational models, as was previously done for the Gamma strain in Brazil and proven to be correct. Similar analysis, by colleagues in UK, shows that both factors are likely to be true. The range of estimates was similar to above and one likely combination that would fit the data is 20-40% reduction in neutralization and about 30-60% higher transmissibility compared to previous background (B.1.1.7 + B.1). The increased Rt of 0.5 also suggests a similar range.

3. The reason for these properties is not clear but there appears to be lower Ct and higher viral load in the samples. Additional molecular properties such as reduced immune neutralization have been reported by NIV and University of Oxford collaborators

4. It is noted that our findings of higher viral load could also potentially explain a greater severity, oxygen need, and hospitalization. Another possible mechanism is increased Syncytium formation by P681R

In summary, as per the findings of member laboratories of INSACOG, the surge of SARS-CoV2 infections in Delhi and the second wave in general is best explained by the introduction of Delta variant, social behaviour that promoted transmission, and insufficient immunity against the variant from previous infections. The increased transmissibility of the Delta variant is driven by a combination of evasion of neutralising antibodies and increased virus infectivity. It is noted that vaccines still protect against infection and severe disease by Delta variant.

These findings of INSACOG members will be submitted for peer-review and publication. Some of the work has been uploaded as pre-prints in view of global urgency associated with Delta.

As a next step, INSACOG would trace the molecular evolution and spread of variants in the time leading up to, during and after the second wave. It would also step up innovative surveillance strategies and determine relevance and spread of new mutations such as K417N that are being seen in Delta.