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Identification of Major SARS CoV-2 Variants in India using in silico PCR-RFLP analysis

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Purpose: As of June 30, 2021, WHO reported 181,344,224 confirmed cases worldwide and 3,934,252 deaths due to SARS CoV-2. India accounts almost 10% of the total mortality. The mutated Variants of Concern (VoC) and Variants of Interest (VoI) has acquired non-synonymous amino acid substitutions in Spike (S), ORF1a, ORF1b, Nucleocapsid (N), Membrane (M), Envelope (E), ORF6, ORF7a, ORF3a and ORF8 regions exhibiting more virulence and higher transmission rate. The S gene displaying nucleotide polymorphisms are studied for identification of major SARS CoV-2 variants circulating in India using *in silico* PCR-RFLP analysis.

Methods & Materials: DNA sequences of major SARS CoV-2 variants [alpha (B.1.1.7), beta (B.1.351), delta (B.1.617.2), epsilon (B.1.429+B.1.427), eta (B.1.525) and zeta (P.2)] prevailing in India retrieved from GISAID database were annotated using VIPR-VIGOR4 genome annotator tool. The S gene of variants were aligned using CLUSTALW algorithm in MEGA-X with default parameters and analyzed for sequence identity using Geneious Prime v2019.2.1. Primers were designed using Primer-BLAST tool, proof-read using FastPCR v6.7.46 and PCR-amplified using Snapgene v.3.2.1. Unique restriction sites in S amplicons of each variant were subjected to online Restriction Analyzer tool. The S amplicons were digested with restriction endonucleases and the band profile of each variant were visualized in the gel simulation tool using Snapgene v.3.2.1.

Results: The percentage of identical sites present in S region among SARS CoV-2 variants was found to be 98.6%. The amplified products were in the length of 3,689 and 3,698 bp. Out of 400 restriction endonucleases identified, 14 buffer-compatible enzymes were selected for single-step restriction digestion to generate unique RFLP profiles for each variant. The *BsaXI-XcmI-AcuI* triple digest showed unique banding pattern identifiers on 2% agarose gel for individual variants including alpha, eta, delta and zeta. The *ApaLI-BsaI* double digest produced distinct band profiles for beta and epsilon variants.

Conclusion: Our study strongly suggests PCR-RFLP analysis of Spike region can differentiate major SARS CoV-2 variants that are circulating in India. Further, quadruple digestion based wet lab experiments are underway to explore the possibility of surveillance of the major variants using a single-tube reaction followed by agarose gel-based profiling.

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Public Search Interests Related to COVID-19: Insights from Google Search Trends in Bangladesh

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Purpose: Public response monitoring is critical to reducing COVID-19 infections and developing effective public health strategies. This study explored Google search trends to understand public responses to COVID-19 concerns in Bangladesh.

Methods & Materials: We used country-level Google search trends data to examine the association between Google search terms related to COVID-19 deaths, face masks, and COVID-19 vaccines and the actual and one-week lagged actual COVID-19 death counts from February 2, 2020, to December 19, 2020, in Bangladesh.

Results: Search terms related to COVID-19 deaths, face masks, and COVID-19 vaccines increased and peaked during March and April, but then began declining gradually after June 2020. The mean relative search volume for face masks (35 points) was higher than for death information (8 points) and vaccines (16 points) throughout the study period, and searching for masks peaked (100 points) during the third week of March. Search interests for death information and face masks were negatively correlated with the actual and one-week lagged actual COVID-19 death counts.

Conclusion: In response to declining trends in COVID-19-related google search terms, policymakers should strengthen ongoing risk communication and preventive information dissemination programs to control and prevent COVID-19 cases and deaths.

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Detection and Contact Tracing of COVID-19 in Indonesia through a Red Cross Community Based Surveillance System

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Purpose: Epidemics and pandemics begin and end in communities. Communities play a critical role in prevention, detection, and response to infectious disease threats when meaningfully engaged. Surveillance and contact tracing are core components of effective public health preparedness and response efforts. But, surveillance systems that rely on case detection via healthcare facilities may fail or be slow to detect and trace many cases. Communities are often the first to identify unusual disease patterns in humans and animals. Community-Based Surveillance (CBS) gives a voice to communities, enables them to communicate unusual health events to authorities, and complements facility-based surveillance systems.

Methods & Materials: Since 2019, Indonesia Red Cross (PMI) has piloted CBS in 8 villages within four provinces. Initiated for passive surveillance, the volunteers trained to report 'alerts' of epidemic-prone diseases to health authorities. The concept was then adapted to an active approach focussing on COVID-19 and scaled to 74 villages across eight provinces. Partnering with local authorities, volunteers supported COVID-19 screening, detection, tracing, and local response, all while implementing volunteer safety standards. The reporting system was created and coordinated by a structure within PMI.

Results: From August 2020-June 2021, community volunteers made 1,079 alerts: 99% of the alerts were reported to health authorities, with 71.1% followed up within 24 hours. After alert detection, volunteers sensitized family members on preventive measures while individuals awaited follow-up visits for diagnosis and management by health authorities. 64% of alerts were PCR positive for COVID-19, 12% were negative, 14% did not undergo testing, and test results for 10% of the alerts were unavailable. Of 979 contacts traced by volunteers, authorities followed up 99%, confirming