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Identification of SARS-CoV-2 clusters from symptomatic cases in India

Sir,

In December 2019, cases of pneumonia-like illness due to an unknown aetiological agent were reported in Wuhan city, Hubei province of China[1]. The aetiological agent was identified as a member of the *Coronaviridae* family and was termed the 2019 novel Coronavirus. Due to its genetic similarity with the severe acute respiratory syndrome (SARS) of 2003, the International Committee on Taxonomy of Viruses renamed it as SARS coronavirus 2 (SARS-CoV-2)[2]. The first case of SARS-CoV-2 was reported from Kerala, India, on January 30, 2020[3] and since then, the numbers are increasing continuously. The present study is a retrospective analysis of two clusters of laboratory-confirmed coronavirus disease 2019 (COVID-19) patients from India and highlights their series of events, clinical features and sequence analysis. The present study is a retrospective analysis of two clusters of laboratory-confirmed coronavirus disease 2019 (COVID-19) patients from India and highlights their series of events, clinical features and sequence analysis.

This study was conducted in the departments of Microbiology and Medicine, King George's Medical University (KGMU), Lucknow, Uttar Pradesh (UP), India. The study protocol was approved by the Institutional Ethics Committee (251/Ethics/2020).

People in close contact with a laboratory-confirmed case and persons who had undertaken international travel within the last 14 days and had developed symptoms, were taken as SARS-CoV-2 suspects as per the prevailing recommendations of the Ministry of Health and Family Welfare, Government of India[4]. These cases were either admitted in the medical wards of KGMU or other district hospitals or were traced by the team of UP Integrated Disease Surveillance Programme (UP-IDSP) during March 2020. Nasal and throat swabs were collected from these individuals and transported to the virology laboratory at the department of Microbiology, KGMU, in a virus transport medium maintaining a cold chain. RNA was extracted using the commercial kits as per the manufacturer's instructions (Thermo Fisher Scientific, USA) and screened for the SARS-CoV-2 specific *E* gene using real-time reverse transcription-polymerase chain reaction (RT-PCR)[5]. The *E* gene-positive samples were confirmed by real-time RT-PCR assay targeting the *HKU-ORF1b* and *RdRp* genes[5]. Clinical, demographic, contact and international travel details of the individuals were recorded from the clinical record forms.

Statistical analysis was done using GraphPad Prism v5.0 (GraphPad Software Inc., San Diego, CA, USA). Inter-group comparisons of continuous variables were done using Chi-square test.

Extensive contact tracing was done by employing personal and family interviews of the SARS-CoV-2-positive patients along with a check on their travel history. All the known contacts were quarantined and monitored for the development of disease symptoms, and their samples were also subjected to real-time RT-PCR testing.

A total of 1,473 patients were referred from various districts of Uttar Pradesh to the department of Microbiology, KGMU, where they were tested for SARS-CoV-2, of whom 29 (1.96%) patients tested positive from March 3 to 31, 2020. The hierarchy of contact tracing for the two clusters of cases, one from Agra and the other from Lucknow, was observed from the positive cases detected, which is depicted in Figure 1.

The two index cases of the first cluster from Agra (TCO461, -462) had sore throat and travel history to Milan, Italy; Budapest, Hungary; Amsterdam (Holland) and Iceland. Three of the 10 samples (TCO464, -465, -467) from the family members of

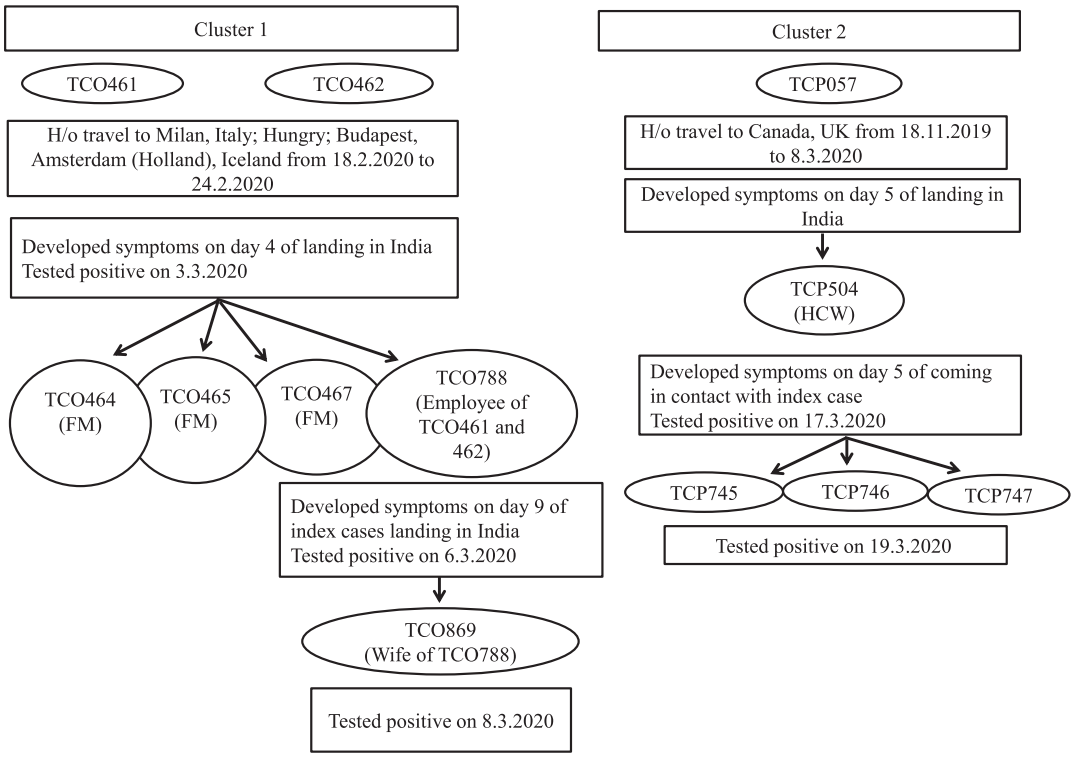

Fig. 1. Depiction of the two different clusters studied. The first cluster had travel history to Budapest, Hungary; Amsterdam (Holland), Iceland. The second cluster had travel history to Canada and the UK along with their contact cases. TCO and TCP numbers represent patient ID; FM, family member; HCW, healthcare worker.

these patients tested positive for SARS-CoV-2. A total of 233 contacts of these five positive cases were also tested, of whom two five were found to be positive within 14 days of coming in contact with the index cases. Of these, one (TCO788) was symptomatic and the other was asymptomatic (TCO869). No further cases could be linked to this cluster. The index case of the second cluster (TCP057) was a symptomatic traveller from Canada. The healthcare worker (TCP504) who attended the index case without appropriate personal protective equipment developed symptoms on day 5 of attending the index case and tested positive. Later, three family members of TCP504 (TCP745, -746, -747) also tested positive (Fig. 1), but no other contact was found to be positive. All the positive cases were hospitalized, and their contacts were home quarantined for 14 days.

The age, sex, history of travel or contact and their symptoms are mentioned in the Table. The median age of the infected patients was 37 yr (age range: 15-72). The majority of the patients (7, 58.3%) were found to be asymptomatic (did not develop any symptom over a 10 day follow up period); four (33.3%) had mild symptoms such as sore throat, body ache or fever and only one (8.3%) had breathlessness. The cyclic threshold values (C_t values) for the *E* gene of SARS-CoV-2 are shown in the Table. No significant difference was observed among the C_t values of the symptomatic (range: 18.89 to 26.93) and asymptomatic (range: 15.23 to 35.39) patients. The symptomatic cases could be linked to new cases. However, none of the asymptomatic patients could be linked to a new case. Recent studies have observed that the percentage of asymptomatic and symptomatic cases infecting others varied from 0 to 2.2 per cent and 0.8 to 15.4 per cent, respectively[6,7].

Clinical samples that formed two different clusters were sequenced using next-generation sequencing at the ICMR-National Institute of Virology, Pune[8,9]. The complete genome sequences retrieved from this study were aligned with the SARS-CoV-2 sequences downloaded from the Global Initiative on Sharing All Influenza Data (GISAID) database[10]. The alignment was done in the CLC Genomics Workbench (Qiagen Aarhus, v11.0, Aarhus C, Denmark), and the tree was generated (Fig. 2) using the MEGA software v7.0[11] and visualized in the Figtree v1.4.2 (*http://tree.bio.ed.ac.uk/software/figtree/*).

Table. Demographic and clinical details of the index cases and their contacts demonstrating two clusters and details of whole genome (WG) sequencing

Patient ID	Gender	Age (yr)	Travel history/ history of contact	C_t values for the *E* gene	Symptomatic/ asymptomatic	Symptoms (duration in days)	Total reads in WG sequencing	Per cent of relevant reads	Complete/ partial genome
Cluster 1									
TCO461/03/20	Male	44	Milan, Italy; Budapest, Hungary; Amsterdam (Holland); Iceland	20.16	Symptomatic	Sore throat (6)	1,407,528	44.70	29878
TCO462/03/20	Male	38	Milan, Italy; Budapest, Hungary; Amsterdam (Holland); Iceland	26.93	Symptomatic	Sore throat (6)	1,444,826	0.97	29855
TCO464/03/20	Male	15	Close contact	24.37	Asymptomatic	None	5,579,730	2.13	29860
TCO465/03/20	Female	37	Close contact	15.23	Asymptomatic	None	2,772,158	88.50	29903
TCO467/03/20	Male	72	Close contact	35.39	Asymptomatic	None	NA	NA	NA
TCO788/03/20	Male	45	Close contact	18.89	Symptomatic	Body ache (3)	3,039,526	86.82	29858
TCO869/03/20	Female	38	Close contact	24.00	Asymptomatic	None	NA	NA	NA
Cluster 2									
TCP057/03/20	Female	38	Canada, UK	20.00	Symptomatic	Fever (5)	3,832,806	30.30	29898
TCP504/03/20	Male	30	Close contact with confirmed case	19.00	Symptomatic, HCW	Fever, cough, breathlessness, sore throat, nasal discharge, body ache (5)	1,688,252	54.12	29875
TCP745/03/20	Female	20	Close contact with confirmed case	32.00	Asymptomatic	None	NA	NA	NA
TCP746/03/20	Male	35	Close contact with confirmed case	24.00	Asymptomatic	None	NA	NA	NA
TCP747/03/20	Male	37	Close contact with confirmed case	21.00	Asymptomatic	None	NA	NA	NA

NA, not available; C_t values, cycle threshold; HCW, healthcare worker

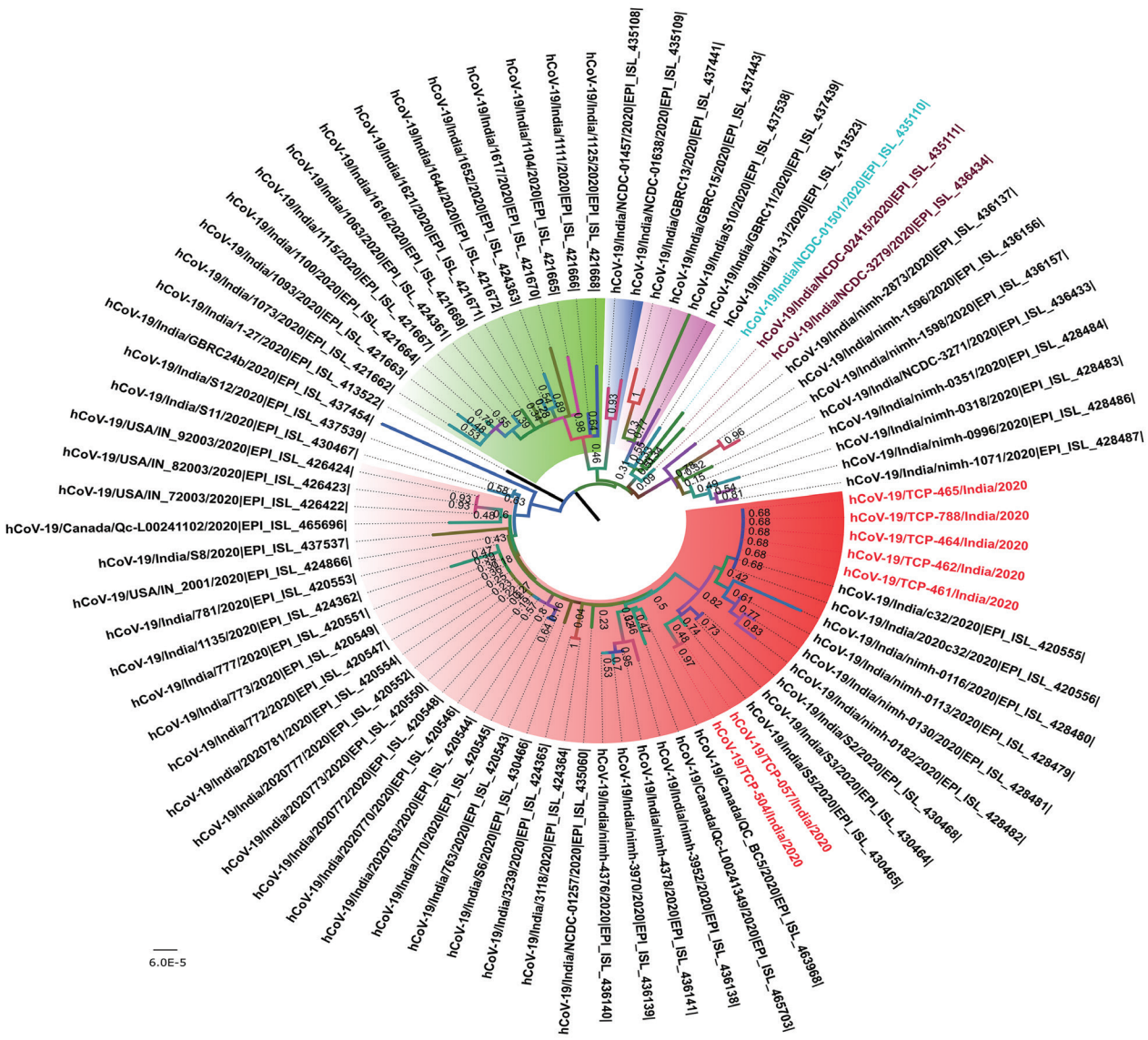

Fig. 2. Phylogenetic tree for the SARS-CoV-2 sequences from India: A phylogenetic tree based on the Kimura-2-parameter model is generated using the MEGA software. A bootstrap replication of 1000 cycles was performed to assess the statistical robustness of the tree generated. The figure is displayed using Figtree v1.4.2. Different clades are marked using colours on branches and taxa. Branch colours: light red colour → A2a clade, pink colour → B4, violet → A1a, green colour → A3. Taxa colour: brown colour → B clade and blue colour → B1 clade, red colour → sequences in this study.

The C_t values for the *E* gene of the seven genomes using real-time RT-PCR ranged from 15.23 to 26.93. The details of C_t values, along with the percentage of relevant reads mapped and genome size recovered, are given in the Table. The phylogenetic analysis of the retrieved sequences demonstrated that the sequences formed two different groups that matched sequences from Italy and Canada. Despite segregating into different groups, the sequences studied clustered in genotype A2a, as observed in the Nextstrain site (*https://nextstrain.org/ncov*), analyzed for the other Indian SARS-CoV-2 sequences. Further comparison of the amino acid variation between the sequences retrieved in this study demonstrated a single change at *N* gene. The hCoV-19/TCP-057/India/2020 and hCoV-19/TCP-504/India/2020 had histidine at amino acid position 343, whereas the other sequences had aspartic acid.

In conclusion, the present pilot study showed that the majority of the SARS-CoV-2-positive patients were asymptomatic. Chances of asymptomatic cases infecting others were less likely as compared to that of symptomatic cases. The analysis of a larger sample of patients needs to be conducted to confirm this interpretation.

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