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Molecular epidemiology of a familial cluster of SARS-CoV-2 infection during lockdown period in Sant Kabir Nagar, Uttar Pradesh, India

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Abstract

We report a familial cluster of 24 individuals infected with severe acute respiratory syndromecoronavirus-2 (SARS-CoV-2). The index case had a travel history and spent 24 days in the house before being tested and was asymptomatic. Physical overcrowding in the house provided a favourable environment for intra-cluster infection transmission. Restriction of movement of family members due to countrywide lockdown limited the spread in community. Among the infected, only four individuals developed symptoms. The complete genome sequences of SARS-CoV-2 was retrieved using next-generation sequencing from eight clinical samples which demonstrated a 99.99% similarity with reference to Wuhan strain and the phylogenetic analysis demonstrated a distinct cluster, lying in the B.6.6 pangolin lineage.

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) was first reported from Wuhan, China [1]. Several studies have reported the transmission of SARS-CoV-2 from human to human, asymptomatic transmission and transmission in family and hospital settings [2, 3]. The first case in eastern Uttar Pradesh, India was reported from Basti town on 31 March 2020 [4]. Following this, the next case in this region was reported from Sant Kabir Nagar (SKN), a district adjacent to Basti [5]. The first case in SKN was reported on 15 April 2020 where a 71-year-old male who had visited New Delhi was found positive. The infection in this case was limited with no positive contacts. Following this a second case was noticed from SKN who was identified as the index case of the present cluster and he had returned home due to countrywide lockdown. Although he remained asymptomatic during his stay, the infection spread further leading to a family cluster. The present study describes in detail the asymptomatic transmission and delineates the transmission dynamics using next-generation sequencing (NGS).

Study setting

A 23-year-old male student suspected to be infected with SARS-CoV-2 along with two of his co-travellers on the same bus returned to SKN from Deoband, Uttar Pradesh during the lockdown. He was tested positive for SARS-CoV-2 on 17 April 2020, following which he was quarantined. As part of contact tracing, all of his family members (28 individuals) and seven relatives who were residing in the same house were tested. In all the individuals, nasopharyngeal and nasal swab samples were collected by the state government health department and sent to our laboratory for testing. All the samples were processed as per the standard protocol and stored at -80 °C. Eighteen out of 35 members were found positive. The positive and negative tested family members were quarantined separately. On repeat testing of negative cases on 02 and 03 May 2020, five more members tested positive for SARS-CoV-2. Overall 12 out of 36 people in the house remain infection-free throughout their quarantine period (median quarantine period: 18 days, range 16–26 days). The secondary attack rate in this familial cluster was found to be 65.7% (assumption: all people got infected in the house before quarantine).

The median age in this cluster was 20.5 years (range: 2 months–72 years). The cluster had 10 family members below 18 years of age. This cluster was composed of 19 males (52.8%) and

		Frequency (column %)		
Characteristics		Infected, $n = 24$	Non-infected, $n = 12$	P value (Fisher's exact test)
Age in years	≼9	4(16.7)	1(8.3)	0.37
	10-19	8(33.3)	2(16.7)	
	20-39	9(37.5)	5(41.7)	
	≥40	3(12.5)	4(33.3)	
Gender	Female	15(62.5)	2(15.4)	<0.01
	Male	9(37.5)	10(84.6)	
Marital status	Married	6(25.0)	5(41.7)	0.10
	Unmarried	18(75.0)	6(50.0)	
	Widowed	0(0.0)	1(8.3)	
Primary occupation	Unemployed	5(20.8)	2(16.7)	0.75
	Housewife	3(12.5)	3(25.0)	
	Student	13(54.2)	4(33.3)	
	Labourer	2(8.3)	2(16.7)	
	Other	1(4.2)	1(8.3)	
Symptoms	Symptomatic	3(12.5)	1(8.3)	0.65
	Asymptomatic	21(87.5)	11(91.7)	
Co-morbid illnesses	Present	3(12.5)	0(0.0)	0.54
	Absent	21(87.5)	12(100.0)	

Table 1. Personal and clinical characteristics of the familial cluster in Sant Kabir Nagar, Uttar Pradesh, India

17 females (47.2%). To obtain the '*P* value' for the association, Fisher's exact test was applied (Table 1). The family lived in a pukka (cemented) house with a total of eight living rooms and a separate kitchen. Overcrowding was noted in the family. Among the individuals tested positives, only three out of 24 developed mild symptoms. Details of the chronological events and family structure are shown in (Fig. 1). Three members in this cluster had co-morbid conditions and were also found infected. All 24 infected individuals recovered from infection and no case fatality occurred in this cluster.

Next-generation sequencing

NGS was conducted by preparing RNA libraries from the extracted RNA. The RNA libraries prepared were sequenced using the Illumina platform (Qiagen, Germany) to delineate the transmission dynamics using the positive samples to retrieve the complete genomic sequence of the SARS-CoV-2. The detailed protocol of the method used is described elsewhere [6]. The pipe-line used to obtain the SARS-CoV-2 sequences is depicted in Figure 2a. The retrieved sequences were aligned using the representative GISAID Indian SARS-CoV-2 sequences. A neighbour-joining tree was generated using the best model in MEGA version 7.0 [7]. A bootstrap replication of 1000 replication was used to assess the statistical robustness. Amino acid variations were also observed for the different proteins encoded by SARS-CoV-2.

The percentage of genome recovered from 24 samples ranged from 1.49 to 99.99 and the relevant reads mapped lay between 0.0 and 92.35%. The details of the percentage of relevant reads mapped and the percentage of the genome retrieved for all the

24 samples are tabulated in Table 2. Eight genomic sequences were retrieved with a genome coverage ranging between 99.94 and 99.96% while the other 16 sequences were below 95.5%. The neighbour-joining tree as generated using the Kimura-2parameter model demonstrated that the retrieved sequences lay in the B.6.6 pangolin lineage (https://pangolin.cog-uk.io/). Two distinct clusters were observed for the generated tree, one with the B pangolin lineage variants comprising of B.B.4, B.6, B.6.6 lineages and other with B.1 pangolin variants (B.1, B.1.1.306, B.1.36.8) (Figure 2). The amino acid variation analysis for different structural and non-structural proteins demonstrated the presence of multiple variant amino acid sites in the ORF1ab at position nsp2(G519S), nsp3(P1010S,T2016 K), nsp4(N2767 T) nsp6 (L3606F), nsp12(A4489 V), nsp13(G5411 V), whereas protein S, ORF3a and ORF8 showed no distinct variation (Technical Appendix Table 1). The amino acid position of ORF1ab protein L3606F (nsp6) is shared with clade O and A3i, and substitution at position A4489 V (nsp12) is shared with clade A3i. The nucleocapsid protein of the studied strain showed a single variable site in amino acid sequence at position P9265L. The percentage of nucleotide and amino acid similarity for different genes are tabulated in the Technical Appendix Table 2.

Discussion

We describe here a familial cluster (n = 24), out of 36 who were found to be infected with SARS-CoV-2. The index case had a travel history and spent 24 days in the house before being tested and was asymptomatic. Physical overcrowding in the house provided a favourable environment for intra-cluster infection transmission. Restriction of movement of family members due to countrywide

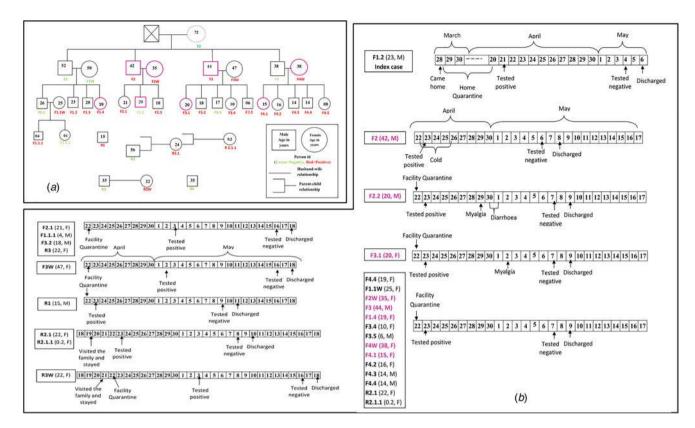


Fig. 1. (a) Diagrammatic representation of family structure and their RT-PCR status for SARS-CoV-2 infection. (b) Chronological events with symptoms, date and duration of symptoms in each infected individual. *In the figure letter 'F' denotes family member, the letter 'R' denotes relative and the letter 'W' means wife. Numbers after letter represent generation separated by a dot (.) and chronological order among siblings; for example, if code is F2 this means a family member of the first-generation and second among siblings according to chronological order. Code F2W means the wife of F2. Similarly, F1.1.1 means the first member of the third generation of F1. We have denoted the only survivor of the oldest generation in the family as F0.

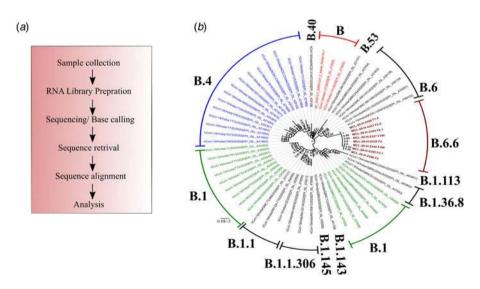


Fig. 2. Phylogenetic tree of the coding region of the SARS-CoV-2. *A neighbour-joining tree of the SARS-CoV-2 sequences retrieved from the clinical samples. The tree was constructed using the representative GISAID clade sequences with the Tamura–Nei parameter model with a gamma distribution as the rate parameter. The bootstrap replication of 500 cycles was used to assess the statistical robustness of the generated tree. The scale depicts the number of base substitutions per site. The tree is coloured as per the GISAID clade and taxa. Taxa colour: (blue: B.4; red: B and green: B.1). Sequences retrieved in this study are marked in brown colour and are bold faced.

lockdown limited the spread in the community. Most of the infected individuals were asymptomatic and those having symptoms had only mild ones.

Of the negative family members, five turned positive on repeat sampling. The possibility of asymptomatic and reverse transcription-polymerase chain reaction (RT-PCR) negative individuals transmitting the infection has been shown in several studies with possible reasons for false-negative results of RT-PCR due to insufficient viral specimens and the low load of virus in the upper respiratory tract infection [8]. The viral load in symptomatic and asymptomatic individuals is shown to be similar [9] and they serve as potential source of infection in the community and hospital settings [3, 10].

Females were infected more than males. Other factors like age, occupation, co-morbidity and marital status were not found statistically significant. The present cluster had 10 members of age

% of Sr Е ORF S % of genome Person Total relevant no. MCL no. ID Gender gene gene reads reads retrieved Age gene 1 MCI -20-H-2336 F1.2 34 37 1 690 508 0.000769 4.330669 23 М 36 2 MCL-20-H-2328 1 507 078 9.841822 F3.4 10 F 32 30 30 0.00292 З MCL-20-H-2329 F3 2 18 М 23 24 22 2 999 722 0.002767 15.77768 4 78.99542 4 MCL-20-H-2330 F1.1.1 Μ 22 24 24 1 324 178 0.05309 5 MCL-20-H-2331 R2.1.1 0.2 F 32 31 31 1 168 428 0.000599 1.498177 6 MCL-20-H-2332 F4.5 F 730 536 0.031073 46.83811 8 36 34 34 7 MCL-20-H-2333 F3.5 6 F 33 31 31 1 198 500 0.083605 85.89105 8 MCL-20-H-2334 22 F 0.009205 26.45554 R2.1 34 32 33 1 184 166 9 MCL-20-H-2335 R3W 22 F 17 17 15 1826444 0.027923 67.2976 F2W E 1 382 850 11.18733 10 MCL-20-H-2337 35 25 24 24 99.94984 11 MCL-20-H-2327 F2.1 21 F 27 28 26 1 123 962 0.310153 98.70582 F 12 MCL-20-H-2338 F1.1W 25 34 31 31 3 079 116 0.038095 76.32345 13 MCL-20-H-2339 F2 42 м 21 19 19 5 830 376 72.60216 99.99666 14 MCL-20-H-2340 F4W 38 F 17 16 15 1 186 078 92.3591 99.95987 1 080 084 0.233315 94.26813 15 MCL-20-H-2341 F4.2 16 F 34 33 32 16 MCL-20-H-2342 R1 15 М 33 31 32 1814786 0.052844 74.56442 10.5289 17 MCL-20-H-2343 F4.1 15 F 21 18 19 3 114 826 99.95653 18 MCL-20-H-2344 F4.4 14 Μ 34 32 32 1 259 566 0.028026 37.8758 19 MCL-20-H-2345 F3.1 20 F 19 17 17 1 963 880 28.5716 99.95653 1 069 632 60.88253 99.95653 20 MCL-20-H-2346 F3 44 М 18 17 16 21 MCL-20-H-2347 22 20 20 7 057 582 6.97756 99.95653 F2.2 20 М MCL-20-H-2348 F4.3 М 26 25 1 724 272 0.20281 97.61228 22 14 26 F 23 4 528 836 23 MCL-20-H-2349 F1.4 19 22 21 17.57688 99.96321 MCL-20-H-2350 0.147909 95.29144 F3W 47 F 34 1 688 878 24 32 32

Table 2. Details of the samples collected and the RT-PCR value for different genes of the 24 clinical samples tested along with the percentage of relevant reads mapped and percentage of genome retrieved

Bold values in the table 2 reflect the eight clinical samples in whom the genomic sequences were retrieved by NGS and used in the phylogenetic analysis.

<18 years, of which two were <5 years. Children being infected from the family members given the proximity and asymptomatic nature of the illness has been documented [8].

In India, multiple SARS-CoV-2 clades are reported to be circulating. The phylogenetic analysis demonstrated a distinct cluster, lying in the B.6.6 pangolin lineage. Further, genetic analysis of the sequences in this study demonstrated conserved amino acid variation in the ORF1ab regions. The variations were observed in the amino acid that contains trans-membrane domain (nsp3, nsp4 and nsp6), RdRp (nsp12) and helicase (nsp13). The implications of these changes need to be further explored.

This study provides insight into transmission of SARS-CoV-2 within a crowded household setting using genome sequencing, which is of notable value when assessing cluster outbreaks and transmission dynamics. With novel variants requiring attention and resources, the window period for such cluster analysis should be considered timely. However, of the 24 samples, genome was retrieved in only eight samples which might be due to low viral load; this highlights the limitation of sequencing in such situations.

Conclusion

This study encourages future implementation of genome sequencing when addressing outbreaks in real-time, particularly in crowded household settings, and tangentially highlights the effectiveness of lockdown measures.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0950268821001989.

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Conflict of interest. The authors do not have any conflict of interest.

Ethical standards. Ethical approval for this study was taken from the ICMR-RMRC Gorakhpur Human Institutional Ethical Committee.

Data. All data generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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