Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Original Research Article

Molecular epidemiology of SARS-CoV-2 in healthcare workers and identification of viral genomic correlates of transmissibility and vaccine break through infection: A retrospective observational study from a cancer hospital in eastern India

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ABSTRACT

Purpose: Despite COVID vaccination with ChAdOx1 ncov-19 (COVISHIELD®) (ChAdOx1 ncov-19) a large number of healthcare workers (HCWs) were getting infected in wave-2 of the pandemic in a cancer hospital of India. It was important therefore to determine the genotypes responsible for vaccine breakthrough infections.

Methods & Objectives: Retrospective observational study of HCWs. Whole genome sequencing of SARS CoV-2 using Illumina NovaSeq was done. Mutations from both waves were compared to identify genomic correlates of transmissibility and vaccine breakthrough infections.

Results: Vaccine breakthrough infections were seen in 127 HCWs out of 1806 fully vaccinated staff (7.03%). Median number of HCWs infected per day in wave-1 was 0.92 versus 3.25 in wave-2. Majority of wave-1 samples belonged to B.1 and B.1.1 lineage. Variant of concern- Delta variant (90%), and variant of interest- Kappa variant (10%), was seen in only wave-2 samples. Total mutation observed in wave-2 samples (median = 44) was 1.8 times than wave-1 sample (median = 24). Spike protein in wave-2 samples had 13 non-synonymous mutation as compared to 8 seen in wave-1 samples. E484Q-vaccine escape mutant was detected in five samples of wave-2; T478K – highly infectious mutation was seen in 31 samples of wave-2. We identified a novelcoding disruptive in-frame deletion (c.467_472delAGTTCA, p. Glu156_Arg158delinsGly) in the Spike protein. This mutation was seen only in wave-2 (78%, n = 39) samples.

Conclusion: The circulating virus strains in wave-2 infections demonstrated a greater degree of infectivity. There was a significant change in the genotypes observed in wave-1 and wave-2 infections along with almost twice the number of mutations. We noted that vaccine breakthrough infections (although mostly mild).

1. Introduction

The COVID-19 pandemic has caused morbidity, mortality, socio-economic catastrophe, and increased healthcare burden on a global scale [1]. Questions have emerged with regard to transmissibility, virulence, vaccine efficacy in evolving variants [2–4]. Although HCWs have received prioritized vaccinations against this virus in India, infections have happened in partially immunized and even those receiving two doses of vaccine. ChAdOx1 ncov-19 (Covishield®) (Serum Institute India) has been the principal vaccine administered in the Indian

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population. The reported vaccine efficacy of ChAdOx1 ncov-19 (Covishield®) was between 91.4% and 94.9% in one study s [5]. In a study from Belgium COVID vaccine breakthrough infections were reported with an incidence of 11.2 per 100 person years. Vaccination with Adenovirus vectored vaccine compared to mRNA vaccines was associated with greater risk of vaccine breakthrough infections, and natural infection was found to be protective and was associated with less incidence of vaccine breakthrough infections [6].

In our study we have examined the molecular epidemiology of SARS CoV-2 infection among HCWs in a cancer hospital in eastern India across the first and second wave of the pandemic. Genomic surveillance by WGS of SARS-Co-2 provides insights into (a) transmissibility; (b) virulence; (c) ability to evade detection by specific diagnostic tests (e.g. when RT-PCR target genes are affected); (d) provide information about decreased susceptibility to medical therapies (e.g. monoclocal antibodies); (e) ability to evade natural or vaccine-induced immunity (vaccine escape mutants); (f) tracking of antigenic evolution for vaccine development; (g) virus variant identification and characterization.

2. Objectives

To investigate: (a) whether the increase in transmissibility of infection observed in wave-2 was related to differences in viral genomic characteristics (b) the specific regions of the viral genome that may have changed resulting in alteration of transmissibility and vaccine break through infections.

3. Materials and methods

3.1. Study design

Retrospective observational study which compares mutations in SARS-CoV-2 genome in samples from wave-1 and wave-2 of the pandemic.

Inclusion criteria: HCWs of any gender or age of this hospital with RT-PCR confirmed SARS-CoV-2 infection of any severity including asymptomatic positives (in wave-1). Periods of recruitment for wave-1: April 06, 2020 to Jan 13, 2021; and for wave-2: March 31, 2021 to May 24, 2021.

Exclusion criteria: SARS-CoV-2 RT-PCR cycle threshold value (CT) > 30.

3.2. Sample collection, storage

Samples of nose swab and throat swab (HiMedia Laboratories, Mumbai, India) were collected in VTM (Viral Transport Medium) and stored at minus 80 °C till the time of processing.

3.3. COVID RT-PCR testing strategy

Wave-1: Symptomatic HCWs and high risk contacts of positive cases (as per WHO definition).

Wave-2: Symptomatic HCWs only.

RT-PCR test methodology: Real-time PCR for the SARS CoV-2 was performed targeting the ORF1ab and N gene on respiratory samples using the Bio-Rad CFX real-time PCR platform. RNA extraction was done using QIAamp Viral RNA Mini Kit (Qiagen, Cat# 52,904).

3.4. Study cohort for whole genome sequencing (WGS)

Ninety-seven samples which met the inclusion criteria (47 from wave-1 and 50 from wave-2) were randomly selected for whole genome sequencing.

3.5. RNA extraction, library preparation and whole genome sequencing

RNA was extracted from the VTM containing nose and throat swab. First-strand cDNA was prepared from extracted RNA. Targeted enrichment was performed by amplifying the entire SARS-CoV-2 genome using a set of commercially available PCR primers. The final library distribution was assessed on Tape Station. The libraries were quantified using Qubit High Sensitivity Assay and were pooled, diluted to final optimal loading concentrations for cluster amplification on Illumina flow cell followed by sequencing on Illumina NovaSeq® instrument to generate 150bp paired-end reads [7].

3.6. Quality control and contamination control

Sample identification was done using barcode labels. Negative controls were used to rule out PCR contamination. External Quality Assurance of tests were performed by the Health Department of Government of West Bengal and Indian Council of Medical research.

3.7. Mapping of reads, variant calling, lineage, clade classification, phylogenetic tree construction

The sequenced data of all samples were analysed using an in-house pipeline. For the low quality read (<Q30), sequencing adapter, and host reads (mapping to human) were filtered out. The remaining reads were aligned to the SARS-CoV-2 reference genome downloaded from NCBI RefSeq (NC_045512.2) using BWA v.0.7.12 aligner [8]. The depth and the alignment percentage of the reference were calculated using Samtoolsflagstat options.

The consensus sequence was called using samtoolsmpileup option. Variant calling was performed using GATK (v4.1.0.0) variant caller. After variant calling, variants with read depth (<30) and allele frequency <0.10 were removed from further analysis.

The variants identified were then annotated to the SARS-CoV-2 genes using SnpEff 4.5COVID19. The variant impact on genes, codon changes and amino acid changes were added to the variant annotation. The variants were further annotated using various data bases including CDC, GISAID and GESS [9]. Lineage and clade classification were performed using Pangolin 3.1.4 and NextClade1.5.1 respectively. Variant surveillance containing non-synonymous variants for each sample was downloaded from GISAID database (filename = variant_surveillance_tsv_2021_06_26. tar.gz). The gene names and position used by GISAID were normalized to the gene model gff file that was used for the variant annotation by SnpEff 4.5COVID19. Delta variant (B.1.617.2) was defined by the Spike Protein Substitutions: T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417 N*), L452R, T478K, D614G, P681R, D950 N. Kappa variant (B.1.617.1) was identified by the Spike Protein Substitutions: (T95I), G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H.Phylogenetic tree was constructed using NextClade1.5.1 and the Auspice 2.23.0. The tree plot was exported as JSON for further improving the visualization.

3.8. STROBE and STROME-ID checklist

Checklists from Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases (STROME-ID), an extension of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) was used to collect, analyse and present data [10].

3.9. COVID case definitions

Case definitions of mild, moderate and severe COVID were as per “clinical management protocol: COVID-19 version 3 June 13, 2020. Government of India. Ministry of Health and Family Welfare [11]. Based on the definition and conceptual understanding provided by Zhang et al., in 2021 we have defined a COVID wave as an epidemiological phenomenon constituting an upward and a downward period, and each

period being sustained over a period of time. A "wave" has been distinguished from a "spike"- the latter being a short lived phenomenon [12].

CDC and published definition was used to define re-infection. Reinfection with the virus that causes COVID-19 means a person was infected, recovered, and then later became infected again. A period of 3 months was used to define re-infection [13,14].

3.10. Bias

Selection bias and effect of confounding was addressed through randomization of sample selection from wave-1 and wave-2 cohort. Study population were from same hospital to minimize heterogeneity of exposure and infection control practices. Discovery or ascertainment bias was addressed by checking significance of findings (virological/clinical/epidemiological) in multiple database such NCBI, GESS, GISAID. Significance of findings were also checked by two teams independently:

3.11. Statistics and sample size calculation

The study used dichotomous endpoints: mutations present or absent and investigated two independent cohort in wave-1 and wave-2. We used alpha error (Type 1 error) = 5%, power = 90%, beta error (Type 2 error) = 10%, and an enrolment ratio: 1: 1 (number of samples in wave-1 and wave-2) to ascertain minimum number of subjects for adequate study power to be 46 in each group. Sample size calculation was done using online calculator: https://clincalc.com/stats/samplesize.aspx (www.ClinCalc.com). Sample size was restricted to 100 samples only (50 samples per wave) in view of funding limitations. Statistical methods used to calculate p values was un-paired two tailed *t*-test using online calculators: https://www.graphpad.com/quickcalcs/ttest1/?invalidNumbers=1&format=50. No sensitivity analysis was done (www.Graphpad.com).

3.12. Ethics

The study approved by institutional review board of Tata Medical Center, Kolkata, India (reference number: 2020/Govt/33/IRB46 dated August 14, 2021).

4. Results

The epidemiological and virological characteristics (Table 1) of COVID 19 cases in wave 1 and 2 in India showed that the wave 1 was significantly prolonged at the time of analysis (283 vs 55 days). The mean number of tests positive per day was higher in wave 2 as compared to wave 1 (13.47/day vs 5.09/day) and the mean number of HCWs who tested positive per day was higher in wave 2 compared to wave 1 (3.25 vs 0.92; $p < 0.0001$). Combined proportion of moderate and severe cases was more in wave 2 (2.24% vs 1.5%). We tested asymptomatic staff with significant contact history (along with symptomatic staff) in wave-1 but only symptomatic staff in wave-2. Moreover, staff vaccination had started during wave-2. These two reasons could have contributed to less number of staff being tested in wave-2.

After WGS of the viruses we noted that the median variant count was more in wave 2 (44 vs 24), so were the median coding variants. We detected five asymptomatic healthcare workers in the wave-1. All of these asymptomatic HCWs had Clade 20 A and B.1 lineage. The rest 41 HCWs were symptomatic. Among the symptomatic HCWs in wave-1 Clade 20 A was detected in 27 cases and 14 belonged to Clade 20 B. Twenty-six out of 41 were from non B.1 lineage. There were slightly more number of coding and total variants in the asymptomatic group compared to the symptomatic group. We observed that in wave-2 HCWs who were vaccinated and had mild disease all had infections with Clade 20 A (Delta lineage 35 and Kappa lineage 5). The median number of total variants and coding variants in wave-2 were more than in wave-1 (44 and 35 compared to 24 and 17 respectively).

Table 1
The epidemiological and virological characteristics of the COVID cases in wave-1 and wave-2.

	wave-1	wave-2	*P*-value (unpaired *t*-test)
Start Date	April 06, 2020	March 31, 2021	
End Date	January 13, 2021	May 24, 2021	
No. Of Days	283	55	
Total COVID Positive HCWs	261	179	
Mean no. Testis positive per day (HCW + patients)	5.09	13.47	
Mean no. Of HCWs testing COVID positive per day	0.92	3.25	<0.0001
Fully vaccinated HCWs among COVID positive	0%	70.95% (127/179)	
HCWs who received one dose of COVID vaccine among COVID positive	0%	7.82% (14/179)	
Un-vaccinated HCWs among COVID positive	100% (261/261)	21.23% (38/179)	
Mean age	31.06 years	29.02 years	0.0076
Male: Female (HCW)	141:120 (1.18:1)	82: 97 (0.85:1)	
Asymptomatic infection (HCW) (PCR confirmed)	32.2% (84/261)	Not assessed	
Mild illness (HCWs)	66.3% (173/261)	97.76% (175/179)	
Moderate illness (HCW)	1.5% (4/261)	1.12% (2/179)	
Severe illness (HCW)	0	1.12% (2/179)	
Moderate and severe (HCW)	1.5%	2.24%	
E gene median RT-PCR CT (HCW)	26	16.99	
S/N/RDRP gene median RT-PCR CT (HCW)	27.4	23.87	
Cumulative median RT-PCR CT (HCW)	26.65	20.43	E: < 0.001 RDRP/N/S < 0.0001
Median variant count in the WGS subset	24 (14–43)	44 (37–58)	
Median coding variants	18	34	

We noted three sub-clusters during wave-1 (Fig. 1a) involving operation theatre staff, nursing hostel residents and post-festival peaks.

In wave-2 symptomatic vaccine breakthrough infections were seen in 127 out of 1806 fully vaccinated HCWs (7.03%). The median number of days after which SARS-CoV-2 infection occurred after the last COVID vaccine was 70 days for the fully vaccinated cohort and 96 days for those who had received one dose of ChAdOx1 ncov-19 (Covishield®) vaccine. Eight patients had evidence of re-infection in wave-2 after an episode of infection in wave-1.

The eligibility for WGS and the exact number of tests performed have been depicted in Fig. 1b. The distribution of the unique coding mutations in wave 1 and 2 in SARS CoV2 genome demonstrated five types of genetic mutations i.e. missense, frame-shift mutations, conservative in-frame insertions, disruptive in-frame deletions and stop changes (Fig. 2, Table 2). The commonest among these were missense mutations in ORF1b while frame-shift mutations were very rare. In wave 2 delta variant (B.1.617.2) was commonest, followed by Kappa (B.1.617.1). In contrast, wave 1 had B.1 as the commonest variant (Fig. 2).

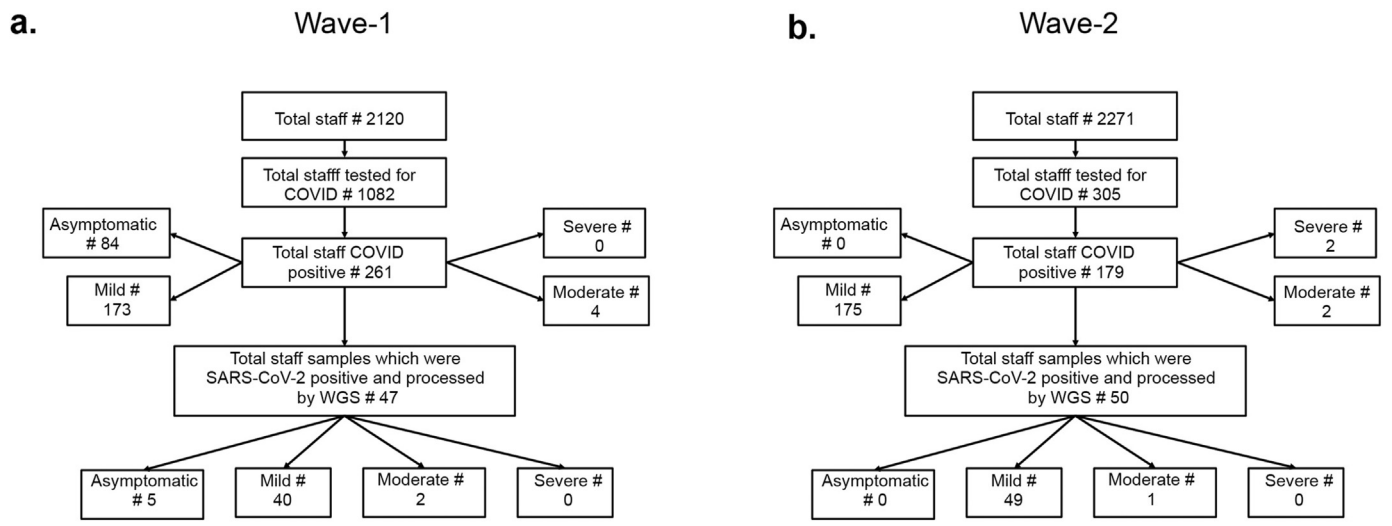

Fig. 1. Staff infected with SARS-CoV-2 in wave-1 (a) and wave-2 (b). Disease severity was classified as per MoHFW guidelines (Govt. Of India).

Analysis of genes of wave 1 samples (Fig. 3) showed that maximum mutational changes were in ORF1b followed by ORF 3a and S genes. Wave 2 samples showed ORF1b to be the commonest site for changes followed by *S*-gene (which were more common than ORF 3a changes).

Supplementary Fig. 2 shows the radial phylogenetic tree and clustering of wave 2 samples. The quality of genome analysis has been demonstrated in Supplementary Fig. 1. Supplementary Fig. 3 depicts trend of wave specific mutation in GISAID.

5. Discussion

A typical SARS-CoV-2 virus accumulates 1–2 single-nucleotide mutations in its genome per month, which is half the rate of influenza virus and one-fourth of the rate of HIV [15]. Nucleotide deletions, unlike substitutions, cannot be corrected by this proofreading mechanism of SARS-CoV-2 exonuclease (ExoN), which is a factor that may accelerate adaptive evolution to some degree.

Previous studies have shown how mutations can affect RT-PCR based diagnostic tests. FDA reported the SARS-CoV-2 test Accula was affected by a genetic variant at position 28,881 (GGG to AAC) [16]. We noted G > A mutation at this position for 12 samples in wave-1 and a G > T mutations in 49 samples in wave-2. Another study demonstrated that the RT-PCR target, gene length, mutation ratio and h-index are factors which potentially affect molecular tests. Among the targets for RT-PCR spike gene is the longest (3819 base pairs) and E gene is the shortest (225 base pairs). However, the mutation ratio is maximum in case of the nucleocapsid gene (mutation ratio is 0.558 and h-index is 44) whereas, the mutation ratio is the least in case of RdRp gene followed by E-gene [4].

In this study, we did not find any evidence of Remdesivir resistance mutations as previously reported (F480L, V557L & D484Y mutation of RdRp gene). We detected five cases of missense mutation at the same site but with different amino acid substitution [17]. The mutations which have been associated with resistance to monoclonal antibody therapies were also not detected (e.g K417 N/T, E484K, N501Y affect efficacies of Caserivimab –Indevimab cocktail or that of Bamlanivimab) [18]. However, in five cases we found the mutation E484Q which is a mutation in the receptor binding domain (RBD) and is associated with immune escape [19]. Mutation in the receptor binding domain (RBD) of S gene and the E484K mutation, is also associated with immune escape, reduced susceptibility to mAbs and increased ACE2 affinity [18,20]'. We also noted 48 cases in wave-2 to have P681R mutation at the furin cleavage site (previously reported from Uganda – A.23.1) [21]. The delta plus variant defined by the mutation K417 N (and associated with high transmissibility, stronger binding to receptors on lung cells and potential reduction in mAb response) was not seen in our cohort.

Deletions in the spike protein gene have been previously reported to be associated with antibody escape and increased infectivity. In our study we detected two deletions in wave-2 samples: a conservative in-frame deletion at base position 28,247 (AGATTTC) and disruptive in-frame deletion at base position 22,028 (GAGTTCA). Previous reports suggested that infection with the deletion Δ382 variant was associated with lower odds of developing hypoxia requiring supplemental oxygen compared with infection with wild-type virus [22,23]. In our study we found that in the ORF gene was affected in 25 samples wave-2 (ORF1ab in 20 samples; ORF3a in two samples and ORF7a in 3 samples), and five samples in wave-1 (ORF1ab gene in four, ORF3a in one).

Previous reports from this region of India has shown A2a clade to be common. Maitra et al. reported mutations in the RDRP gene to be common, but also present were occasional mutations in S, N, ORF3a, ORF8, ORF9, NSP3 and 5′- UTR gene [24]. We found the ORF8 deletion Asp 119 - Phe120del (ΔD119-F120). Deletions in the ORF8 gene reported to be associated with milder symptoms and better prognosis [25,26].

Vaccine breakthrough infections among HCWs as seen in our study have been previously investigated in India. Philomina et al. detailed six cases of such infections among HCWs from Kerala, who had received the ChAdOx1 NCOV-19 (Covishield®) vaccine as in our study [27]. The breakthrough occurred with B.1.1.7 (four cases) and one each due to B.1.1.306 and B.1.1. In our study 35 vaccine breakthrough cases were due to Delta variant and 5 due to Kappa variant. Another study from the United States reported two cases of vaccine breakthrough infections among 417 persons who had received the second dose of BNT162b2 (Pfizer–BioNTech) or mRNA-1273 (Moderna) vaccine [28]. The strains from the New York report were related to B.1.1.7 and B.1.526, had deletion del 142–145, mutations at T95I, E484K, A570D, D614G, P681H, D796H. We detected D614G mutation in all cases; P681R in 48 cases, E484Q in five cases and deletion 156–158 in 39 cases we sequenced. In a study from South India, the protective efficacy of two doses of COVID vaccine was 65%. Our vaccine breakthrough infection rate was slightly lower than that reported from Vellore (7.81% versus 9.81%) and our breakthrough infections occurred later (70 and 96 days in our study compared to 69 and 77 days in Vellore after receiving two or one dose of vaccine) [29].

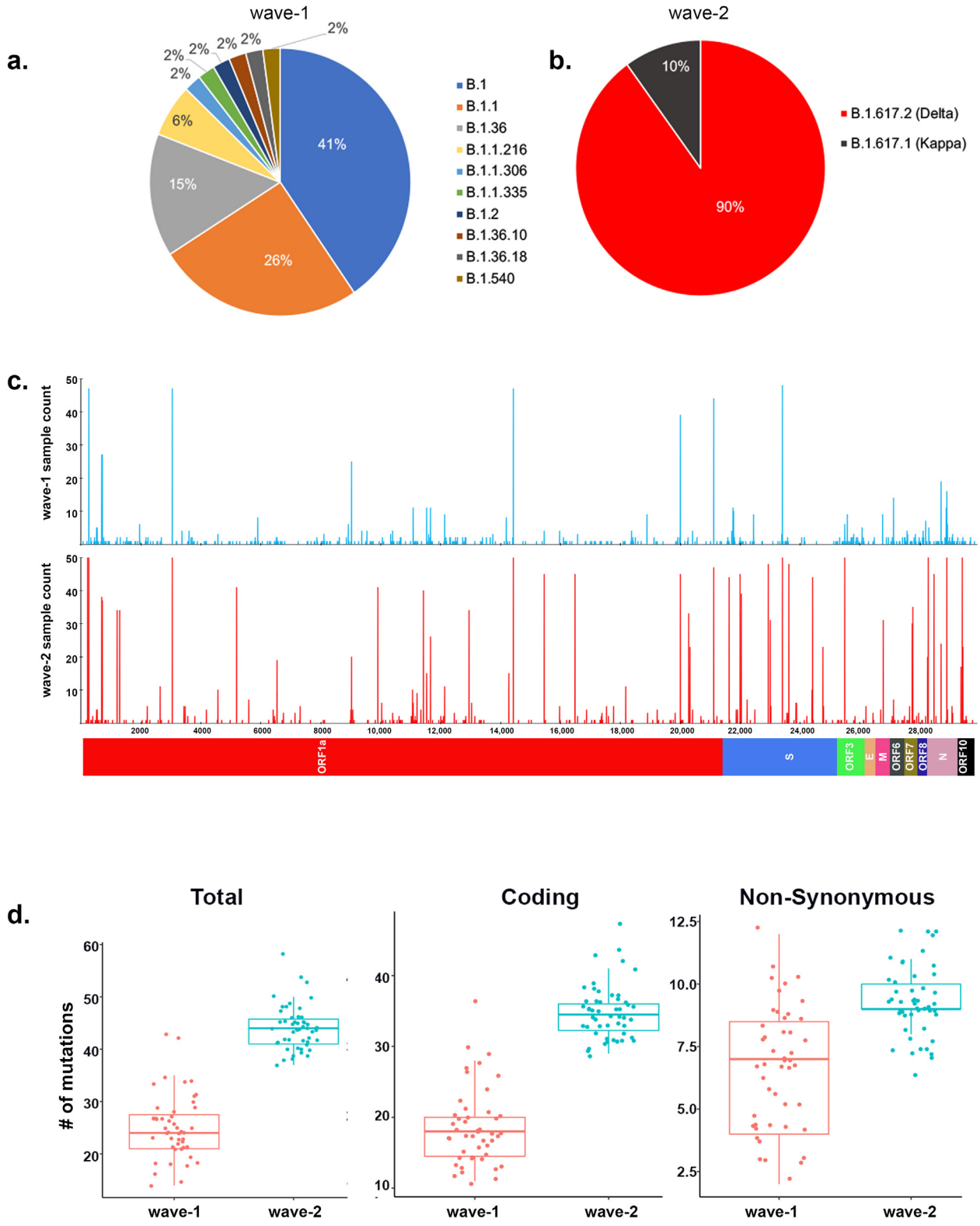

Fig. 2. Contrasting difference between wave-1 and wave-2. Lineage distribution in wave-1 (a) and wave-2 (b), Distribution of mutations identified in wave-1 and wave-2 samples (c), Total, coding and non-synonymous mutation load in wave-1 and wave-2 samples (d).

Table 2
Distribution of unique coding mutations in wave-1 and wave-2 among the genes in SARS-CoV2 genome in our study.

	Missense		Frameshift		Conservative in-frameinsertion		Stop gained		Disruptivein-frame deletion		Total	
Gene	wave-1	wave-2	wave-1	wave-2	wave-1	wave-2	wave-1	wave-2	wave-1	wave-2	wave-1	wave-2
E	4	0	0	0	0	0	0	0	0	0	4	0
M	1	0	0	0	0	0	0	0	0	0	1	0
N	11	3	0	0	0	0	0	0	0	0	11	3
ORF1ab	53	35	0	0	0	0	0	0	0	0	53	35
ORF3a	17	7	0	0	0	0	0	0	0	0	17	7
ORF6	0	0	0	1	0	0	0	0	0	0	0	1
ORF7a	3	2	0	0	1	0	0	0	0	0	4	2
ORF7b	2	1	0	0	1	0	1	0	0	0	4	1
ORF8	4	1	1	0	0	0	1	0	0	0	6	1
S	7	12	0	0	1	0	0	0	0	1	8	13

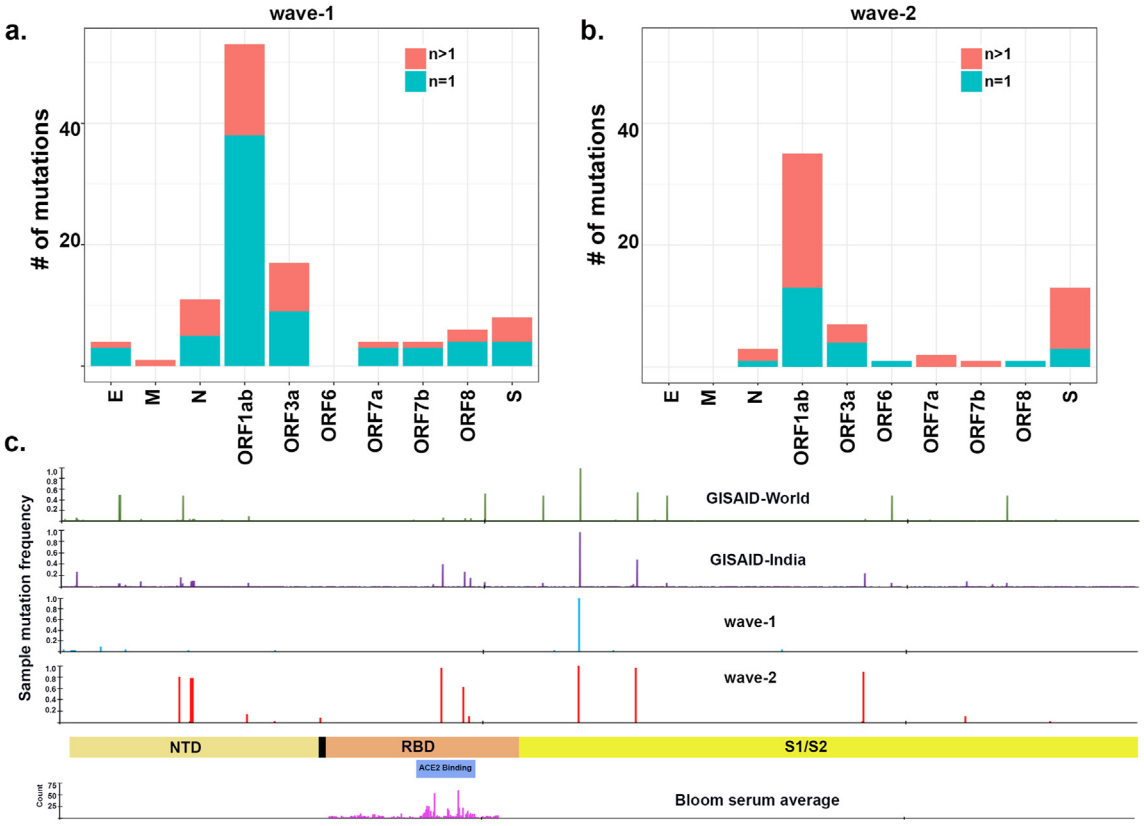

Fig. 3. Mutation distribution in genes. (a) Count of unique wave specific singleton and non-singleton mutations in various genes of wave-1 (a) and wave-2 (b) samples, (c) mutation distribution Spike (S) gene.

6. Conclusion

Our study has the following limitations: (a) small sample size; (b) very few cases of severe/moderate illness; (c) effect of viral mutations on treatment with Remdesivir or monoclonal antibody could not be assessed as very few HCWs required specific treatment; (d) we did not test for neutralizing antibodies or T-cell immunity in staff; (e) the role of host genetics (e.g. HLA types) was not assessed.

The significance of our study are: (1) different SARS-CoV-2 lineages caused wave-2 infections as compared to wave-1 infections, (2) the average and total number of mutations was much more in wave-2 compared to wave-(1, 3) mutations associated with high infectivity (T478K, P681R) and immune escape (L452R and E484Q) were detected in wave-2 samples, (4) eight new non-synonymous mutations never previously reported (as per GISAID database), (5) vaccine breakthrough infections were not uncommon and occurred despite receiving two doses of ChAdOx1 NCOV-19 (Covishield®) vaccine and even after 14 days from the last dose of the vaccine.

CRediT author statement

Conceptualization: SC, SB; Methodology: SB,PD; Software: PD, mc; Validation: PD, SM; Formal analysis: SC, SB, MC; Investigation: SB, SC, PD, SM; Resources: MC, AYM, DM, SB, GG, PV; Data Curation: SB, SC, AYM; Writing - Original Draft: SB, SC; Writing - Review & Editing: SB, SC, PD, MC; Supervision: MC, SB, SC; Project administration: MC, SB, GG, DM; Funding acquisition: MC, SB.

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Ethics approval

Approved by Institutional Review Board, Tata Medical Center, Kolkata.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijmmb.2022.09.010.

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