Impact of COVID-19 vaccination on transmission risk of breakthrough infections: Lessons from adapted N95 mask sampling for emerging variants and interventions

Kalpana Sriraman1 | Ambreen Shaikh1 | Smriti Vaswani1 | Tejal Mistry1 | Grishma Patel1 | Shalini Sakhivel1 | Vikas Oswal2 | Pratibha Kadam1 | Kayzad Nilgiriwala2 | Daksha Shah3 | Mangala Gomare3 | Nerges Mistry1

1The Foundation for Medical Research, Mumbai, Maharashtra, India
2Vikas Nursing Home, Mumbai, Maharashtra, India
3Municipal Corporation of Greater Mumbai (MCGM), Mumbai, Maharashtra, India

Correspondence
Kalpana Sriraman, The Foundation for Medical Research (FMR), Dr. Kantilal J. Sheth Memorial Bldg, 84-A, R.G. Thadani Marg, Worli, Mumbai, Maharashtra 400018, India.
Email: fmr@fmrindia.org

Abstract
This study used an adapted N95 mask sampling to understand the effect of COVID-19 vaccination in the context of circulating variants on infected individuals to emit the virus into the air, a key risk factor of transmission. Mask, swab, and blood samples were collected from 92 COVID-19 patients vaccinated (Covishield/COVAXIN-partial/fully) or unvaccinated between July and September 2021 during the Delta-dominated period in Mumbai. Mask/swab samples were analyzed by reverse transcription polymerase chain reaction for viral RNA. Blood was evaluated for SARS-CoV-2 anti-spike and nucleocapsid antibody responses. At <48 h of diagnosis, 93% of the patients emitted detectable viral RNA, with 40% emitting >1000 copies in 30 min (high emitters). About 8% continued to be high emitters even after 8 days of symptom onset. No significant difference was observed in emission patterns between partial, full, and unvaccinated patients. However, when vaccinated patients were stratified based on spike protein neutralization and nucleocapsid immunoglobulin G (IgG), the group with moderate/high neutralization showed a significantly lower proportion of high emitters and viral RNA copies than the group with no/low neutralization, which further reduced in the group having antinucleocapsid IgG. In conclusion, mask sampling showed that Delta infections were associated with greater virus emission in patients, which was significantly reduced only in vaccinated patients with moderate/high SARS-CoV-2 neutralization, especially with evidence of past infection. The study demonstrated that mask sampling could be useful for understanding the transmission risk of emerging variants, screening vaccine/booster candidates, and guiding control interventions.

KEYWORDS
Delta variant, mask sampling, neutralizing antibodies, SARS-COV-2, vaccination
INTRODUCTION

The course of any disease among a population depends on its transmission dynamics. There is clear evidence that SARS-CoV-2, responsible for the ongoing COVID-19 pandemic, predominantly spreads through the air.1 The dynamics of airborne disease transmission are complex, but understanding them has implications for disease control interventions, health policies, and messages. It involves many factors, namely, the rate at which an infector produces infectious droplets and aerosols, environmental factors including ventilation and non-pharmaceutical interventions, and the immune resilience of the recipient.2 A modeling study showed that most of these factors could be significantly impacted by measures like vaccination and the emergence of immune escape variants of the virus.3

The year 2021 was marked by the spread of the highly virulent SARS-CoV-2 Delta variant and intense efforts to vaccinate the global population. India deployed two vaccines, Covishield (adenovirus vector vaccine, ChAdOx1 nCoV-19-Serum Institute of India) and COVAXIN (inactivated whole virion vaccine, BBV152-Bharat Biotech). During this period, the Delta variant dominated the breakthrough infections that raised concerns about vaccine efficacy.4,5 Studies started emerging on the real-world efficacy of Indian vaccines on Delta infections.6 From a pandemic control perspective, although understanding vaccine efficacy was important, it was also critical to understand the impact of vaccination on preventing transmission in the context of variants. While extensive and systematic evidence on the impact of mRNA vaccines on the transmission of variants and its risk factors is now available,9–14 such data are still limited for other vaccine types, including evidence for Indian vaccines in Indian settings.15 The UK investigated the effect of AZD1222 (ChAdOx nCoV19-AstraZeneca) on secondary infections and transmission from Delta index cases and transmission risk factors.11,13 The current study was initiated in mid-2021 when no information was available on the impact of Indian vaccines and the Delta variant on transmission risk factors.

Our earlier work in COVID-19 patients showed that measuring SARS-CoV-2 RNA copies in respiratory particles expelled by infected COVID-19 patients at an early and a subsequent (late) stage of infection. As vaccines and past exposure to the virus can affect viral load in an individual through virus-specific immune responses,17,18 we further investigated the relationship of emission pattern to SARS-CoV-2-specific humoral responses in patients’ serum.

MATERIALS AND METHODS

Patient recruitment and sample collection

The study was approved by the Institutional Research Ethics Committee at the Foundation for Medical Research (FMR/IREC/C19/01/2021) and registered in the Clinical trials registry of India (No: CTRI/2021/07/035143). Informed consent was obtained from all individual participants of the study. Laboratory confirmed COVID-19 quantitative reverse transcription polymerase chain reaction (qRT-PCR) positive adults who were reported to the public health department or approached private care were screened via phone. Consenting eligible patients were enrolled within 48 h of diagnosis. A total of 95 vaccinated (Covishield—Cs or COVAXIN—Cx) and unvaccinated adults who had mild disease with SpO2 ≥ 95 at room air and thus were fit for mask testing were enrolled. Among these, 92 were in home isolation, and three were in the Kasturba Hospital COVID care ward due to a lack of isolation facilities at home. Patients were grouped based on vaccination status—fully vaccinated (n = 50: 26 Cs, 24 Cx; ≥14 days from second dose of vaccine), partially vaccinated (n = 31: 28 Cs, 3 Cx; ≥7 days from first dose or <14 days from second dose), and unvaccinated (n = 14; 13 no vaccine taken and 1 <7 days from first dose of vaccine). The fully vaccinated were further grouped based on vaccine taken (Cs or Cx) for subgroup analysis. The number of unvaccinated patients recruited was low due to an exponential increase in vaccination rates in the city during the study period, higher institutional care, and lesser willingness among unvaccinated patients to give consent for the study.

Samples were collected at two time points—(a) mask, nasopharyngeal swab (NPS), and blood samples were collected in tandem at the time of enrollment; (b) only mask and NPS samples were collected between 8 and 12 days from the first reported COVID-19 symptom (or from the date of diagnosis for asymptomatic positives; follow-up sample). Demographics, clinical presentation, treatment, and household information, including their vaccination and self-reported infection status, were recorded for all the study participants at all interaction points. A final telephonic follow-up was conducted between 15 and 21 days from the first reported symptom to document the patients’ disease outcome. The mask sampling involved collecting expelled respiratory particles of patients for 30 min using a modified N95 mask attached with a gelatin membrane as previously described.16 After the sampling, the gelatin membrane was dissolved immediately in RNAzol (Sigma-Aldrich). The NPS sample was collected in viral transport media (VTM; Vi-Trans, Cellkraft Biotech).
Pvt. Ltd.), followed by 5 ml blood in a serum vacutainer. Samples were transported to the FMR laboratory in cold conditions. The serum was separated by centrifugation immediately upon reaching the laboratory and stored at −20°C until analysis. Mask and swab samples were stored for not more than 24 h at 4°C before further processing.

### 2.2 Sample processing and qRT-PCR

RNA was extracted from VTM using QiaAmp viral RNA mini kit (Qiagen GmBH) as per the manufacturer’s protocol, while RNA from RNAzol containing dissolved gelatin membrane was extracted as previously described. The qRT-PCR was carried out in a CFX 96 real-time thermal cycler (Bio-Rad Laboratories) with COVIpak COVID-19 Kit (Invitrogen Bio Services India Pvt. Ltd.) as per the manufacturer’s protocol. The kit detects the N and O genes specific to SARS-CoV-2. COVID-19 negative NPS samples and RNA isolated from tuberculosis patients’ mask samples collected before December 2019 (pre-COVID) were used as negative controls. As the samples were from qRT-PCR confirmed COVID-19 patients, detection of both N and O genes or the O gene with visible sigmoidal PCR amplification curves and detectable Ct value (<40 as against <35 used for diagnosis) were considered positive. A standard curve was generated by performing 10-fold serial dilutions of the commercially available IVT RNA kit (TaqPath COVID-19 Control kit; Thermo Fischer Scientific) to determine the viral load (RNA copies) in SARS-CoV-2 positive samples. In mask samples, viral RNA copies of >1000 in the 30-min collection time defined the patient as a high emitter. All NPS samples with Ct value <33 (n = 83) were subjected to whole-genome sequencing (WGS) by the Oxford Nanopore sequencing using MinION, and viral lineage was determined in 75 of 83 samples using the PANGOLIN tool (v3.1.17) as described earlier.

### 2.3 SARS-CoV-2-specific humoral responses

All sera samples were tested for anti-IgG against SARS-CoV-2 spike protein S1 antigen (IgG-S) and nucleocapsid protein (IgG-N) for ancestral strain, and neutralizing antibodies against RBD of both ancestral strain (nAb-AS) and Delta (nAb-D) variant. IgG-S was measured by chemiluminescent VITROS reagent according to the manufacturer’s protocol on the VITROS XT 7600 Integrated Systems (Ortho Clinical Diagnostics). Signal cut-off ratio (S/C) >1 was considered reactive/positive. IgG-N was measured using the indirect ELISA method (Raybiotech) according to the manufacturer’s protocol. The titers were extrapolated from the positive-only calibration provided by the manufacturer, and the specified cut-off value for reactivity/positivity was >30.1 U/ml. The nAb-AS and nAb-D were measured by the SARS-CoV-2 surrogate virus neutralization test (GenScript), and the inhibition rate (%) was estimated as per the manufacturer’s protocol. As specified in the manufacturer’s datasheet, an inhibition rate of <30% was considered as no neutralization, 30%–60% was low neutralization, 61%–90% was moderate neutralization, and >90% was high neutralization.

### 2.4 Statistical analysis

Results were statistically analyzed using Graph Pad Prism software (version 9). Percentages were calculated for categorical variables, and Fisher exact test was applied. For continuous variables, the median with interquartile range (IQR) was reported, and statistical tests of Mann–Whitney unpaired test or Wilcoxon rank-sum were applied. A p < 0.05 was considered significant for both tests. Multivariate analysis was carried out by logistic regression analysis. Probit modeling was performed with MedCalc version 20.019 (MedCalc Software Ltd.). Where necessary, a power analysis was carried out to evaluate the impact of sample size on the reported results using the online tool OpenEpi.

### 3 RESULTS

Of the 95 COVID-19 patients enrolled in the study, 3 patients (one Cs, two Cx) were NPS and mask qRT-PCR negative at the first sample collection and thus were excluded from the analysis. A total of 84 patients provided both enrollment and follow-up samples. Follow-up samples could not be collected from eight patients due to patient refusal or admission to a hospital beyond the study jurisdiction (Figure S1). The median age of the enrolled patients was 40 (IQR: 30–50), and 55.5% were males (Table 1). Of the 92 patients analyzed, 89 had a mild disease as per the disease severity definition of the Government of India. Three patients’ disease status changed after enrollment into the study to moderate. Tables 1 and S1 depict the demographics, COVID-19 clinical characteristics, and mask sampling scores based on the vaccination status. WGS data analysis confirmed that the SARS-CoV-2 positive samples were either Delta (88%) or Delta derivative (Table S2).

#### 3.1 Emission pattern in mask samples at the enrollment and at the follow-up stage

The overall proportion of patients expelling the virus (mask positives) was 93% in this cohort, ≈ two-fold more than the mask positivity rate observed in our previous study (Table 2). The proportion was similar in partially or fully vaccinated and unvaccinated groups (Table 2) and between Covishield or COVAXIN fully vaccinated groups, suggesting that the vaccination may not have impacted the patient’s virus emission patterns. All patients with NPS Ct value <30 were mask positive (100%), while the mask positivity reduced to 61% for NPS Ct > 30 (Figure S2A). Also, the overall percentage of high emitters in the current study was 40%, about three-fold more than the percentage observed in the previous study cohort (13% Table 2), supporting the higher transmission of SARS-CoV-2
observed during the Delta wave in 2021. The proportion of high emitters was marginally higher in the fully vaccinated group; however, it was not statistically different compared to partial and unvaccinated groups (Table 2).

At follow-up, the proportion of mask positivity remained high (>50%) even after 8 days of symptom onset, irrespective of the vaccination status, although less than swab positivity (Table 2). Despite high mask positivity, partially and fully vaccinated patients displayed a significant reduction in mask viral RNA copy numbers (Figure 1). The data also showed that the mask positivity at follow-up did not correlate to the NPS Ct value as observed in enrollment samples (Figure S2B). Notably, about 8% of all patients continued to be high emitters even after 8 days of symptom onset and were more likely to have had a cough as a symptom (odds ratio [OR] = 8.323; p = 0.0434). When compared based on the vaccine taken, unlike the Covishield group, the mask positivity of fully vaccinated COVAXIN patients may be clearing the virus slower than the Covishield group; however, the sample size was small to arrive at a definitive conclusion (power = 57%).

To predict the expelling pattern and understand the potential contribution to further transmission on the tenth day of symptom onset (when general recommendations at the time of study advised the ending of patient isolation), Probit modeling was applied. Enrollment and follow-up data were segregated day-wise based on the duration of symptom onset to sampling. The probability of being mask positive decreased from the predicted 100% on Day 1 to 64.9% on the 10th day (FigureS3A), while the swab positivity reduced to 83% (Figure S3B). In contrast, the probability of being a high emitter significantly reduced from a predicted 90% on Day 1 to 7% on Day 10 (Figure S3C).

### 3.2 SARS-CoV-2-specific antibodies and relationship to emission pattern

Table 3 depicts the proportion of patients detected with IgG-S, nAb-AS, and nAb-D. The proportion (Table 3) and similar

---

**Table 1** Comparison of patient demographics, COVID-19 symptom characteristics, and mask sampling characteristics at enrollment and follow-up stratified based on vaccination status.

| Description                        | Overall | Partial | Full | Unvacc | Cs          | Cx          | p Value<sup>a</sup> | p Value<sup>b</sup> |
|------------------------------------|---------|---------|------|--------|-------------|-------------|---------------------|---------------------|
| Number of patients                 | 92      | 31      | 47   | 14     | 25          | 22          |                     |                     |
| Patient characteristics            |         |         |      |        |             |             |                     |                     |
| Male                               |         |         |      |        |             |             |                     |                     |
| Male                               | 51 [55] | 18 [58] | 24   | 9 [64] | 15 [60]     | 9 [41]      | 0.753               | 0.247               |
| Female                             | 41 [45] | 13 [42] | 23   | 5 [36] | 10 [40]     | 13 [61]     |                     |                     |
| Age                                | 40 (30–50) | 32 (28–42) | 45 (34–60)<sup>c</sup> | 42 (32–55) | 51 (41–72) | 39 (34–47) | 0.971              | 0.146              |
| Number of patients with comorbidities | 32 [35] | 6       | 21   | 5 [36] | 12 [48]     | 9 [41]      | 0.761               | 0.777               |
| Number of comorbidities            | 0 (0–1) | 0       | 0 (0–1) | 0 (0–1) | 0 (0–1)     | >0.99       | >0.99               | >0.99               |
| Duration between vaccination and COVID-19 diagnosis (days) | 58 (21–82) | 59 (38–76) | 67 (30–103) | NA | 72 (29–96) | 62 (43–121) | >0.99               | >0.99               |
| Symptom characteristics            |         |         |      |        |             |             |                     |                     |
| Days since the onset of the first symptom | 4 (3–5) | 4 (3–5) | 4 (3–5) | 3.5 (2.5) | 3 (2.5) | 4 (4–5) | >0.99              | 0.115               |
| Number of symptoms                 | 3 (2–5) | 3 (2–4) | 3 (2–5) | 3 (1–5) | 2 (1–4) | 5 (3–6) | >0.99              | 0.02                |
| Number of patients prescribed Antivirals<sup>d</sup> | 54 [59] | 20 [65] | 28 [60] | 7 [50] | 15 [60] | 13 [59] | 0.553              | >0.99               |
| Mask sampling characteristics      |         |         |      |        |             |             |                     |                     |
| Sampling Score – First sampling    | 6 (5–8) | 6 (4–7) | 7 (6–8) | 7 (6–8) | 7 (5–8) | 7 (6–8) | >0.99              | >0.99               |
| Sampling Score – Follow-up         | 6 (5–7) | 5 (4–7) | 6 (5–7) | 6 (5–7) | 6 (5–7) | 7 (5–8) | >0.99              | >0.99               |

Note: All values are represented as median (IQR) unless specified otherwise. Numbers in the square bracket represent percentages.

Abbreviations: Cs, two doses of Covishield and ≥14 days from second dose. Cx, two doses of COVAXIN and ≥14 days from second dose; Full, two doses of Covishield/COVAXIN and ≥14 days from second dose; IQR, interquartile range; NA, not applicable; Partial, ≥7 days from first dose or <14 days from second dose of Covishield/COVAXIN; Unvacc, unvaccinated.

<sup>a</sup>Significance calculated on comparing vaccinated groups with unvaccinated.

<sup>b</sup>Significance calculated on comparing Cs with Cx.

<sup>c</sup>Significant on comparing fully and partially vaccinated groups.

<sup>d</sup>Drugs like Fabiflu, Ivermectin, or Remdesivir with demonstrated in vitro antiviral activity were considered as antivirals.

---

---
The magnitude of inhibition (Figure S4A) of nAb-AS (vaccine-specific) and nAb-D (the strain of the current infection) in vaccinated patients indicated a high degree of cross-protection. The proportion of fully vaccinated Covishield patients with SARS-CoV-2-specific antibodies was 1.8 times more than those in the COVAXIN group (p < 0.01; Table 3). Nevertheless, the magnitude of the detected antibodies was not significantly different between the two vaccine groups (Figure 4B,C). The partially vaccinated group in this cohort had a slightly higher proportion of patients positive for antibodies than the fully vaccinated group (Table 3). This is most likely due to the over-representation of Covishield vaccinated patients (28/31 vs. COVAXIN 3/31) in this group and a higher proportion of Covishield vaccinated patients showing neutralization. Among Covishield vaccinated (partial or full) and unvaccinated patients, 14% of patients were reactive for IgG-N (evidence of recent past infection). It was anticipated that COVAXIN (a whole virion-inactivated vaccine) would produce detectable IgG-N in all its vaccinees. However, only 13.6% of the fully vaccinated COVAXIN patients were IgG-N reactive, similar to other groups (Table 3).

**TABLE 2** Comparison of mask and NPS positivity at enrollment and follow-up.

| Stage          | Category                  | This study | Previous study (2020) |
|----------------|---------------------------|------------|-----------------------|
|                |                           | Overall    | Partial | Full | Unvacc | Cs | Cx | Unvacc Cs Cx |
| Enrollment     | Total enrolled            | 95         | 31      | 50   | 14    | 26 | 24 |
|                | Total patients after exclusion | 92        | 31      | 47   | 14    | 25 | 22 | 31 |
|                | Swab positives            | 91         | 31      | 47   | 13    | 25 | 22 | 29 |
|                | Swab positivity (%)        | 99         | 100     | 100  | 92    | 100| 100| 94 |
|                | Mask positives            | 86         | 29      | 44   | 13    | 23 | 21 | 13 |
|                | Mask positivity (%)        | 93         | 94      | 94   | 92    | 92 | 95 | 42 |
|                | High emitters (%)          | 40         | 29      | 49   | 36    | 48 | 50 | 13 |
| Follow-up      | Lost to follow-up         | 8          | 3       | 4    | 1     | 2  | 2  |
|                | Total followed-Up         | 84         | 28      | 43   | 13    | 23 | 20 |
|                | Swap positives            | 72         | 22      | 39   | 11    | 22 | 17 |
|                | Swab positivity (%)        | 86         | 79      | 91   | 85    | 96 | 85 |
|                | Mask positives            | 55         | 15      | 32   | 8     | 15 | 17 |
|                | Mask positivity (%)        | 65         | 54      | 74   | 62    | 62 | 90 |
|                | High emitters (%)          | 8          | 7       | 9    | 8     | 4  | 15 |

Note: Percentages are indicated in bold. The previous study—results are from Sriramam et al.16
Abbreviations: Cs, two doses of Covishield and ≥14 days from second dose; Cx, two doses of COVAXIN and ≥14 days from second dose; Full, two doses of Covishield/COVAXIN and ≥14 days from second dose; Partial, ≥7 days from first dose or <14 days from second dose of Covishield/COVAXIN; Unvacc, unvaccinated.

**FIGURE 1** Mask viral load reduction from enrollment (Enr) to follow-up (FU) in the various vaccine groups. Partial, ≥7 days from first dose or <14 days from second dose of Covishield/COVAXIN; Full, two doses of Covishield/COVAXIN and ≥14 days from second dose; Cs, two doses of Covishield and ≥14 days from second dose; Cx, two doses of COVAXIN and ≥14 days from second dose; Unvac, unvaccinated; Enr, enrollment; FU, follow-up. **p < 0.01; ***p < 0.001.
TABLE 3 Proportion of patients with SARS-CoV-2-specific antibody response.

| Study groups | Inhibition against | IgG-specific antibodies |
|--------------|-------------------|-------------------------|
|              | Ancestral strain  | Delta variant | Spike protein | Nucleocapsid protein |
| Partially vaccinated | 74% | 87% | 87% | 6% |
| Fully vaccinated | 66% | 74% | 70% | 19% |
| Unvaccinated | 43% | 64% | 43% | 14% |
| Cs           | 84% | 96% | 92% | 24% |
| Cx           | 45% | 50% | 45% | 12% |

Abbreviations: Cs, two doses of Covishield and ≥14 days from second dose; Cx, two doses of COVAXIN and ≥14 days from second dose; Full, two doses of Covishield/COVAXIN and ≥14 days from second dose; Partial, ≥7 days from first dose or <14 days from second dose of Covishield/COVAXIN.

Neutralizing antibodies generated from vaccination and/or infection is considered a correlate of the protection against SARS-CoV-2 infection17,18; however, it is unclear how nAbs and past infection can influence the emission of the virus by infected patients. Therefore, vaccinated patients (partially/fully) were grouped based on the inhibition percentages of nAbs-AS and D (please refer to methods) and reactivity for IgG-N for comparing the emission pattern, as follows.

1. Poor neutralization group (PN; n = 33): Low or no neutralization (<60% inhibition) for both AS and D + nonreactive for IgG-N.
2. Good neutralization without IgG-N group (GN-IgG-N; n = 33): Moderate or high neutralization (60%–100% inhibition rate) for both AS and D + nonreactive for IgG-N.
3. Good neutralization with IgG-N group (GN + IgG-N; n = 11): Moderate or high neutralization (60%–100% inhibition rate) for both AS and D + reactive for IgG-N (evidence of recent past exposure).

The three groups had similar demographics (Table S3) but had significant differences in IgG-S levels [median S/C (IQR): 3.9 (2.6–8) PN, 15 (13–17.8) GN-IgG-N, 20 (18.5–21.3) GN + IgG-N; p < 0.001]. The increased IgG-S response in the GN groups further attests to a broader breadth of SARS-CoV-2-specific response compared to those with PN. One-way ANOVA was used to analyze the expelled mask and swab viral RNA copies at enrollment for the three groups. The GN – IgG-N group had 1.4 log-fold fewer viral copies in mask samples than the PN group (p = 0.009; Figure 2A). The GN + IgG-N group had 1.3 log fold (p = 0.036) and 2.6 log fold (p < 0.0001) lower viral RNA copies in the mask than GN – IgG-N and PN groups, respectively. A similar trend was observed for NPS viral RNA copy numbers between the three groups (Figure 2B), although there was a more marked decrease in mask viral RNA copies than in swabs. The proportion of high emitters was significantly lower (1.8-fold) in the GN – IgG-N group compared to the PN group (p = 0.026; Figure 2C).

Interestingly, there were no patients with high emission patterns in the GN + IgG-N group (Figure 2C). For the sample size indicated, the reduction in the high emitter pattern in the GN+/−IgG-N groups compared to the PN group was powered at 96.4% and 60.6%, respectively. A similar trend in the number of high emitters was observed in unvaccinated patients (n = 14; 4/8 for PN, 1/6 for GN), but the sample size was too small for meaningful analysis. Collectively, results indicated that vaccinated patients with good neutralization capacity and more with IgG-N response are likely to emit lower viral RNA copies and thus may have a low risk for transmission.

3.3 Factors associated with a high emission pattern

Univariate logistics regression analysis for the overall cohort (n = 92) showed that cough as a symptom at enrollment, shorter duration between symptom onset and enrollment, presence of comorbidity, poor neutralization, no IgG reactivity for S/N protein, and presence of symptoms beyond 8 days from symptom onset were independent predictors (higher OR) of patients being high emitters (Figure S5). However, in multivariate logistics analysis, after adjusting for assortative factors, only the presence of comorbidities, cough as a symptom at enrollment and poor neutralization continued to be independent and significant predictors of high emission pattern (Figure S5). Moreover, patients who had all of these three risk factors had eight times higher odds of being high emitters (OR = 8.833; p = 0.008).

4 DISCUSSION

In this study, we used a simple adapted N95 mask sampling combined with qRT-PCR to measure the impact of Delta variant and COVID-19 vaccination on the rate at which individuals infected with SARS-CoV-2 emitted virus into the air, an important risk factor of disease transmission.2 To our knowledge, this is the first study that investigated the impact of Indian vaccines on transmission risk factors in the context of SARS-CoV-2 variants. Here, we discuss the key results and their learnings for applications in transmission risk assessments relevant for guiding disease control interventions, policy designs, and new vaccine testing.

4.1 Learning 1: Mask sampling supports increased emission of the virus by Delta variant infected patients: Relevance for understanding transmissibility of emerging variants

In this study, 93% of the people infected with the Delta variant emitted qRT-PCR detectable levels of virus in respiratory particles within 48 h of diagnosis. This proportion was about two-fold more
than people infected with SARS-CoV-2 before the emergence of Delta in 2020, noted by us and others. Noticeably, the proportion of high emitters was three-fold more than in our earlier 2020 study conducted in Mumbai with the same sampling method, in a population with similar age, comorbidities, and COVID-19 characteristics, the change in emission rates observed can be attributed to the Delta variant itself, thereby explaining its high rates of transmission. These results align with other laboratory and epidemiological studies supporting increased transmissibility of the Delta variant. More importantly, similar to our previous study, the proportion of high emitters (40%) observed in this study also correlated to the reported Delta variant-related secondary attack rate (30.8%; 95% confidence interval, 23.5%–39.3%) derived from a meta-analysis of household contact studies. The high emitter proportion also matched a South Korean Delta outbreak study which showed that only 40% of the individuals caused all secondary infections. Although direct relation to the actual transmission was not established in this study, a correlation of emission quantity from similar mask sampling to transmission has been shown for other airborne diseases and very recently for SARS-CoV-2. The UK study showed that for every log increase in peak exhaled SARS-CoV-2 RNA by the index case, the probability of household transmission increased by 5–20-fold. A similar analysis of our dataset with the same definition for household transmission showed three-fold more household transmission in our high emitter group. However, the number of potential index patients (24/92) and their households with secondary infections (6/24) were too low to show a statistical correlation with transmission (data not shown). Overall, the alignment of the high emitter pattern to epidemiologically observed transmission rates both for ancestral strains and the Delta (current study) suggests that tracking high emitter patterns through mask sampling can serve as a quick tool to understand real-world transmission risks from any new emerging variants or even any novel respiratory viruses, useful for timely guiding of disease control policies.
4.2 Learning 2: Only vaccinated patients with good SARS-CoV-2 neutralizing antibodies have a lower risk of being high emitters: Relevance for boosters and new vaccine development

In this study, mask sampling initially showed that the proportion of people who were emitters (Table 2) and the magnitude of the viral load (Figure 1) was similar in partial, full, and unvaccinated individuals. It suggested that vaccinated individuals were equally likely to emit the virus and carry forward the transmission risk. The proportion of high emitters among fully vaccinated was marginally higher than in partial and unvaccinated groups. However, the difference was not statistically significant, probably because of the smaller sample size of the latter groups. Nevertheless, the results were congruent with other early studies that reported marginally different but statistically nonsignificant swab viral loads in vaccinated and unvaccinated individuals. However, studies based on contact tracing and infectious virus measurements, primarily in mRNA vaccinated individuals, showed that vaccinated individuals infected with Delta had marginally lower secondary transmission rates and significantly lower culturable/infectious virus. Despite these studies indicating that vaccination reduced transmission, a high degree of variability was noted. Eyre et al. observed that Delta transmission among fully vaccinated individuals was similar to that in unvaccinated persons by 12 weeks of ChAdOx-nCoV-19 (AZD1222) vaccination, attributing to waning vaccine immunity with time. In support of these observations, our results showed that the emission of virus required for transmission from vaccinated infected individuals depended on the levels of variant-specific neutralizing antibodies at the time of infection, a known correlate of protection from infection for vaccines. The current results show that only vaccinated individuals having good virus neutralizing capacity had a lower propensity to be high emitters with a potentially lower risk for transmission (Figure 2). These patients exhibited similar levels of cross-protection to both ancestral and Delta variants (Table S3) and also showed increased levels of IgG-S antibodies (Table S3). This was further augmented in fully vaccinated individuals who showed evidence of past infection (IgG-N reactive; Table S3).

Our findings suggest that only vaccines that elicit broad neutralization against various variants (including immune evasive variants like Omicron) would significantly impact breaking transmission. Therefore, from a pandemic control perspective, accelerated efforts are urgently required to develop booster strategies like heterologous boosters that likely increase neutralizing antibody titers and the development of new/multivalent vaccines with broad neutralizing antibodies. Furthermore, incorporating mask sampling during clinical trials of new vaccine development along with measuring antibody outcomes can help identify vaccine candidates that would have a greater impact on reducing disease transmission.

4.3 Learning 3: Longitudinal follow-up of patients with mask sampling shows that a subset of patients continue to be high emitters: Relevance for patient isolation policies

In late 2021 until Omicron emerged, guidelines across various countries, including India, recommended that mild patients’ isolation be terminated at 10 days from symptom onset, provided they did not have fever for about 24 (USA) to 72 (India) hours. These guidelines were initially framed based on contact tracing and laboratory studies that looked at culturable viruses from 2020, which showed less than 5% risk for transmission at the late stage. Even though the Delta variant emerged to be more virulent and transmissible, the same isolation policies continued. One study that tracked infectious viruses for up to 15 days showed that infectious viral shedding was longer for the Delta than non-Delta infections. In this study, we have shown that the likelihood of being a high emitter was 7% at a late stage of infection (Figure S3C). This suggests a theoretical risk for transmission for Delta even at this stage. Notably, except for two (0.02%), all patients, including high emitters, had no fever in the follow-up period (Table S1). However, the high emitters were significantly associated with having cough as a symptom, suggesting that relying on a single symptom of fever may overlook the possible risk for transmission. Even though our results show that guidelines of 10 days’ isolation were applicable in most cases, a subset (7%) may continue to carry high risk and may need more prolonged isolation. In contrast, Siedner et al. showed that they could not detect the culturable virus in all NPS-positive samples of vaccinated patients on the 10th day or earlier if the fever resolved. This difference in infection risk may be because the Siedner study was conducted in the USA in the context of mRNA vaccines, while the present study was conducted in India in the context of inactivated and adenovirus-based vaccines. In essence, our results support that if mask sampling is used in a pilot surveillance mode at regular intervals, it can help frame a more rational approach to patient isolation policies as the disease situation changes with emerging variants.

5 CONCLUSION

In conclusion, our study provided evidence for increased transmission of the Delta variant and conditions in which vaccination can reduce the risk of transmission using an adapted N95 mask sampling. It is to be noted that the study measured one of the known risk factors for transmission (virus production rate by infector) and could not directly link emission pattern to transmission. Many household members of the study patients tested positive at the same time or within 3 days. Hence, we could neither define study patients as an index in many cases nor establish a definite relationship between their viral load on household transmission. Moreover, the study measured the transmission of relevant viral RNA copies emitted through qRT-PCR, which does not differentiate between active and inactive viruses.
Despite these limitations, the study results were consistent with those of other studies that showed that the mask viral load could indicate transmission risk, and the Delta variant had a propensity for higher transmission, and vaccination helps in reducing transmission, suggesting that the mask sampling approach can be used for understanding the transmission risk of SARS-CoV-2.

With the constant threat of the emergence of highly transmissible new variants and the introduction of mass-scale interventions like vaccination and boosters, studies like this become critical for continuously understanding transmission patterns of the ongoing pandemic. The tool and methods used in this study have many applications, including (a) understanding the potential transmission risk of any new variants or interventions that can guide the development of patient isolation policies or disease control strategies, (b) screening new vaccines or therapeutic candidates for their ability to block transmission. Though the findings from this study are specific to the Delta variant and India-approved vaccines, the method has applications for future pandemics and can be extended to testing other airborne respiratory viruses.

AUTHOR CONTRIBUTIONS
Kalpana Sriraman and Ambreen Shaikh contributed to conceptualization, project development and management, study design, patient recruitment, data analysis and interpretation, and drafting and revising of the manuscript. Smriti Vaswani, Tejal Mestry, Grishma Patel, and Shalini Sakhthivel contributed to investigations, data acquisition, data management, and visualization. Smriti Vaswani also contributed to the revision and editing of the manuscript. Vikas Oswal contributed to conceptualization, providing resources, and patient recruitment. Pratibha Kadam contributed to the sequencing analysis. Kayzad Nilgiriwala contributed to sequencing resources and the revision of the manuscript. Daksha Shah and Mangala Gomare provided resources and approvals for the successful conduct of the study. Nerges Mistry contributed to conceptualization, study design, data interpretation, manuscript revision, and overall supervision. Kalpana Sriraman and Nerges Mistry acquired funding for the study.

ACKNOWLEDGMENTS
The authors are deeply thankful to Mr Nadir Godrej, Mr Aditya Berlia, Mr Rakesh Agarwal, Mr Pranav Kothari, Mr Sandeep Chopra, Mr Anantnarayan Sundresan, and others for their donations. The authors thank Dr Chandrakant Pawar, Medical Superintendent, Kasturba Hospital for Infectious Diseases, for his initial support of the study. The authors sincerely appreciate the contribution of Ms Niharika Shinde, the field researcher who enrolled the patients and collected the samples. Finally, the authors would like to thank all the participants of this study for their cooperation, patience, and support, without which this study would not have been possible. The study was funded by individual donations from members of the Harvard Business School Alumni Club of India and general donations from Zoroastrian Charity Funds of Hongkong, Canton and Macau to the Foundation for Medical Research.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Kalpana Sriraman http://orcid.org/0000-0001-8012-0149
Ambreen Shaikh http://orcid.org/0000-0001-6037-9137
Kayzad Nilgiriwala http://orcid.org/0000-0002-5458-4916
Nerges Mistry http://orcid.org/0000-0003-3509-4994

REFERENCES
1. Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. The lancet. 2021;397(10285):1603-1605.
2. Wang CC, Prather KA, Sznitman J, et al. Airborne transmission of respiratory viruses. Science. 2021;373(6558):eabd9149.
3. Rowe BR, Canosa A, Meslem A, Rowe F. Increased airborne transmission of COVID-19 with new variants, implications for health policies. Build Environ. 2022;219:109132.
4. Mlcocoha P, Kemp SA, Dhar MS, et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. Nature. 2021;599(7883):114-119.
5. Singh UB, Rophina M, Chaudhry R, et al. Variants of concern responsible for SARS-CoV-2 vaccine breakthrough infections from India. J Med Virol. 2022;94(4):1696-1700.
6. Bhatnagar T, Chaudhuri S, Ponnaiah M, et al. Effectiveness of BBV152/Covaxin and AZD1222/Covishield vaccines against severe COVID-19 and B.1.617.2/Delta variant in India, 2021: a multicentric hospital-based case-control study. Int J Infect Dis. 2022;122:693-702.
7. Ghosh S, Shankar S, Chatterjee K, et al. COVISHIELD (AZD1222) VaccNe effectiveness among healthcare and frontline Workers of Indian Armed Forces: interim results of VIN-WIN cohort study. Med J Armed Forces India. 2021;77:S264-S270.
8. Singh C, Naik BN, Pandey S, et al. Effectiveness of COVID-19 vaccine in preventing infection and disease severity: a case-control study from an Eastern State of India. Epidemiol Infect. 2021;149:e2224.
9. Ng OT, Koh V, Chiew CJ, et al. Impact of Delta variant and vaccination on SARS-CoV-2 secondary attack rate among household close contacts. Lancet Reg Health West Pac. 2021;17:100299.
10. de Gier B, Andeweg S, Backer JA, et al. Vaccine effectiveness against SARS-CoV-2 transmission to household contacts during dominance of Delta variant (B.1.617.2), the Netherlands, August to September 2021. Euro Surveill. 2021;26(44):2100977.
11. Eyre DW, Taylor D, Purver M, et al. Effect of Covid-19 vaccination on transmission of alpha and delta variants. New Engl J Med. 2022;386:744-756.
12. Acharya CB, Schrom J, Mitchell AM, et al. Viral load among vaccinated and unvaccinated, asymptomatic and symptomatic persons infected with SARS-CoV-2 delta variant. Open Forum Infect Dis. 2022;9:ofac135.
13. Singanayagam A, Hakki S, Dunning J, et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated Individuals in the UK: a prospective, longitudinal, cohort study. Lancet Infect Dis. 2021;22:183-195.
14. Puhach O, Adea K, Hulo N, et al. Infectious viral load in unvaccinated and vaccinated individuals infected with ancestral, Delta or Omicron SARS-CoV-2. Nat Med. 2022;28:1491-1500.
15. Kemp SA, Cheng MTK, Hamilton WL, et al. Transmission of B.1.617.2 delta variant between vaccinated healthcare workers. Sci Rep. 2022;12:10492.

16. Sriraman K, Shaikh A, Parikh S, et al. Non-invasive adapted N-95 mask sampling captures variation in viral particles expelled by COVID-19 patients: implications in understanding SARS-CoV2 transmission. PLoS One. 2021;16(4):e0249525.

17. Anichini G, Terrosi C, Gandolfo C, et al. SARS-CoV-2 antibody response in persons with past natural infection. New Engl J Med. 2021;385(1):90-92.

18. Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates of postvaccination SARS-CoV-2 infections during the Delta dominated second wave of COVID-19 pandemic, from Mumbai Metropolitan Region (MMR), India. J Med Virol. 2022;94:4206-4215.

19. Nilgiriwala K, Kadam P, Patel G, et al. Genomics of postvaccination SARS-CoV-2 infections during the Delta dominated second wave of COVID-19 pandemic, from Mumbai Metropolitan Region (MMR), India. J Med Virol. 2022;94:4206-4215.

20. Dean A, Sullivan K, Soe M, Mir R. OpenEpi: open source epidemiologic statistics for public health. 2013.

21. Ministry of Health and Family Welfare. ICMR Clinical Guidance for Mask Sampling for Mycobacterium Tuberculosis: Insights Into Detection and Transmission. Thesis. University of Leicester; 2021.

22. Williams CM, Pan D, Decker J, et al. Exhaled SARS-CoV-2 quantified by face-mask sampling in hospitalised patients with COVID-19. J Infect. 2021;82(6):253-259.

23. Adenaiye OO, Lai J, Bueno de Mesquita PJ, et al. Infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in exhaled aerosols and efficacy of masks during early mild infection. Clin Infect Dis. 2022;75:e241-e248.

24. Li B, Deng A, Li K, et al. Viral infection and transmission in a large, well-traced outbreak caused by the SARS-CoV-2 Delta variant. Nat Commun. 2022;13:460.

25. Liu Y, Rocklov J. The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. J Travel Med. 2021;28:aabb124.

26. Madewell ZJ, Yang Y, Longini IM Jr, Halloran ME, Dean NE. Household transmission of SARS-CoV-2: a systematic review and meta-analysis. JAMA Netw Open. 2020;3(12):e2031756.

27. Lee YS, Kim S, Kim GJ, et al. Transmission dynamics of the Delta variant of SARS-CoV-2 infections in South Korea. J Infect Dis. 2021;201(3):5327-5342.

28. Williams CM. Mask Sampling for Mycobacterium Tuberculosis: Insights Into Detection and Transmission. Thesis. University of Leicester; 2021.

29. Huynh KN, Oliver BG, Stolzer S, Rawlinson WD, Tovey ER. A new method for sampling and detection of exhaled respiratory virus aerosols. Clin Infect Dis. 2008;46(1):93-95.

30. Pan DWC, Decker J, Fletcher E, et al. Exhaled SARS-CoV-2 RNA viral load kinetics measured by facemask sampling associates with household transmission. Clin Microbiol Infect. 2022;14:S1198-743X(22)00369-X.

31. Riemersma KK, Hadock III LA, Wilson NA, et al. Shedding of infectious SARS-CoV-2 despite vaccination. medRxiv. 2021. doi:10.1101/2021073121261387

32. Pena-Hernandez MA, Klein J, Malik A, et al. Comparison of infectious SARS-CoV-2 from the nasopharynx of vaccinated and unvaccinated individuals. medRxiv. 2021.

33. Despres HW, Mills MG, Shirley DJ, et al. Measuring infectious SARS-CoV-2 in clinical samples reveals a higher viral titer: RNA ratio for Delta and Epsilon vs. Alpha variants. Proc Natl Acad Sci U S A. 2022;119:e2116518119.

34. Earle KA, Ambrosino DM, Fiore-Gartland A, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. Vaccine. 2021;39(32):4423-4428.

35. van Kampen JAA, van de Vijver DAMC, Fraaij PLA, et al. Duration and key determinants of infectious virus shedding in hospitalised patients with coronavirus disease-2019 (COVID-19). Nat Commun. 2021;12:267.

36. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of the Delta variant of SARS-CoV-2 in exhaled aerosols and efficacy of masks during early mild infection. Clin Infect Dis. 2022;75:e241-e248.

37. Cheng H-Y, Jian S-W, Liu D-P, et al. Contact tracing assessment of COVID-19 transmission dynamics in Taiwan and risk at different exposure periods before and after symptom onset. JAMA Intern Med. 2020;180(9):1156-1163.

38. Siedner MJ, Boucau J, Gilbert RF, et al. Duration of viral shedding and culture positivity with post-vaccination SARS-CoV-2 delta variant infections. JCI insight. 2022;7:e155483.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sriraman K, Shaikh A, Vaswani S, et al. Impact of COVID-19 vaccination on transmission risk of breakthrough infections: Lessons from adapted N95 mask sampling for emerging variants and interventions. J Med Virol. 2022;95:e28188. doi:10.1002/jmv.28188