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Impact of COVID-19 vaccination on transmission risk of breakthrough infections: Lessons from Adapted N95 Mask Sampling for Emerging Variants and Interventions

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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 analysed by RT-PCR for viral RNA. Blood was evaluated for SARS-CoV-2 anti-spike and nucleocapsid antibody responses. At < 48 hours of diagnosis, 93% of the patients emitted detectable viral RNA, with 40% emitting >1000 copies in 30-minutes (high emitters). About 8% continued to be high emitters even after eight days of symptom onset. No significant difference was observed in emission patterns between partial, full and un-vaccinated patients. However, when vaccinated patients were stratified based on spike protein neutralisation and nucleocapsid IgG, the group with moderate/high neutralisation showed a significantly lower proportion of high emitters and viral RNA copies than the group with no/low neutralisation, which further reduced in the group having anti-nucleocapsid 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-CoV2 neutralisation, 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 - Mask sampling, SARS-COV-2, Vaccination, Delta variant, Neutralizing Antibodies

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 modelling 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 [6-8]. 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 are 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-Astra Zeneca) 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 patients while talking, coughing, and breathing using a non-invasive adapted N-95 mask sampling could be useful in understanding transmission risk [16]. In that study, it was observed that only a subset of patients emitted the virus. Moreover, the proportion of patients who emitted more than 1000 RNA copies in 30 minutes (termed high emitters) reflected the known secondary attack and

transmission rates in the community better than standard swab viral load, indicating that high emitters have a high risk of causing secondary transmission [16]. These findings prompted us to use the mask sampling method to understand the impact of the Indian vaccines and circulating variant on the individual emission pattern and thereby its potential effect on individual transmission risk.

Here we report the results of a prospective observational study conducted in Mumbai during the Delta-dominated period of July to September 2021. We demonstrated the effect of the variant and vaccination on the emission pattern of the viral 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-CoV2 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 (No: CTRI/2021/07/035143). Informed consent was obtained from all individual participants of the study. Laboratory confirmed COVID-19 RT-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 hours 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 2nd dose of vaccine), partially vaccinated (n=31:28 Cs, 3 Cx; ≥ 7 days from 1st dose or <14 days from 2nd dose), and unvaccinated (n=14: 13 no vaccine taken, 1 <7 days from 1st dose of vaccine). The fully vaccinated were further grouped based on vaccine taken (Cs or Cx) for sub-group 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 to give consent for the study among the unvaccinated patients.

Samples were collected at two time points – a) Mask, nasopharyngeal swab (NPS) and blood samples were collected in tandem at the time of enrolment; 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 minutes 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, MO, USA). The NPS sample was collected in viral transport media (VTM; Vi-Trans, Cellkraft Biotech Pvt. Ltd, Bengaluru, India), followed by 5ml 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^{0}C until analysis. Mask and swab samples were stored for not more than 24 hours at 4^{0}C before further processing.

Sample Processing and RT-PCR:

RNA was extracted from VTM using QiaAmp viral RNA mini kit (Qiagen GmBH, Hilden, Germany) as per the manufacturer's protocol, while RNA from RNAzol^{TM} was extracted as previously described [16]. The RT-PCR was carried out in a CFX 96 real-time thermal cycler (Bio-Rad Laboratories, California, USA) with COVIPath^{TM} COVID-19 Kit (Invitrogen Bio Services India Pvt Ltd., Bengaluru, India) 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 RT-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, USA) to determine the viral load (RNA copies) in SARS-CoV-2 positive samples. In mask samples, viral RNA copies of >1000 in the 30-minute collection time defined the patient as a high emitter [16]. 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 75/83 samples using the PANGOLIN tool (*v3.1.17)* as described earlier[19].

SARS-CoV2 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 neutralising 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, Mumbai, India). Signal cut-off ratio (S/C) >1 was considered reactive/positive. IgG-N was measured using the indirect ELISA method (Raybiotech, GA, USA) 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 >30.1U/ml. The nAb-AS and nAb-D were measured by the SARS-CoV-2 surrogate virus neutralisation test (sVNT) (GenScript, NJ, USA), 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 neutralisation, 30%-60% was low neutralisation, 61%-90% was moderate neutralisation, and >90% was high neutralisation.

Statistical analysis:

Results were statistically analysed 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-value of < 0.05 was considered significant for both tests. Multivariate analysis was carried out by logistic regression analysis. Probit modelling 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 [20].

Results

Of the 95 COVID-19 patients enrolled in the study, three patients (1Cs, 2Cx) were NPS and mask RT-PCR negative at the first sample collection and thus were excluded from the analysis. A total of 84 patients

provided both enrolment 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 (Supplementary Fig. 1). The median age of the enrolled patients was 40 (IQR 30-50), and 55.5% were males (Table 1). Of the 92 patients analysed, 89 had a mild disease as per the disease severity definition of the Govt. of India [21]. Three patients' disease status changed after enrolment into the study to moderate. Table 1 and Supplementary Table 1 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 (Supplementary Table 2)[19].

Emission pattern in mask samples at the enrolment and at the follow-up stage:

The overall proportion of patients expelling the virus (mask positives) was 93% in this cohort, ≈ 2-fold more than the mask positivity rate observed in our previous study [16] (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 value >30 (Supplementary Fig. 2a). Also, the overall percentage of high emitters in the current study was 40%, about 3-fold more than the percentage observed in the previous study cohort (13% [16], 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 eight 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 (Fig. 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 (Supplementary Fig. 2b). Notably, about 8% of all patients continued to be high emitters even after eight days of symptom onset and were more likely to have had a cough as a symptom (OR=8.323, p-value =0.0434). When compared based on the vaccine taken, unlike the Covishield group, the mask positivity of fully vaccinated COVAXIN patients at follow-up (90%) was similar to their swab positivity (85%; Table 2). In addition, COVAXIN patients had no statistically

significant reduction in mask viral RNA copy numbers (Fig. 1) and had a higher high emitter proportion (15%; Table 2) compared to fully vaccinated Covishield patients (4%). This finding suggested that COVAXIN vaccinated 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 modelling was applied. Enrolment 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 tenth day (Supplementary Fig. 3a), while the swab positivity reduced to 83% (Supplementary Fig. 3b). In contrast, the probability of being a high emitter significantly reduced from a predicted 90% on day 1 to 7% on day 10 (Supplementary Fig. 3c).

SARS-CoV2 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 magnitude of inhibition (Supplementary Fig. 4a) 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-value < 0.01, Table 3). Nevertheless, the magnitude of the detected antibodies was not significantly different between the two vaccine groups (Supplementary Figs. 4b and 4c). 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 neutralisation. 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).

Neutralising antibodies generated from vaccination and/or infection is considered a correlate of the protection against SARS-CoV-2 infection [17,18]; 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 + Non-reactive for IgG-N
2. Good neutralisation without IgG-N group (GN-IgG-N; n=33): Moderate or high neutralisation (60%-100% inhibition rate) for both AS and D + Non-reactive for IgG-N
3. Good neutralisation with IgG-N group (GN+IgG-N, n=11): Moderate or high neutralisation (60%-100% inhibition rate) for both AS and D + Reactive for IgG-N (evidence of recent past exposure)

The three groups had similar demographics (Supplementary Table 3) 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 in the patients in GN-/+IgG-N compared to those with PN. One-way ANOVA was used to analyse the expelled mask and swab viral RNA copies at enrolment 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-value=0.009, Fig. 2a). The GN+IgG-N group had 1.3 log fold p-value-0.036) and 2.6 log fold (p-value<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 (Fig. 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-value - 0.026, Fig. 2c). Interestingly, there were no patients with high emission patterns in the GN+IgG-N group (Fig. 2c). For the sample size indicated, the reduction in 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 neutralisation capacity and more

with IgG-N response are likely to emit lower viral RNA copies and thus may have a low risk for transmission.

Factors associated with high emission pattern:

Univariate logistics regression analysis for the overall cohort (n=92) showed that cough as a symptom at enrolment, shorter duration between symptom onset and enrolment, presence of comorbidity, poor neutralisation, no IgG reactivity for S/N protein and presence of symptoms beyond eight days from symptom onset were independent predictors (higher odds ratio) of patients being high emitters (Supplementary Fig. 5). However, in multivariate logistics analysis, after adjusting for associative factors, only the presence of comorbidities, cough as a symptom at enrolment and poor neutralisation continued to be independent and significant predictors of high emission pattern (Supplementary Fig. 5). Moreover, patients who had all of these three risk factors had eight times higher odds of being high emitters (OR-8.833, p-value 0.008).

Discussion

In this study, we used a simple adapted N95 mask sampling combined with RT-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.

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 RT-PCR detectable levels of virus in respiratory particles within 48 hours of diagnosis. This proportion was about 2-fold more than people infected with SARS-CoV-2 before the emergence of Delta in 2020, noted by us and others [16,22,23]. Noticeably, the proportion of high emitters was 3-fold more than in our earlier study conducted in 2020 [16]. As both studies were 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 [4,24,25]. More importantly, similar to our previous study [16], the proportion of high emitters (40%) observed in this study also correlated to the reported Delta variant–related SAR (30.8%, 95%CI, 23.5%-39.3%) derived from a meta-analysis of household contact studies [26]. The high emitter proportion also matched a South Korean Delta outbreak study which showed that only 40% of the individuals caused all secondary infections [27]. 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 [28,29] and very recently for SARS-CoV-2 [30]. 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 3-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 [16] 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.

Learning 2: Only vaccinated patients with good SARS-CoV-2 neutralising 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 (Fig. 1) was similar in partial, full and un-vaccinated 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 non-significant swab viral loads in vaccinated and

unvaccinated individuals [12,13,31]. However, studies based on contact tracing [10,11,13] and infectious virus measurements, primarily in mRNA vaccinated individuals, [14,32,33] 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. [11] 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 neutralising antibodies at the time of infection, a known correlate of protection from infection for vaccinees [18,34]. The current results show that only vaccinated individuals having good virus neutralising capacity had a lower propensity to be high emitters with a potentially lower risk for transmission (Fig. 2). These patients exhibited similar levels of cross-protection to both ancestral and Delta variants (Supplementary Table 3) and also showed increased levels of IgG-S antibodies (Supplementary Table 3). This was further augmented in fully vaccinated individuals who showed evidence of past infection (IgG-N reactive, Supplementary Table 3).

Our findings suggest that only vaccines that elicit broad neutralisation 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 neutralising antibody titers and the development of new/multivalent vaccines with broadly neutralising antibodies. Further, 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.

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 ten days from symptom onset, provided they did not have fever for about 24 (USA)-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 [35-37]. 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 [38]. In this study, we have shown that the likelihood of being a high emitter was 7% at a late stage of infection (Supplementary Fig. 3c). 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 (Supplementary Table 1). 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. [38] 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.

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 three days. Hence, we could neither define study patients as an index in many cases nor establish a definite relationship between their viral load on the household transmission. Moreover, the study measured the transmission of relevant viral RNA copies emitted through RT-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 mask viral load could indicate transmission risk [30], the Delta variant had a propensity for higher transmission [4,25], and

vaccination helps in reducing transmission [11], 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 used has applications for future pandemics and can be extended to testing other airborne respiratory viruses.

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Author Contributions

KS and AS contributed to conceptualisation, project development and management, study design, patient recruitment, data analysis and interpretation, and drafting and revising the manuscript. SV, TM, GP and SS contributed to investigations, data acquisition, data management and visualisation. SV also contributed to the revision and editing of the manuscript. VO contributed to conceptualisation, providing resources and patient recruitment. PK contributed to sequencing analysis. KN contributed to sequencing resources and **the** revision of the manuscript. DS and MG provided resources and approvals for the successful conduct of the study. NFM contributed to conceptualisation, study design, data interpretation, manuscript revision, and overall supervision. KS and NFM acquired funding for the study.

Statements and Declarations

Competing Interest - The authors have no relevant financial or non-financial interests to disclose.

Consent to Publish- The authors affirm that all research participants provided informed consent to publish.

Data Availability statement - The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure Legends

Fig. 1 – Mask viral load reduction from enrolment (Enr) to follow-up (FU) in the various vaccine groups, Partial -≥ 7 days from 1st dose or <14 days from 2nd dose of Covishield/COVAXIN; Full-Two doses of Covishield/ COVAXIN and ≥14 days from 2nd dose; Cs- Two doses of Covishield and ≥14 days from 2n dose and Cx- Two doses of COVAXIN and ≥14 days from 2nd dose, Unvac- Unvaccinated, Enr-Enrolment and FU- Follow up. ** indicates statistical significance with p<0.01; *** indicates statistical significance with p<0.001

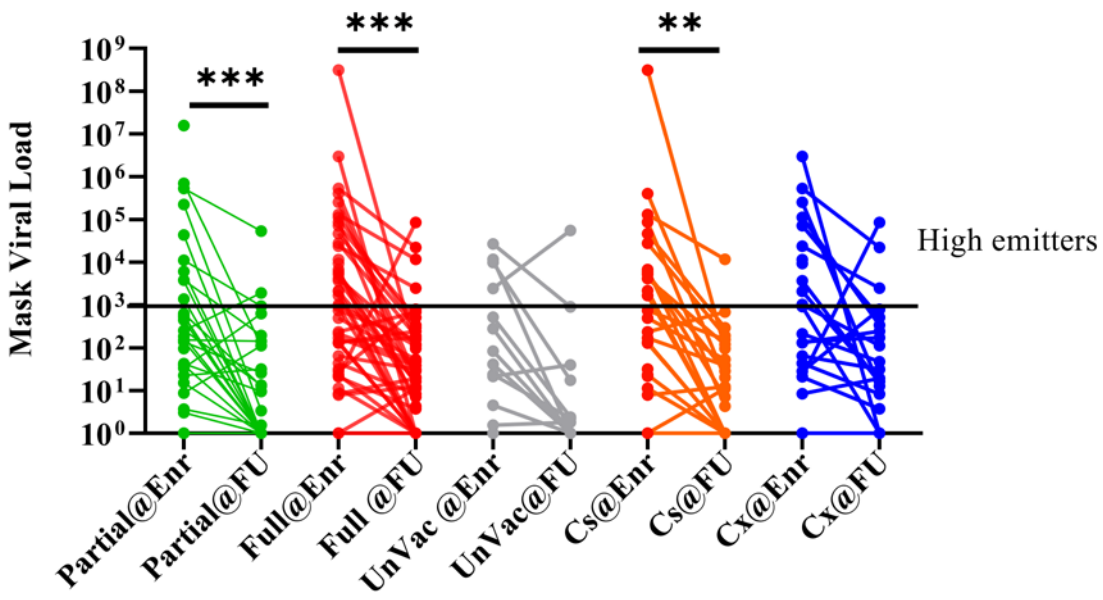

Fig. 2 –Comparison of SARS-CoV-2 emission pattern based on neutralisation and anti-nucleocapsid IgG – A: Comparison of mask viral RNA copy numbers, B: Comparison of swab viral RNA copy numbers, C: Comparison of proportion of high emitters. PN: Poor neutralisation group with non-reactive IgG-N. GN-IgG-N: Good neutralisation group with non-reactive IgG-N, GN+IgG-N: Good SARS-CoV-2 neutralisation group with reactive IgG-N, ** indicates statistical significance with p<0.01; *** indicates statistical significance with p<0.001

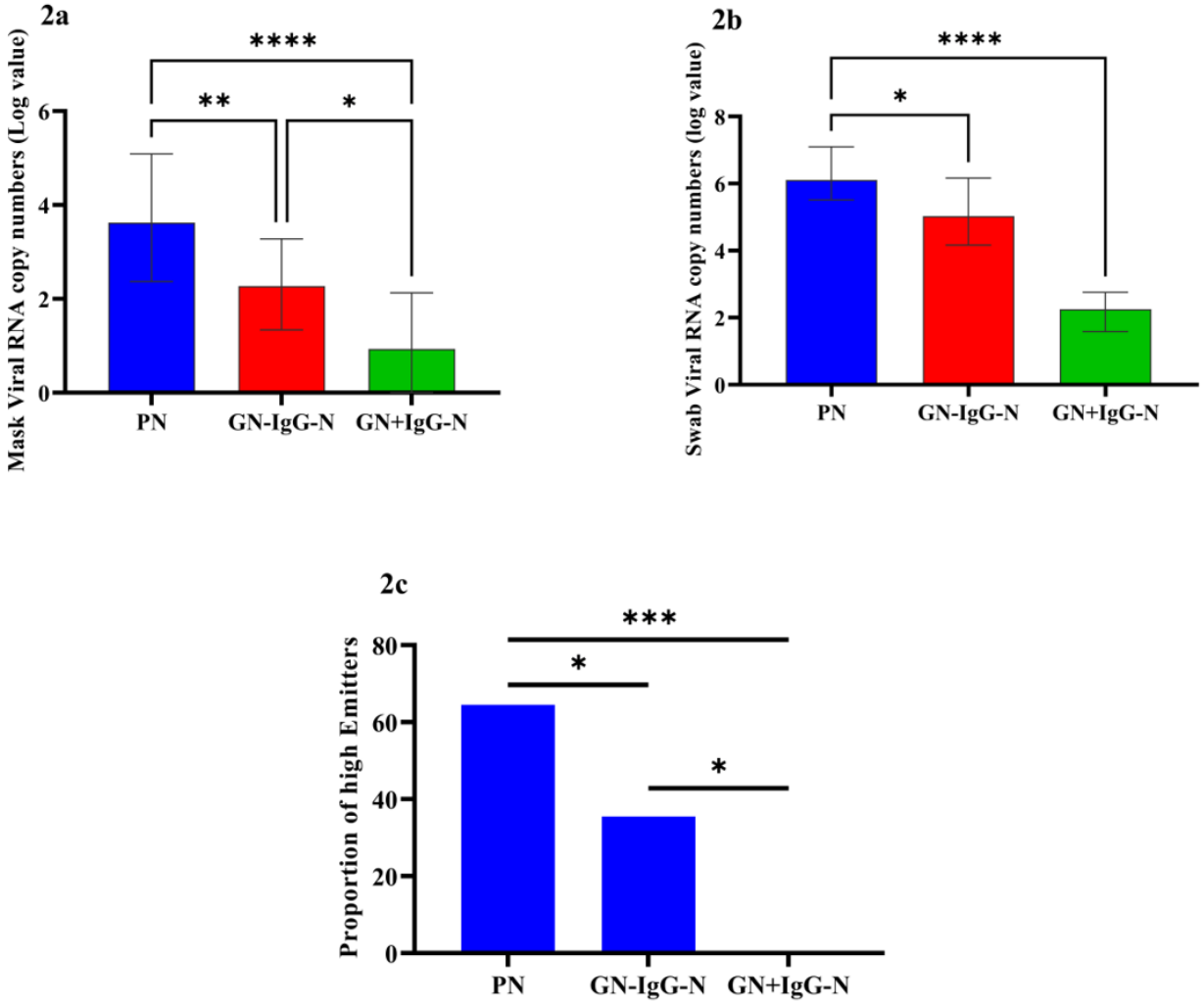

Table 1 – Comparison of patient demographics, COVID-19 symptom characteristics and mask sampling characteristics at enrolment and follow-up stratified based on vaccination status

All values are represented as median (IQR) unless specified otherwise. Numbers in the square bracket represent percentages. $Drugs like Fabiflu, Ivermectin or Remdesivir with demonstrated *in vitro* anti-viral activity were considered as anti-virals. Partial -≥ 7 days from 1st dose or <14 days from 2nd dose of Covishield/COVAXIN; Full-Two doses of Covishield/ COVAXIN and ≥14 days from 2nd dose; Cs- Two doses of Covishield and ≥14 days from 2nd dose and Cx- Two doses of COVAXIN and ≥14 days from 2nd dose, Unvacc- Unvaccinated; IQR – interquartile range, p-value **(a)** – significance calculated on comparing vaccinated groups with unvaccinated. p-value **(b)** – significance calculated on comparing Cs with Cx, * - significant on comparing fully and partially vaccinated groups, NA - Not Applicable

Table 2 – Comparison of mask and NPS positivity at enrolment and follow-up

Percentages are indicated in bold; partial -≥ 7 days from 1st dose or <14 days from 2nd dose of Covishield/COVAXIN; Full-Two doses of Covishield/ COVAXIN and ≥14 days from 2nd dose; Cs- Two doses of Covishield and ≥14 days from 2nd dose and Cx- Two doses of COVAXIN and ≥14 days from 2nd dose, Unvacc-Unvaccinated. The previous study – results are available at https://doi.org/10.1371/journal.pone.0249525;

Table 3 – Proportion of patients with SARS-CoV-2 specific antibody response

Partial -≥ 7 days from 1st dose or <14 days from 2nd dose of Covishield/COVAXIN; Full-Two doses of Covishield/ COVAXIN and ≥14 days from 2nd dose; Cs- Two doses of Covishield and ≥14 days from 2nd dose and Cx- Two doses of COVAXIN and ≥14 days from 2nd dose