

Differential aerosol shedding of SARS-CoV-2 Delta and Omicron variants during respiratory activities

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Brief Communication

Keywords: SARS-CoV-2, COVID-19, aerosol transmission, airborne transmission, Delta, Omicron variants of concern

Posted Date: February 11th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1348915/v1>

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Abstract

Data on the viral loads in respiratory aerosols from patients infected with Delta and Omicron variants are limited. In this study, we used an exhaled breath bioaerosol collector to collect aerosol samples in coarse ($> 5\mu\text{m}$) and fine ($\leq 5\mu\text{m}$) fractions from COVID-19 patients infected with these VOCs while doing various respiratory activities. Samples were tested via SARS-CoV-2 RT-qPCR and virus culture. Nine patients (4 Delta and 5 Omicron) were included. Viral RNA was detectable in seven participants, with greater viral loads in fine aerosols. Notably SARS-CoV-2 RNA was consistently detectable in respiratory samples of all Omicron patients despite them being fully vaccinated and mostly asymptomatic in contrast with Delta patients. Singing and talking without mask generated the greatest viral loads underscoring the transmission potential of SARS-CoV-2 and its variants via respiratory aerosols. The more consistent detection of viral RNA in Omicron-infected patients may account for its greater transmissibility.

Background

Periodic emergence of novel variants of concern (VOCs) has confounded efforts to eliminate SARS-CoV-2 transmission, due largely to changes in transmissibility, virulence, and immune evasion. Transmission may occur across a range of distances via respiratory particles including finer aerosols – although the precise extent and proportion of aerosol transmission remain uncertain. The rapid spread of more recent VOCs such as the Delta and Omicron variants has raised the question as to whether aerosol transmission is more efficient with these variants, as this would have a significant impact on public health interventions.

We have previously demonstrated that SARS-CoV-2 virus and its viral components can be shed via aerosols emitted from activities such as breathing, talking and singing (1), while others have managed to culture viable virus in similar fine aerosol samples in a small number of subjects (2). Both studies were conducted early in the Delta wave and before the Omicron wave which has rapidly spread globally, and there are limited data examining the impact of these variants on the extent of aerosol shedding and transmission. In this follow-up study, we employed the same sampling methodology to collect aerosol samples from patients infected with Delta and Omicron VOCs, and to evaluate viral loads and infectivity during different respiratory activities.

Methods

We recruited patients with SARS-CoV-2 infection confirmed by reverse transcription-polymerase chain reaction (RT-PCR) and hospitalized at the National Centre for Infectious Diseases. There were no specific inclusion criteria, but we attempted to select patients as early into the infection phase as possible and with an index nasopharyngeal swab PCR cycle threshold (Ct) value of < 25.0 . Patients requiring supplemental oxygen or with severe disease were excluded as they were likely to be unfit for completion of study procedures. Patients with co-infections were also excluded to minimize confounding effects from other pathogens. Using the Gesundheit G-II exhaled breath collector (3), we collected aerosol samples from participants while they were breathing, talking, and singing without wearing a mask as previously described (1). We further assessed the impact of mask-wearing by repeating the talking segment with subjects wearing a standard surgical mask (ASSURE, Pharmex Healthcare, Singapore). Aerosols were collected as fractions of two sizes, namely coarse ($> 5\mu\text{m}$) and fine ($\leq 5\mu\text{m}$). We also collected nasal swabs prior to and on the same day of sampling. Samples were processed in a biosafety level-3 laboratory for virus culture and RT-qPCR (for N1 gene targets) to assess infectious viral load and viral RNA load, respectively. Samples were inoculated onto VeroE6 TMPRSS2 cells (for both passages of 14 days each) which represent the more suitable cell line for SARS-CoV-2 culture (4). Whole viral genome sequencing was conducted by the National Public Health Laboratory for VOC identification. Due to the relatively small sample size, statistical tests of significance were not performed, and our results are reported descriptively. This study was approved by the National Healthcare Group Domain Specific Review Board (reference number 2020/01113). All study participants provided written informed consent.

Results

Nine patients were recruited – 4 were infected with Delta VOC and 5 with Omicron VOC. Table 1 summarizes the aerosol test results, together with participant demographics, vaccination status, and symptoms on the day of sampling. Viral RNA was detectable in seven (77.8%) participants in at least one aerosol fraction including all patients with Omicron. Fine aerosols exhibited higher positivity (77.8% vs 44.4%) as well as greater viral loads (median 774.6 vs 354.2 copies, summing all respiratory activities) compared to coarse aerosols. The range of viral RNA copies per respiratory activity (99.2 to 5964.9 copies) was similar to our previous study (63.5 to 5821.4 copies) which included individuals infected with wild-type SARS-CoV-2 as well as variants including Alpha, Beta, and Kappa and one Delta (1). All viral cultures of aerosol samples were negative after two consecutive passages. However, cytopathic effect was observed for viral cultures of nasal swab samples from participants 5 and 6 (Supplementary Figure S1).

Table 1
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in respiratory aerosols emitted by coronavirus disease

No	Age	Sex	Number of vaccine doses		Symptoms	Clinical Ct value from index nasal swab	Ct value from nasal swab on sampling day		Variant	CPE (Viral culture) from nasal swab		Viral Load		
			Fine	Coarse			Fine	Coarse		Fine	Coarse	Nasal swab (sampling day)	Breathing without mask	Singing without mask
1	64	M	0	6	Fever	12.10		N.A	Delta	N.A.	N.A	368.9	N.D.	1
2	47	F	2	11	Fever, cough	24.80	28.88		Delta	No	26166.5	N.D.	N.D.	1
3	49	M	2	5	Cough	24.40	N.D.		Delta	No	N.D	N.D.	N.D.	1
4	57	M	2	9	Asymptomatic	22.00	23.43		Delta	No	1096691.5	N.D.	N.D.	1
5	41	F	2	7	Cough	23.10	14.57		Omicron	Yes	477804799.1	139.2	N.D.	1
6	37	M	2	5	Asymptomatic	19.70	14.61		Omicron	Yes	464557034.5	1069.8	275.5	3
7	44	M	2	11	Asymptomatic	20.80	30.73		Omicron	No	7370.3	232.4	375.8	4
8	46	F	3	8	Asymptomatic	23.10	29.15		Omicron	No	21728.6	N.D.	N.D.	1
9	36	M	2	5	Asymptomatic	21.30	N.A		Omicron	N.A.	N.A	1616.8	N.D.	1

Ct = cycle threshold; CPE = cytopathic effect; N.A. = not available; N.D. = not detected; M = male; F = female

^a Data not available as patients did not complete these study procedures.

^b Excluding incomplete respiratory activities (singing without mask, talking with mask)

Participants 1 and 9 did not complete all the study procedures, resulting in missing data for singing without mask and talking with mask. Comparing the mean viral load (summing fine and coarse samples) per respiratory activity per participant (among participants with detectable virus for that activity), singing without mask and talking without mask generated higher viral loads (mean viral copies of 1327.0 and 1336.7, respectively) compared to breathing (mean viral copies of 815.7). Comparing individuals with data available for talking with and without mask (participants 2, 5, 6, 7, and 8), talking with and without surgical mask generated similar viral RNA amounts in collected aerosols (total copies of 1487 vs 1239.4; mean copies of 297.4 vs 247.9).

Viral RNA in aerosol samples was consistently detectable in all 5 participants infected with Omicron variant as opposed to only 2 out of 4 participants infected with Delta variant, despite similarity in day of illness during sampling. Furthermore, this consistent detection of Omicron occurred despite all 5 subjects receiving two vaccine doses, with one subject inoculated with an additional booster dose.

Discussion

In this study, we found that patients infected with Delta and Omicron variants generated detectable viral particles in respiratory aerosols, similar to other findings in patients with previous SARS-CoV-2 variants. Consistent with previous reports, fine aerosols accounted for a larger proportion of the total viral load, raising the possibility of transmission over greater distances. The negative viral cultures from aerosols may continue to reflect the technical difficulties in viral aerosol sampling as opposed to true non-infectivity of exhaled viral particles (5). However, viable virus was cultured from nasal swabs of two Omicron-infected participants, which may corroborate what others found where Omicron is concentrated in the upper airway in animal models (6).

In this pilot study, we observed similar viral loads generated from aerosols of Delta and Omicron variants during talking with and without surgical masks. This finding may be attributed to the relatively small sample size and single mask type studied, and limited by the inability to quantify viable viruses. Other modelling studies have also shown less efficient reduction of infectious aerosols as well as leakage from surgical masks (7, 8). Furthermore, our sampling method induces suction of aerosols from the facial region, whether emitted through or leaked around the mask. Future studies should systematically investigate the effects of various mask types on infectious respiratory aerosol production. These will be critical to help craft public health policies to reduce aerosol transmission including respiratory protection, social distancing, ventilation and air filtration and disinfection.

The significantly enhanced transmissibility of Delta variants was hypothesized to be related to increased respiratory viral loads (9). which was thought to lead to increased aerosol transmission via greater respiratory shedding (10). On the other hand, emerging data shows that respiratory viral loads are comparatively lower for the Omicron variant, despite its greater transmissibility (11–13). Our preliminary data suggest that while Omicron infected patients have similar individual viral loads with Delta and other variants, Omicron virus were detected more consistently in respiratory aerosols even in vaccinees – albeit limited by the small sample size and inability to adjust for confounders such as day of illness and clinical symptoms. Our data indicated no difference in the magnitude of aerosol emissions between the variants tested suggesting that it is not aerosol emissions per se that accounts for the dominance of the Omicron variant over delta. Rather, the more consistent presence of Omicron variant RNA in aerosols from all five fully vaccinated patients studied, suggests that perhaps better adaptation to different hosts, enhanced receptor binding or greater immune and vaccine evasion, may explain its increased transmissibility (14–17).

We could not statistically analyze the effects of vaccination status and clinical symptoms on respiratory viral shedding. However, we observed that fully vaccinated patients with the Omicron variant including one subject with a booster could generate detectable viral RNA copies in fine respiratory aerosols unlike two of our Delta patients. This suggests that vaccination alone is insufficient to stop secondary spread, as was seen in reports of secondary household transmission from vaccinated individuals infected with both variants (18–20). Nonetheless, broad community vaccination with effective vaccines will remain a key public health intervention to mitigate the impact of future waves on healthcare systems as they remain effective in preventing severe disease.

As observed previously, we also noticed a significant divergence of viral RNA copies between patients, with participants 6 and 9 accounting for 74.4% of the total detectable viral aerosols. This is consistent with the “super-spreader” phenomenon and inter-individual heterogeneity observed in COVID-19 transmission (21), where a minority of infected patients contributes to a majority of secondary transmission (22). This also needs to be further explored.

Conclusion

Our preliminary study underscores the transmission potential of Delta and Omicron variants via fine and coarse aerosols from infected patients during normal respiratory activities. The more consistent detection of Omicron viral RNA despite vaccination and minimal symptoms may contribute to its higher transmissibility. More detailed studies exploring molecular, physical, and host responses to these variants and sub-variants (20) will be critical to elucidate the mechanisms underpinning their increased transmissibility, and to formulate targeted public health interventions.

Declarations

Funding

This work was supported by the Singapore National Medical Research Council [MOH-000443 to KWT, and NMRC/CG/M009/2017 NUH/NUHS to JJHC] and the National University of Singapore [NUS Reimagine Research Grant to JJHC].

Acknowledgments

We thank the NUS Medicine BSL-3 Core Facility team for their support in BSL-3 procedures. We gratefully acknowledge the University of Maryland for the availability of the G-II machine for the study. We also thank Lin Cui and Raymond Lin from the National Public Health Laboratory for sharing whole genome sequence data for VOC identification. Lastly, we thank Mark Chen I-Cheng, Margaret Soon, Phoon Long Yoke, Loh Kyun Yen, Pang Jia Xin, and all nursing, infection control, and operational staff at the National Centre for Infectious Diseases for their support.

Role of team members

Conceptualization: KWT, VTKC, PAT, KST, SWXO; Investigation and methodology: KST, SWXO, MHK, DJWT, DZHA, YWN, MRBA; Formal analysis: KST, SWXO, MHK, DJWT, DZHA, YWN, KWT. Resources: KST, SWXO, MRBA, JJHC, KWT, VTKC; Writing: KST, SWXO, MHK, VTKC, PAT, KWT; Funding acquisition and supervision: JJHC, PAT, VTKC, KWT. All authors have read and agreed to the final version of the manuscript.

Conflict of Interest Statement

Author PAT reports receiving grants from Roche, Arcturus, Johnson and Johnson, Sanofi Pasteur, and personal fees from AJ Biologicals, outside the submitted work. The remaining authors declare no conflicts of interest. All authors have completed the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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