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Letter to the Editor

High amounts of SARS-CoV-2 in aerosols exhaled by patients with Omicron variant infection

Dear Editor,

In this Journal, Migueres and colleagues commented on the influence of immune escape and nasopharyngeal virus load on the spread of SARS-CoV-2 Omicron variant.[1] Their data demonstrated that the increased transmissibility of the Omicron variant was not explained by higher nasopharyngeal viral load. In this study, we explored the relationship between the high amounts of SARS-CoV-2 in aerosols exhaled by patients with Omicron variant infection and the increased transmissibility. Our findings may help to explain the rapid spread of the Omicron variant.

Omicron variant of SARS-CoV-2, which was designated as a variant of concern (VOC) by the World Health Organization on November 26, 2021,[2] is causing the new wave of COVID pandemic. To date, Omicron variant has been found in more than 40 countries around the world and accounts for 99.9% of new COVID-19 cases in the US.[3] The rapid spread of Omicron variant indicates its high transmissibility. Recently, cell-based pseudovirus experiment showed that infection rate of the Omicron variant was four times higher than that of the wild-type virus, and was twice as high as that of the Delta variant, suggesting higher transmissibility of Omicron than other variants.[4,5] Omicron is the most heavily mutated variant among all the VOCs so far, likely contributing to its enhanced transmissibility.[6,7] However, the airborne transmissibility of Omicron variant remains unclear.

In this study, we monitored the viral aerosols exhaled by patients infected with Omicron variant and analyzed the release of Omicron viral aerosols with COVID-19 development, which may provide the evidence for the high transmissibility of Omicron variant.

36 patients from different foreign countries who were diagnosed with Omicron infection were recruited in this study (Supplementary Table 1). The throat swab COVID tests at the first day after admission were performed by using a SARS-CoV-2 test kit (Liferiver, Shanghai ZJ Bio-Tech Co., Ltd, Shanghai, China). Exhaled breath condensate (EBC) samples were collected 5 min from all the 36 patients by using a BioScreen device (Dingblue Technology Co., LTD, Beijing, China) and 800 μL EBC samples was collected form each patient. Firstly, all collected samples were examined for SARS-CoV-2 using reverse-transcription polymerase chain reaction (RT-PCR), targeting both the ORF1ab and N genes (RT-PCR assay kit from Shanghai ZJ Bio-Tech CO., LTD.). Then the viral load in all SARS-CoV-2 positive EBC samples were further confirmed with absolute quantitative experiments by using standard curve based on plasmid containing S gene (experimental and calculation details are provided in Supplementary Information). The virus breath emission rate (BER) and virus concentration in exhaled air of each patient were calculated by the following equation[8] (detailed method described in Supplementary Information):

Breath emission rate (copies/hour) = $C_{cDNA} \times V_{cDNA} \times (V_{RNA}/V_{RNA\ for\ reverse}) \times (V_{EBC}/V_{EBC\ for\ RNA\ Extraction}) \times (60\ min/T_{EBC})$

Concentration in exhaled air (copies/m^3)=Breath emission rate/Breathing rate

C_{cDNA}: the concentration of cDNA (copies/μL); V_{cDNA}: the overall volume of cDNA; V_{RNA}: the overall volume of RNA; $V_{RNA\ for\ reverse}$: the volume of RNA used for reverse transcription reaction; V_{EBC}: the overall volume of EBC; $V_{EBC\ for\ RNA\ Extraction}$: the volume of EBC used for RNA extraction; T_{EBC}: the time for EBC collection. The breathing rate was assumed to be 12 L/min on average for an adult.[8]

36 patients were divided into three groups for EBC collection. As shown in Fig. 1A, the throat swab COVID tests at the first day after admission of all 36 patients were positive, and no significant differences of Ct value were found among the three groups (for N gene, $P = 0.3866$, 0.2959, 0.8630; for ORF1ab gene, $P = 0.1722$, 0.3114, 0.9237), suggesting the virus loads in these patients were similar. EBC samples were collected at different disease development stages: the early (1–5 days), middle (6–10 days) and late (11–19 days) stages after the onset of COVID-19 symptom (Fig. 2B). RT-PCR results of EBC samples showed that up to 40% of patients with Omicron infection released viral aerosols when they breathed. The positive rates of EBC samples were 41.7% (5/12) in early stage, 38.5% (5/13) in middle stage, and 9.10% (1/11) in late stage.

The virus amounts in the 11 SARS-CoV-2 positive EBC samples were further quantified by absolute quantitation using standard curve. The quantitative data showed that, although the virus positive rates were decreased in EBC samples of the three stages, the virus amounts were similar ($P = 0.1588$). The average viral concentration of EBCs in each group were 4801.99 copies/L (Early), 3699.64 copies/L (Middle), 4736.43 copies/L (Late) (Supplementary Table 1).

The BERs of SARS-CoV-2 from Omicron variant patients, calculated from quantitative data of EBC samples, were shown in Fig. 1C. At the early and middle stages, Omicron variant patients exhaled ten million viral particles per hour, with the average value of 5.8×10^7 copies/hour and 4.4×10^7 copies/hour, respectively. Specially, one patient in the late stage group was even able to exhale 5.7×10^7 copies/hour on the 15th day after the onset of COVID-19 symptom (Supplementary Table 1). The overall BER was from 9.31×10^6 to 2.01×10^8 copies/hour and the virus concentration in exhaled air was from 1.29×10^7 to 2.79×10^8 copies/m^3 (Supplementary Table 1).

In this study, we quantified the viral aerosols released into the air by monitoring the breathing of the patients infected with Omicron variant. The results showed that patients at the early stage of infection (1–5 days) were able to exhale up to billions of viral particles per hour (2.01×10^8 copies/hour). It was ten-fold

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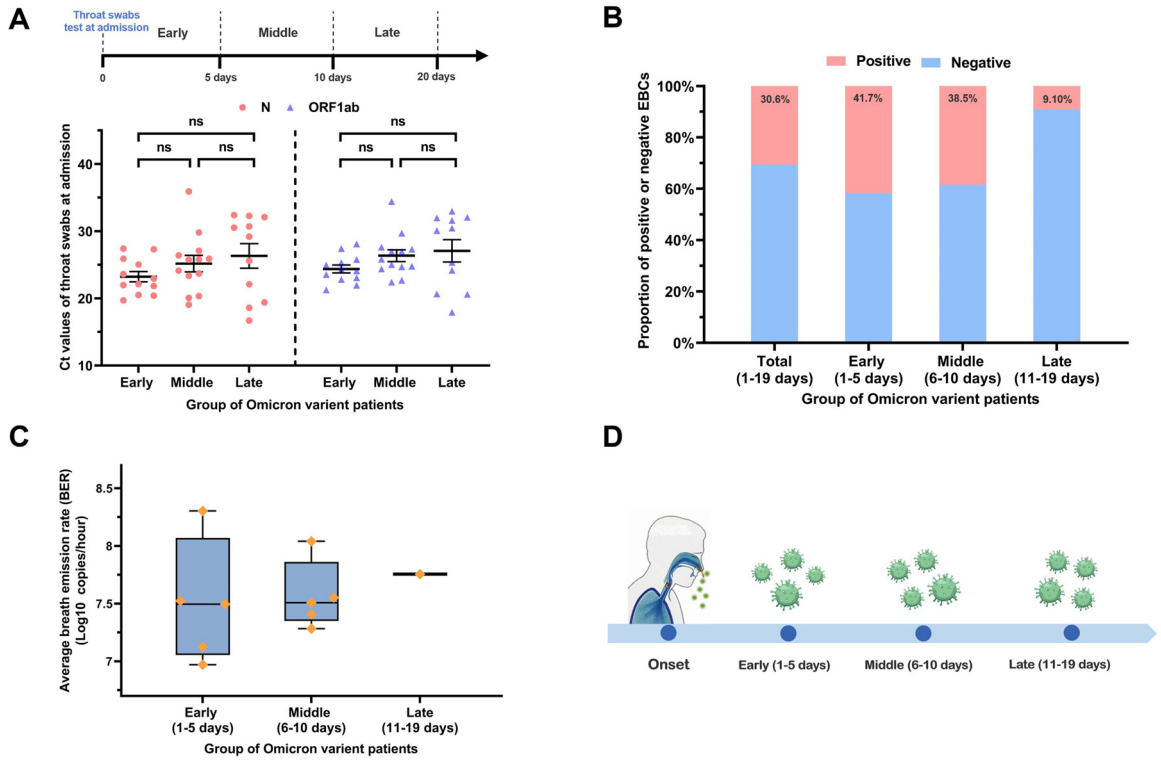

Fig. 1. The viral aerosols exhaled by Omicron variant patients after the onset of COVID-19 symptom. (A) Ct values of throat swabs at admission. Statistical significance between two groups was calculated by unpaired Student's *t*-test. Comparisons among more than two groups were analysed by one-way ANOVA followed by Dunnett's multiple comparison. Values are expressed as the mean ± standard error of the mean (SEM). NS: no significant difference; (B) Proportion of positive or negative EBCs in total groups (1–19 days), early stage (1–5 days), middle stage (6–10 days) and late stage (11–19 days); (C) Breath emission rate (copies/hour) and Ct value of exhaled breath condensate. (D) Omicron variant patients exhaled high amounts of SARS-CoV-2 from early stage to late stage after the onset of COVID-19 symptom.

higher than the highest monitoring data of 2.25×10^7 copies/hour reported in a 2020 study,[8] which used the same method as in this study. Also, the SARS-CoV-2 positive rates in the exhaled air of patients in early and middle stages were higher than that reported in 2020 year (41.7% and 38.5% vs 26.9%).[8] All the data indicated that the Omicron variant might have higher transmissibility than other variants. Furthermore, this study suggested that someone infected with Omicron was able to release viral aerosols even two weeks after the onset of COVID-19 symptom, without decreasing number of virus particles when breathing. Importantly, all the patients recruited in this research have been fully vaccinated.

A recent study reported that Omicron variant showed reduced replication in Calu3 and Caco2 cells and was markedly attenuated in both the upper and lower respiratory tract of infected K18-hACE2 mice in comparison to that of WT or Delta variant, which resulted in its dramatically ameliorated lung pathology.[9] However, our study suggested that the reduced replication of Omicron might not decrease its transmissibility. Extended studies on Omicron infectivity are extremely needed, and special attention should be paid to its sub-variants.

Declaration of Competing Interest

The authors declare no competing interests.

Ethics statement

Sample collection and all experiments in the present study were performed with Ethical Approval given by Ethics Committee of the Center for Disease Control and Prevention of Xiamen.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.02.015.

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