Omicron Positivity in Air of Hospital Settings Gathered COVID-19 Patients, Vaccinated/Unvaccinated Populations

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ABSTRACT

The Omicron variant spreads quicker than the earlier variants and can evade the immune response. The behavior changes and waning immunity could cause large numbers of COVID-19 infections and potential hospitalizations.

Here, we present a cross-sectional/longitudinal study conducted in a tertiary hospital in Istanbul. In the cross-sectional part, we collected aerosol samples from clinical and public areas in the hospital. We performed qPCR and viral culture. In the pediatrics, outpatient clinic waiting room, where the children were without masks, and unvaccinated, 66% of the samples were positive for viral RNA. However, the positivity rate was 14% in the staff dining hall where everybody was without masks but fully vaccinated. The viral RNA was positive in 50% of the COVID-19 patient rooms, 33% of the febrile disease outpatient clinic, and 33% of the clinical laboratory waiting room. There was no viral growth in the culture of all samples. The highest viral load was detected in the COVID-19 patient room (3.60 × 10^{10} PFU m^{-3}), followed by the pediatrics outpatient clinic waiting room (2.8 × 10^{8} PFU m^{-3}). In the longitudinal study, samples were collected 0, 2.5, 4.5, and 24 hours after a meeting in which all attendees were wearing masks and three participants were diagnosed with COVID-19. Only one sample collected 24 hours after the meeting was weakly positive for the viral RNA (1.12 × 10^{2} PFU m^{-3}).

In conclusion, mask use and vaccination are still the main effective methods for preventing the COVID-19 Omicron variant in indoor environments. Unvaccinated children are a significant source of air contamination and risk further transmission of COVID-19.

Keywords: COVID-19, Aerosol, Mask, Vaccination, Transmission

1 INTRODUCTION

Since the start of coronavirus disease of 2019 (COVID-19) in Wuhan, China, in December 2019, the pandemic has spread across the globe. The increase in the number of cases continues with the evolution of the virus to several variants (Araf et al., 2022). The viral transmission mainly occurs via the aerosols produced by the infected person in a crowded indoor environment with poor ventilation (Silva et al., 2022). Air and surface contamination with SARS-CoV-2 was detected in both COVID-19 and non-COVID-19 areas of long-term healthcare facilities and hospitals (Silva et al., 2022). Symptomatic and asymptomatic patients may spread infective viral particles of Wuhan type for up 8 days, and the Omicron variant for up to 14 days (Kapmaz et al., 2021; Riddell et al., 2020; Puhach et al., 2022; Keske et al., 2022). The viability of
the virus in the air depends on environmental factors such as relative humidity and temperature (van Doremalen et al., 2020; Kapmaz et al., 2021; Riddell et al., 2020).

To prevent the spread of SARS-CoV-2, wearing masks, maintaining social distancing, and getting vaccinated were the main guiding principles across the globe (Christie et al., 2021). After implementing the different vaccination programs and the widespread use of vaccines globally, countries relaxed the COVID-19 restrictions in public places as a part of their normalization strategy (Massetti et al., 2022). However, indoor environments continue to pose a risk for infection, especially in the presence of unvaccinated persons. Therefore, healthcare workers, particularly pediatricians, are under high risk of infections (WHO, 2021). The higher transmissibility of the Delta and Omicron variants has increased the spread of infection (Fall et al., 2022). Nevertheless, immune-evading variants, behavior changes, and waning immunity could cause large numbers of COVID-19 infections and potential hospitalizations (Callaway, 2022).

In this autumn and winter seasons, a surge of COVID-19 cases started in the northern hemisphere, but nothing appears to be projecting anything yet. Monitoring air in public places such as schools and hospitals could support infection control measures to prevent virus transmission alongside vaccination programs. Therefore, to evaluate the spread risk of the Omicron variant in indoor environments in vaccinated and unvaccinated populations, we studied the presence and viability of SARS-CoV-2 in aerosol samples collected from medical and public indoor areas in the hospital. Although the study was conducted in the hospital setting, we suggest that the results can be used to present the risk of transmission in the overall population.

2 METHODS

The study was performed at the Koç University Hospital in Istanbul, Turkey. The indoor air samples were collected during the peak period of the Omicron variant between the dates 3rd of January 2022 and the 25th of March 2022.

In the first part, air sampling was performed after an on-site meeting with 16 participants, three of whom tested positive for the Omicron variant on the same day. The identification of Omicron variant was made by variant specific PCR detection system (Bioeksen, SARS-CoV-2 Variant Plus, Turkey) (cat.no: BS-SY-WCOR-402-250). The vaccination status of the participants was recorded. All participants were followed up for symptoms of COVID-19 for one week and requested to give a nasopharyngeal sample for PCR test on day 5 of the exposure. The aerosol samples in the meeting room were collected 1 hour, 2.5 hours, 4.5 hours, and 24 hours after the meeting with a Coriolis µ-biological air sampler (Bertin Instruments, France) with a flow rate of 300 L min^{-1} for 10 min (Fig. 1(a)).

In the second part, different indoor areas of the hospital, where the patients and the hospital staff gather, were examined. Consecutive air samples were obtained from the clinical laboratory waiting room (n = 3), staff dining hall (n = 7), COVID-19 patient rooms (n = 4), COVID-19 patient ward corridor (n = 1), febrile diseases outpatient (n = 3), pediatrics outpatient clinic waiting room (n = 3), routine microbiology laboratory (n = 3) and one sample was from COVID-19 ICU (n = 1). The air sample was collected with a Coriolis-µ biological air sampler with a flow rate of 150 L min^{-1} for 1 hour. The localization of the sample collection areas in the hospital is shown in Fig. 1(b). With the working principle of the Coriolis-µ biological air sampler device, the concentrated airborne particles were transferred into a liquid sample by centrifugation. During the hospital sampling, the device was set next to a power supply, for the COVID-19 ward and ICU, approximately 2 meters away from the patient, and the general areas, to the most crowded location. The sampling time, flow rate, and set-up location were selected and optimized based on the previous literature (Mallach et al., 2021; Silva et al., 2022).

Aerosol samples were collected in 10 mL viral transport medium, DMEM High-Glucose (Sigma-Aldrich^®^, cat.no: D6429) supplemented with 5% Fetal Bovine Serum (HyClone™, cat.no:SV30160.03H), 1% Penicillin-Streptomycin (HyClone™, cat.no: SV30010) and Amphotericin B (HyClone™, cat.no: SV30078.01). All samples were examined for viral RNA with quantitative PCR (qPCR) and viral culture for viable viral particles as described previously (Kuloglu et al., 2022).

Viral RNA extraction was performed using the E.Z.N.A.^®^ Viral RNA Kit (Omega, cat.no: R6874) according to the manufacturer’s instructions. Viral RNA was reverse transcribed to cDNA by
Fig. 1. (a) Distribution of COVID-19 positive participants during the onsite meeting and timings. (b) The cross-sectional sampling areas in the hospital. A central air conditioning system was active in each section.

Superscript II reverse transcriptase enzymes (Thermo Fisher Scientific, cat.no: 18064014). Quantitative PCR (qPCR) was conducted with primers and probes which target the N1 and RdRp genes (TaqPCR was performed in Applied Biosystems™ QuantStudio 7 Flex Real-Time PCR System (cat.no: 4485701) with TaqMan Fast Advanced Master Mix (Applied Biosystems™, cat.no: 4444557). The cycle of threshold values (Ct) was analyzed. For quantification of RNA in the samples, RNA standards were prepared from SARS-CoV-2 Wuhan culture stocks at the concentration of $1.0 \times 10^6$ Plaque forming unit (PFU) mL$^{-1}$. The RNA from viral culture was diluted 10-fold and used as a positive control and standard for quantification. The viral RNA titers per m$^3$ were calculated by multiplying the estimated viral load of each sample with the volume of that sampling area (Santarpia et al., 2022).

HEK293 cells expressing TMPRSS2 and ACE2 receptors (NIH No: NR-52511) were grown in 96 well plates by using DMEM High-Glucose (Sigma-Aldrich®, cat.no: D6429) supplemented with 10% FBS (HyClone™, cat.no: SV30160.03HI) and 1% Penicillin - Streptomycin (HyClone™, cat.no: SV30010), Amphotericin-B (HyClone™, cat.no: SV30078.01). In the Biosafety Level-3 (BSL-3) laboratory, 150 µL from SARS-CoV-2 qPCR confirmed positive aerosol samples were inoculated as duplicates on 90–100% confluent HEK293 cells. One hour after the inoculations, culture supernatants were discarded, and fresh cell culture media was added to wells. The cytopathic effect was monitored in the culture for seven days. On day 7 of the culture, supernatants were collected, and viral growth was confirmed with qPCR as described previously (Keske et al., 2022).

### 3 RESULTS AND DISCUSSION

#### 3.1 The Positivity of Viral RNA in Different Settings of the Hospital with the Cross-sectional Sampling

The estimated viral load, positivity rates, mask use information, environmental conditions, and the number of people present in the sampling location were presented in Table 1. In the cross-sectional sampling, two of the four samples (50%) were collected from COVID-19 patient rooms, and one
The environmental conditions, SARS-CoV-2 RNA positivity, rates and viral loads in different hospital settings.

Table 1. The environmental conditions, SARS-CoV-2 RNA positivity, rates and viral loads in different hospital settings.

<table>
<thead>
<tr>
<th>Room Environment</th>
<th>RNA Positivity</th>
<th>Mask Use</th>
<th>Number of People</th>
<th>Ct Value</th>
<th>Viral Load per m³</th>
<th>Volume (%)</th>
<th>Humidity (%)</th>
<th>Temperature (°C)</th>
<th>Ventilation Rate (m³ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19 ICU</td>
<td>1/1</td>
<td>No</td>
<td>1</td>
<td>38.420</td>
<td>2.38 × 10²</td>
<td>83.91</td>
<td>32</td>
<td>21</td>
<td>9.3</td>
</tr>
<tr>
<td>COVID-19 Patient Rooms</td>
<td>2/4 (50)</td>
<td>No</td>
<td>1</td>
<td>17.666</td>
<td>3.60 × 10¹⁰</td>
<td>97.33</td>
<td>44</td>
<td>22</td>
<td>2.5</td>
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<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>2</td>
<td>36.432</td>
<td>1.45 × 10³</td>
<td>68.28</td>
<td>36</td>
<td>24</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>0–5</td>
<td>&gt; 40</td>
<td>N. A</td>
<td>84.24</td>
<td>27</td>
<td>24</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>2</td>
<td>&gt; 40</td>
<td>N. A</td>
<td>84.24</td>
<td>36</td>
<td>24</td>
<td>2.5</td>
</tr>
<tr>
<td>COVID-19 Patient Ward Corridor</td>
<td>1/1</td>
<td>Yes</td>
<td>5–10</td>
<td>37.89</td>
<td>4.12 × 10¹</td>
<td>751.62</td>
<td>37</td>
<td>24</td>
<td>2.5</td>
</tr>
<tr>
<td>Pediatrics Outpatient Clinic Waiting Room</td>
<td>2/3 (66)</td>
<td>No</td>
<td>20–50</td>
<td>38.532</td>
<td>1.80 × 10²</td>
<td>561.50</td>
<td>38</td>
<td>24</td>
<td>41.3</td>
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<td>Febrile Diseases Waiting Room</td>
<td>1/3 (33)</td>
<td>Yes</td>
<td>10–20</td>
<td>37.497</td>
<td>5.92 × 10²</td>
<td>72.00</td>
<td>38</td>
<td>24</td>
<td>1.25</td>
</tr>
<tr>
<td>Clinical Laboratory Waiting Room</td>
<td>1/3 (33)</td>
<td>Yes</td>
<td>10–20</td>
<td>37.972</td>
<td>2.05 × 10²</td>
<td>144.00</td>
<td>38</td>
<td>24</td>
<td>10.5</td>
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<tr>
<td>Meeting Room</td>
<td>1/5 (20)</td>
<td>Yes</td>
<td>10–20</td>
<td>37.371</td>
<td>1.12 × 10²</td>
<td>124.80</td>
<td>38</td>
<td>23</td>
<td>4.5</td>
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<tr>
<td>Staff Dining Hall</td>
<td>1/7 (14)</td>
<td>No</td>
<td>&gt; 50</td>
<td>36.871</td>
<td>4.22 × 10³</td>
<td>1676.91</td>
<td>42</td>
<td>24</td>
<td>275</td>
</tr>
<tr>
<td>Routine Microbiology Laboratory</td>
<td>0/3 (0)</td>
<td>Yes</td>
<td>10–20</td>
<td>&gt; 40</td>
<td>N. A</td>
<td>486.20</td>
<td>28</td>
<td>21</td>
<td>5.75</td>
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<td>Offices</td>
<td>0/3 (0)</td>
<td>No</td>
<td>10–20</td>
<td>&gt; 40</td>
<td>N. A</td>
<td>218.40</td>
<td>42</td>
<td>24</td>
<td>4.5</td>
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</table>

Sample from the ward corridors was positive with qPCR, but there was no growth in the cell culture. In the first room, the patient was without a mask, and the viral RNA load per m³ of the room air was found to be 3.60 × 10¹⁰ (Ct = 17.66), and the 44% humidity was the highest among all other COVID-19 patient rooms. In the other patient’s room, where the patient was wearing a mask during the air sampling, the viral load was calculated as 1.45 × 10² PFU m⁻³ (Ct = 36.432). Although the patient rooms have negative-pressure air ventilation, we also detected viral RNA in the corridor area (Ct = 37.89, 4.12 × 10¹ PFU m⁻³) of the COVID-19 ward (Fig. 2 and Table 1). There was no growth in the cell culture. The existence and stability of viral RNA-containing aerosols exhaled from infected patients were reported by previous studies (Liu et al., 2020; Santarpia et al., 2022; Kayalar et al., 2021).

We detected the highest rate of viral RNA positivity (66%) in the pediatric outpatient clinic waiting room’s air. The calculated viral load per m³ of air in this area was between 1.80 × 10⁷ PFU m⁻³ and 2.80 × 10⁸ PFU m⁻³, comparable to the air in COVID-19 patient rooms (1.45 × 10² PFU m⁻³ and 3.6 × 10⁸ PFU m⁻³). Yet, there was no growth in the cell culture. During the sampling of this area, all the staff wore their face masks, but the children were without masks. (Fig. 2). The COVID-19 vaccination for children was not approved by the Turkish Ministry of Health yet. Therefore, the high viral load in the aerosols from the pediatric outpatient waiting room suggested that the unvaccinated children are a significant source of the spread of the Omicron variant. Likewise, the shedding of less infectious viral particles of the Omicron variant from the boosted patients compared to the unvaccinated patients highlighted the importance of vaccination to prevent the spread of the Omicron variant (Puhach et al., 2022).

As a simulation for an ideal fully vaccinated population, aerosol samples were collected from the staff dining hall of the hospital during lunch times. The dining hall was the only place where the staff took off their masks to have their meals. We performed seven samplings in the dining room during lunchtime for hospital staff, and only 14% of the samples were positive for viral RNA. All staff in the dining room had received at least two doses of the CoronaVac or BioNTech vaccine. Despite the crowdedness of the dining area and lack of mask use, only one sample out of seven was positive for viral RNA with a low viral load of 4.22 × 10¹ PFU m⁻³, Ct = 36.87, and there was no growth in the cell culture. This positive aerosol sample was collected at the end of lunchtime when there was already a circulation of around 1000 people in the dining hall. The positivity was...
probably due to the accumulation of viral RNA. The low positivity rate and low viral load in this area emphasized the protective role of a full dose of vaccination in indoor environments. A recent study reported that fully vaccinated individuals with booster vaccination shed lower amounts of infectious Omicron particles than unvaccinated individuals. Likewise, it was shown that both the duration of the viable virus and the transmission were detected in lower amounts for the vaccinated individuals compared with unvaccinated individuals (Jung et al., 2022).

The viral RNA loads in the samples collected from the COVID-19 patient’s rooms, the waiting rooms of the febrile disease’s outpatient clinic, and the clinical laboratory also emphasized the importance of mask use. In the febrile disease outpatient clinic and the clinical laboratory waiting room, where people were wearing masks and presumably vaccinated, thirty-three percent of the air samples were positive for viral RNA at low concentrations \((5.92 \times 10^{2} \text{ PFU m}^{-3} \text{ and } 2.05 \times 10^{2} \text{ PFU m}^{-3})\) respectively. We detected a significant difference between the viral loads of COVID-19 Rooms with patients wearing masks \((1.45 \times 10^{2} \text{ PFU m}^{-3})\) and without masks \((3.60 \times 10^{10} \text{ PFU m}^{-3})\).

These findings support the knowledge that masks use in indoor environments to reduce the spread risk of SARS-CoV-2 via the aerosol pathway, especially in potentially SARS-CoV-2-positive individuals, is the most effective preventive strategy (WHO, 2020).

### 3.2 The Assessment of Transmission Risk of the Omicron Variant in the Indoor Environment by Longitudinal Sampling

The result of the longitudinal study corresponds to the findings of the cross-sectional part. Three participants in which diagnosed with COVID-19 presented mild-moderate symptoms such as sore throat, weakness, and headache after the meeting. All received two doses of inactivated vaccine (CoronaVac) and a booster dose of mRNA vaccine (BioNTech). Two of them were also volunteers in another study, and the neutralizing antibody levels of these patients were found to be 1/40 by plaque assay during the same period (Kuloglu et al., 2022). Thanks to all the members of the meeting in the room who were fully vaccinated and wore their masks for the whole length of the meeting, the uninfected 13 participants were fully vaccinated with two doses of CoronaVac and one dose of CoronaVac or three doses of BioNTech. Their PCR results were negative at the end of the 5 days of the follow-up period. The qPCR results of air samples collected from the meeting room and corridor area after 1 hour, 2.5 hours, and 4.5 hours of the meeting were all negative. However, the meeting room’s air was found to be positive for SARS-CoV-2 RNA \((\text{Ct} = 38)\) after
24 hours. The meeting room is an unrestricted space in the hospital that everyone has access to. It was assumed that a potential SARS-CoV-2-positive individual had visited the meeting room during the night, accounting for the positivity. There was no growth in the cell culture of all air samples (Fig. 2). This result further emphasizes the protective role of wearing masks and getting vaccinated in the fact of infected individuals, especially in indoor environments (Cheng et al., 2021; Brooks and Butler, 2021).

In most epidemiological studies, Ct values were used to predict the transmissibility of the infection, and the higher viral load was found to be associated with the higher transmission of infections (Marc et al., 2021). The viral culture should be used for confirmation of infectiousness. Unfortunately, the success of viral culture significantly drops when virus concentration is below six log10 copies (Wölfel et al., 2020). In this study, none of the air samples, even those with RNA over this threshold, yielded viral growth. This could be due to the low infectivity of the viral particles in the air or damage that could have occurred to the viral particles during the air sampling. The low correlation between RNA levels and the cell culture growth rate of the Omicron variant was reported (Puhach et al., 2022). In this study, we performed air sampling with a liquid-based collection device at the flow rate of 150 L min⁻¹ and 300 L min⁻¹. These systems were used for the viral culturing of other viruses before (Chamseddine et al., 2021), but no study reported the SARS-CoV-2 culture in the air collected by these systems. The best environmental conditions for the virus stability are 21–23°C and 40% humidity (van Doremalen et al., 2020). During our air sampling, the ventilation rate, temperature, and humidity settings were at the recommended range of 6 m s⁻¹, 21–24°C, and 27–44%, respectively. Also, the estimates suggested that the Delta variant requires the air change rate to be increased over 1000 times compared to the Wuhan type, which is probably true for the Omicron variant, too (Rowe et al., 2022).

This study has several limitations; the hospital’s air conditioning system is better than most public indoor environments, and these results may not accurately represent the infection risk in public indoor environments. Also, we didn’t have the patients’ Ct values of the nasopharyngeal PCR on the sampling day and did not confirm the variant type. As the last limitation, repeated samples were not collected in some locations due to technical difficulties. Therefore, our sample size isn’t the same for all considered areas.

4 CONCLUSIONS

In conclusion, this study presents that mask use and vaccination are still the main effective methods for preventing COVID-19 in indoor environments. The unvaccinated children are a significant source of air contamination and pose a risk for further transmission of COVID-19. Therefore, it is highly encouraged to implement vaccination programs for children soon.

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